ISSN 0104-4230
ISSN 1806-9282 (On-line)

RAMB

Journal of The Brazilian Medical Association

Volume 70, Number 6
June, 2024

AMB
Associação Médica Brasileira

WMA

RAMB

Journal of The Brazilian Medical Association

Volume 70, Number 6, June, 2024

ISSN 0104-4230
ISSN 1806-9282 (On-line)

SECTIONS

ARTICLES

RAMB
Journal of The Brazilian Medical Association

AMB
Associação Médica Brasileira

INTENSIVE MEDICINE
Israel Silva Maia

INTERNAL MEDICINE
Ana Paula de Oliveira Ramos

LEGAL MEDICINE AND MEDICAL EXAMINATIONS
Rosa Amélia Andrade Dantas

MASTOLOGY
Gil Facina

MEDICAL GENETICS
Ida Vanessa D. Schwartz

NEUROSURGERY
Manoel Jacobsen Teixeira

NEPHROLOGY
Lúcio Roberto Requião Moura

NEUROLOGY
Marcondes Cavalcante França Jr.

NUCLEAR MEDICINE
Diego Pianta

NUTROLOGY
Aline Zanetta

OCCUPATIONAL MEDICINE
Andrea Franco Amoras Magalhães

OPHTHALMOLOGY
Eduardo Melani Rocha

ORTHOPAEDICS AND TRAUMATOLOGY
Sergio Luiz Checchia

OTOLARYNGOLOGY
Thiago Freire Pinto Bezerra

PAEDIATRIC
Lilian dos Santos Rodrigues Sadeck

PAEDIATRIC SURGERY
Lisieux Eyer Jesus

PATHOLOGY
Monique Freire Santana

PHYSICAL MEDICINE AND REHABILITATION
Eduardo de Melo Carvalho Rocha

PLASTIC SURGERY
Daniela Francescato Veiga

PREVENTIVE MEDICINE AND HEALTH ADMINISTRATION
Antônio Eduardo Fernandes D´Aguiar

PSYCHIATRY
Leonardo Rodrigo Baldaçara

PULMONOLOGY / PHTHISIOLOGY
Suzana Erico Tanni Minamoto

RADIOTHERAPY
Wilson José Almeida Jr.

RADIOLOGY
Alexandre Bezerra

RHEUMATOLOGY
Ricardo Machado Xavier

SPORTS MEDICINE
Neuza Mitsuanga

SURGICAL ONCOLOGY
Héber Salvador de Castro Ribeiro

TRAFFIC MEDICINE
José Heverardo da Costa Montal

THORACIC SURGERY
Juliana Dias Nascimento Ferreira

UROLOGY
Roni de Carvalho Fernandes

RAMB - REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
(JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION)

Editors-in-Chief: Renato Deláscio Lopes, José Maria Soares Jr and Roseli Nomura.
Managing Editor: César Teixeira
E-mail: ramb@amb.org.br
Website: www.ramb.org.br

ADDRESS: Rua São Carlos do Pinhal, 324
Bela Vista – São Paulo
Postal Code: 01333-903
Phone no.: (+55 11) 3178-6800 Ext. 177
The RAMB, Journal of The Brazilian Medical Association, is an official publication of the Associacao Medica Brasileira (AMB – Brazilian Medical Association), indexed in Medline, Science Citation Index Expanded, Journal Citation Reports, Index Copernicus, Lilacs, and Qualis B1 Capes databases, and licensed by Creative CommonsR.
Registered in the 1st Office of Registration of Deeds and Documents of Sao Paulo under n. 1.083, Book B, n. 2.

Publication norms are available on the website www.ramb.org.br

THE RAMB IS INDEXED IN SCIELO - SCIENTIFIC ELECTRONIC LIBRARY ONLINE.

Editorial Production

ZEPPELINI PUBLISHERS

https://doi.org/10.1590/1806-9282.706EDIT

The challenges of female chronic pelvic pain

Luciano Gibran[1*], Beatriz Martinelli Menezes Gonçalves[1], Edmund Chada Baracat[1], José Maria Soares Júnior[1]

The topic of chronic pelvic pain is of paramount importance to the gynecologist because this entity compromises the quality of life of women with great prevalence. It is a reason for professional absenteeism and psychosocial disorders that affect the relationships of these women in all areas of their daily lives. The great challenge of the gynecologist in dealing with this entity is justified by the fact that the cause is often multifactorial and that commonly the disorders of the reproductive system can be associated with dysfunctions of the digestive system, urinary system, and musculoskeletal system.

Chronic pelvic pain is defined as persistent, acyclic pain perceived through pelvic structures lasting more than 6 months. It affects around 26% of the female population[1], and its etiology may be associated with several diseases, and the concomitant presentation of pathologies is not uncommon, which occurs in up to 50% of cases[2]. Among the main physical causes of pelvic pain, endometriosis, adenomyosis, irritable bowel syndrome, chronic interstitial cystitis, myofascial pain syndrome, and pain associated with venous return insufficiency stand out. However, it is worth noting that among patients who seek medical care for chronic pelvic pain, about half will have some history of sexual, physical, or emotional abuse, with an important effect on pain modulation and perception. In addition, it is important to remember that there is an association between chronic pelvic pain and other non-pelvic disorders such as fibromyalgia and migraine[1].

The International Society of Chronic Pelvic Pain has created a reference of symptoms and changes in physical examination to guide healthcare professionals in possible diagnoses present in women with chronic pelvic pain. After listening carefully to the patient's complaints, characterizing the type of pain, intensity, association with symptoms, triggering, and improvement factors is essential to aid in the diagnosis and consequently in the appropriate therapy[2]. Symptoms and their connection to the main differential diagnoses are highlighted: association of pain with the menstrual period (endometriosis and adenomyosis), cramp-like pain (inflammatory bowel disease), sensations of shock, burning, and heat (nerve compression), voiding pain and urgency (interstitial cystitis and urethral syndromes), postcoital pain and bleeding (cervical neoplasia), postmenopausal bleeding (endometrial neoplasia), weight loss (malignant and systemic diseases), and history of multiple surgical approaches (fibrosis and adhesion)[2,3].

The Visual Analog Scale (VAS) is the most widely used for quantifying pain intensity and is extremely useful, including for post-treatment follow-up and improvement assessment. There are also symptoms considered "red flags" that should be prioritized over the investigation because they may be associated with the presence of neoplasia or serious systemic diseases, such as rectal bleeding, intestinal symptoms after the age of 50 years, pain that arises after menopause, pelvic mass, suicidal ideation, excessive weight loss, significant vaginal bleeding after the age of 40 years, and bleeding after sexual intercourse[3].

It is essential to have discernment of the type of pain that the patient is referring to, if it is a pain related to the illness of some target organ, which in the vast majority of cases is what we think, known as nociceptive pain, if the origin of the pain is central (hypothalamic), known as nociplastic pain, or even if it is caused by irritation of some peripheral nerve, known as neuropathic pain[3,4].

Patients with chronic pain for long periods can be victims of a process of neuroplasticity, thus changing their perception of the painful stimulus and amplifying the symptoms at the level of the central nervous system[4]. In addition, it is very common to come across cases of female pelvic pain, with mixed pain, of multiple origins.

For the treatment of these pains, surgery is often the solution, requiring the use of alternative therapies such as physiotherapy with myofascial release for patients with "trigger points" in the pelvic region, cognitive behavioral therapy, myorelaxant

[1]Lim_58 laboratório de Ginecologia molecular e estrutural, Disciplina de Ginecologia, Departamento de Obstetrícia e Ginecologia, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo – São Paulo (SP), Brazil.
*Corresponding author: gibran.luciano@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on March 11, 2024. Accepted on March 28, 2024.

medications, tricyclic antidepressants, or selective serotonin reuptake inhibitors and GABA inhibitors (gabapentin and pregabalin)[4,5]. Great care should be taken with the improper prescription of opioids for the treatment of chronic pain due to the risk of addiction to drugs.

Among the various causes of chronic pelvic pain, we highlight the importance of three main non-gynecological causes: interstitial cystitis, myofascial pain, and irritable bowel syndrome.

INTERSTITIAL CYSTITIS

Interstitial cystitis is defined as a chronic inflammation of the bladder and urinary tract whose main symptoms are suprapubic pain, dysuria, and urinary urgency. One of the standout features is pain relief on bladder emptying[6]. Symptoms of interstitial cystitis are commonly found in patients with chronic pelvic pain. In three observational studies of women with pelvic pain who sought treatment, around 38–84% had symptoms suggestive of interstitial cystitis[7-9]. The diagnosis is exclusionary, which sometimes delays the implementation of treatment.

Myofascial pain can arise from changes in the musculoskeletal system. Most patients with this etiology for pain have trigger points identified as areas of muscle band contracture that are very painful on palpation. It is believed that the appearance of trigger points may also be associated with pelvic misalignment, secondary to postural changes due to the effect of pain with its perpetuation. That is, they can be both a consequence and a cause of chronic pelvic pain[3].

Irritable bowel syndrome is a disease that alters gastrointestinal functionality and is mostly characterized by abdominopelvic pain associated with changes in the frequency and formation of stool. It most often presents in relapses and remissions and is more common in patients with psychiatric comorbidities. Although there is no complete understanding of the pathophysiology, it is known that there is a hyperstimulus in the central nervous system with feedback of symptoms[10].

Pelvic varicose veins also represent a concern in the workup in patients with pelvic pain, as there is a direct relationship between pelvic pain and venous compression syndromes that may promote pelvic congestion. This presents as a hard type of pain with worsening at the end of the day and associated lower limb edema in most cases[11].

Compressive syndromes may be related to the presence of arteriovenous malformations that hinder the venous return of the female reproductive system, such as Cockett's syndrome (left common iliac vein is compressed between the right common iliac artery and spine) and Nutcracker syndrome (left renal vein and superior mesenteric artery and aorta)[11].

For the diagnosis, the use of magnetic resonance angiography of the abdomen and pelvis with contrast is necessary, and the treatment requires expertise in the application of stents by interventional radiology[11].

Adenomyosis is also a benign gynecological disease of high prevalence and may or may not be associated with endometriosis, which is considered an important cause of chronic pelvic pain with symptoms that vary according to the degree of involvement[1,3]. It evolves from cyclical pain such as dysmenorrhea, which may or may not exacerbate menstrual blood flow, promote dyspareunia and infertility, and also be considered a cause of chronic pelvic pain[3].

Hormonal treatment with progestogens has good results, but it is restricted to women without reproductive desire or with constituted offspring. In cases of pain refractory to medical treatment and without reproductive desire, total hysterectomy can be an excellent alternative, but it is important to make it clear that in cases where there is an association with deep endometriosis, if it is also not removed during surgery, there may be the persistence of all pain symptoms[2,12].

According to epidemiological studies, endometriosis represents around 50% of the causes of chronic female pelvic pain and is therefore considered the main cause of pelvic pain[12]. In the last 20 years, we have been following the evolution of non-invasive diagnostic imaging through ultrasound with bowel preparation and magnetic resonance imaging of cases of deep peritoneal endometriosis[13].

Despite the concept that there is no direct relationship between the degree of endometriosis involvement and the level of pain presented by the patient, information on the presence of the disease through imaging tests is considered a positive predictor that justifies the cause of pain. In these cases, considering the absence of some conditions such as reproductive desire, signs of intestinal or ureteral subocclusion, presence of large-volume endometriomas, appendix, and ileocecal endometriosis, clinical treatment may be chosen instead of immediate surgical treatment[12,13], and surgical treatment is restricted to cases of refractoriness to clinical treatment and to the absolute indications previously mentioned.

Surgical treatment, on the other hand, is still a great challenge because deep endometriosis surgery is highly complex due to the distortion of the anatomy, which can lead to infiltration of the retroperitoneum toward vital structures such as the ureter, large vessels, and autonomic and somatic pelvic innervation, in addition to intestinal involvement (rectum, sigmoid, ileum, cecum, and appendix), bladder, and diaphragmatic[12-14]. Therefore, there is a need for a multidisciplinary team

for complete resection of the disease. For this reason, there is a very large percentage of patients who undergo suboptimal surgeries with the persistence of the disease and therefore the persistence of symptoms.

The use of hormonal therapy in the postoperative period has been pointed out as a fundamental tool in the attempt to prevent the secondary recurrence of lesions and symptoms[14].

Another less frequent cause, but which we cannot rule out, is pelvic-perineal pain related to damage to the pudendal nerve that can be caused by accidents, bruising, or more often by stretching of the nerve associated with childbirth, pelvic organ prolapse, sports such as cycling, or even patients who remain seated for long periods[15]. The pain pattern is often associated with shock, paresthesia, and burning sensations and can be relieved with changes in positioning that tend to decompress the nerve root[3]. Another therapeutic alternative is imaging-guided infiltration of the Alcock canal with local anesthetics by imaging[15].

Female chronic pelvic pain is indeed a huge challenge for the gynecologist. The latter should be aware of several possible etiologies, both gynecological and non-gynecological, and may require expertise in diagnosis that goes beyond organic pain of organs located in the pelvis and multidisciplinary team effort for the best treatment in the search for long-lasting results.

AUTHORS' CONTRIBUTIONS

LG: Data curation, Formal Analysis, Project administration, Writing – original draft. **BMMG:** Data curation, Formal Analysis, Project administration, Writing – original draft. **ECB:** Supervision, Writing – original draft. **JMSJ:** Writing – original draft, Writing – review & editing.

REFERENCES

1. Lamvu G, Carrillo J, Ouyang C, Rapkin A. Chronic pelvic pain in women: a review. JAMA. 2021;325(23):2381-91. https://doi.org/10.1001/jama.2021.2631
2. Speer LM, Mushkbar S, Erbele T. Chronic pelvic pain in women. Am Fam Physician. 2016;93(5):380-7. PMID: 26926975
3. Jarrell JF, Vilos GA, Allaire C, Burgess S, Fortin C, Gerwin R, et al. Consensus guidelines for the management of chronic pelvic pain. J Obstet Gynaecol Canada. 2005;27(9):869-87. ISSN 1701-2163. https://doi.org10.1016S1701-2163(16)30993-8
4. Siqueira-Campos VM, Deus MSC, Poli-Neto OB, Rosa-E-Silva JC, Deus JM, Conde DM. Current challenges in the management of chronic pelvic pain in women: from bench to bedside. Int J Womens Health. 2022;14:225-44. https://doi.org/10.2147/IJWH.S224891
5. Bułdyś K, Górnicki T, Kałka D, Szuster E, Biernikiewicz M, Markuszewski L, et al. What do we know about nociplastic pain? Healthcare (Basel). 2023;11(12):1794. https://doi.org/10.3390/healthcare11121794
6. Clemens JQ, Erickson DR, Varela NP, Lai HH. Diagnosis and treatment of interstitial cystitis/bladder pain syndrome. J Urol. 2022;208(1):34-42. https://doi.org/10.1097/JU.0000000000002756
7. Clemons JL, Arya LA, Myers DL. Diagnosing interstitial cystitis in women with chronic pelvic pain. Obstet Gynecol. 2002;100(2):337-41. https://doi.org/10.1016/s0029-7844(02)02087-2
8. Os-Bossagh P, Pols T, Hop WC, Bohnen AM, Vierhout ME, Drogendijk AC. Voiding symptoms in chronic pelvic pain (CPP). Eur J Obstet Gynecol Reprod Biol. 2003;107(2):185-90. https://doi.org/10.1016/s0301-2115(02)00363-9
9. Reddy J, Barber MD, Walters MD, Paraiso MF, Jelovsek JE. Lower abdominal and pelvic pain with advanced pelvic organ prolapse: a case-control study. Am J Obstet Gynecol. 2011;204(6):537.e1-5. https://doi.org/10.1016/j.ajog.2011.01.015
10. Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. Lancet. 2020;396(10263):1675-88. https://doi.org/10.1016/S0140-6736(20)31548-8
11. Durham JD, Machan L. Pelvic congestion syndrome. Semin Intervent Radiol. 2013;30(4):372-80. https://doi.org/10.1055/s-0033-1359731
12. Mechsner S. Endometriosis, an ongoing pain-step-by-step treatment. J Clin Med. 2022;11(2):467. https://doi.org/10.3390/jcm11020467
13. Horne AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. BMJ. 2022;379:e070750. https://doi.org/10.1136/bmj-2022-070750
14. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: clinical challenges and novel innovations. Lancet. 2021;397(10276):839-52. https://doi.org/10.1016/S0140-6736(21)00389-5
15. Hibner M, Desai N, Robertson LJ, Nour M. Pudendal neuralgia. J Minim Invasive Gynecol. 2010;17(2):148-53. https://doi.org/10.1016/j.jmig.2009.11.003

https://doi.org/10.1590/1806-9282.20231570

Panel testing may not be sufficient to reveal the etiology of suspected genetic epilepsy

Josef Finsterer[1*], Fulvio Alexandre Scorza[2]

Dear Editor,

We read with interest the article by Baris et al., which is a retrospective, cross-sectional cohort study on 198 pediatric patients with refractory epilepsy and global developmental delay enrolled between July 2018 and July 2021 based on the presence of a pathogenic variant, as assessed by a panel for mutations in genes associated with genetic epilepsy[1]. The most commonly mutated genes in this cohort were the *SCN1A* and *TBC1D24* genes, followed by the *CACNA1A* and *KCNQ2* genes[1]. A pathogenic variant was detected only in three patients (*ALDH7A1*, *KCNQ2*, and *SCN1A*)[1]. It has been concluded that gene panels support the diagnosis of refractory epilepsy, whereas the undiagnosed conditions remain[1]. The study is impressive, but some points require discussion.

The major limitation of the study is that the pathogenicity of the detected variants was not confirmed by functional or biochemical analysis but only by in silico testing[1]. No population data, computational data, functional data, or segregation data were described. Before a variant is classified as pathogenic, certain criteria as defined by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology system must be met[2]. There are also other classification models that should be satisfied which classify genetic variants as hypomorphic alleles, imprinted alleles, copy number variants, runs of homozygosity, enhancer variants, and variants related to traits[3].

The second limitation is that a panel testing was performed[1], which has the disadvantage that new mutated genes not included in the panel may be missed. Panel studies allow only confirm what has been reported previously, while whole exome sequencing (WES) or whole genome sequencing (WGS) allows the detection of a broader spectrum of disease-causing variants.

The third limitation is that panel studies for epilepsy-associated mutations included only genes located on nuclear DNA (nDNA). However, a number of mitochondrial disorders (MIDs) due to variants in mitochondrial DNA (mtDNA) and transmitted through the maternal line manifest phenotypically with epilepsy[4]. Therefore, it is recommended to screen epilepsy patients not only for nDNA but also for mtDNA variants in order not to miss a MID with epilepsy. The best known syndromic MIDs with epilepsy include mitochondrial encephalopathy, lactic acidosis, and stroke-like episode (MELAS) syndrome, Leigh syndrome, Kearns-Sayre syndrome (KSS), and some of the mitochondrial DNA depletion syndromes[5].

The fourth limitation is that whether the variants occurred in a homozygous or heterozygous distribution was not reported. Knowledge of allele dosage is crucial for predicting outcome and for genetic counselling.

The final limitation is that it was not clarified whether the detected variants were inherited or occurred sporadically. To find out whether the variants were inherited or sporadic, it is imperative to perform family screening for the causative variants. How many of the 198 patients had a positive family history of epilepsy? How many had other first-degree relatives tested for the detected variant of an index patient?

In summary, the interesting study has limitations that put the results and their interpretation into perspective. Addressing these issues would strengthen the conclusions and could improve the status of the study. To clarify the underlying genetic defect in patients with suspected genetic epilepsy, it is imperative to obtain a thorough family history and perform not only panel tests but also WES and, if inconclusive, WGS.

[1]Neurology and Neurophysiology Center – Vienna, Austria.
[2]Unibersidade Federal de São Paulo/Escola Paulista de Medicinal, Neuroscience Discipline – São Paulo (SP), Brazil.
*Corresponding author: fifigs1@yahoo.de

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on November 16, 2023. Accepted on November 27, 2023.

STATEMENT OF ETHICS

(a) The study was approved by the institutional review board (responsible: Finsterer J.) at November 4, 2022. (b) Written informed consent was obtained from the patient for publication of the details of their medical case and any accompanying images.

DATA AVAILABILITY STATEMENT

Data that support the findings of the study are available from the corresponding author.

COMPLIANCE WITH ETHICS GUIDELINES

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

AUTHORS' CONTRIBUTIONS

JF: Conceptualisation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. **FAS:** Investigation, Validation, Writing – review & editing.

REFERENCES

1. Bariş S, Kırık S, Balasar Ö. Importance of targeted next-generation sequencing in pediatric patients with developmental epileptic encephalopathy. Rev Assoc Med Bras (1992). 2023;69(10):e20230547. https://doi.org/10.1590/1806-9282.20230547
2. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. https://doi.org/10.1038/gim.2015.30
3. Houge G, Laner A, Cirak S, Leeuw N, Scheffer H, Dunnen JT. Stepwise ABC system for classification of any type of genetic variant. Eur J Hum Genet. 2022;30(2):150-9. https://doi.org/10.1038/s41431-021-00903-z
4. Finsterer J, Zarrouk Mahjoub S. Epilepsy in mitochondrial disorders. Seizure. 2012;21(5):316-21. https://doi.org/10.1016/j.seizure.2012.03.003
5. Lim A, Thomas RH. The mitochondrial epilepsies. Eur J Paediatr Neurol. 2020;24:47-52. https://doi.org/10.1016/j.ejpn.2019.12.021

https://doi.org/10.1590/1806-9282.20240005

There is currently no evidence that high serum leptin and low insulin-like growth factor-1 levels characterise fibromyalgia

Josef Finsterer[1*], Fulvio Alexandre Scorza[2]

Dear Editor,

We read with interest Atamer et al.'s article on the serum levels of leptin, growth hormone, insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-3 in 30 patients with primary fibromyalgia (FM), which were compared with 30 healthy controls[1]. Serum leptin, tender point count, visual analogue scale (VAS) score, FM impact questionnaire score (FMIQS) and Beck depression inventory score (BDIS) were found to be increased and IGF-1 decreased in FM patients[1]. Leptin levels were positively correlated with VAS score, FMIQS, BDIS, tender point count, and disease duration[1] but negatively correlated with IGF-1[1]. IGF-1 was negatively correlated with age, VAS, FMIQS, BDIS, disease duration, and tender point count[1]. It was concluded that increased serum leptin and decreased IGF-1 levels may be involved in the pathogenesis of FM[1]. The study is impressive, but some points should be discussed.

A limitation of the study is that factors other than FM that influence the level of the parameters analysed in the study were not sufficiently considered and discussed. For example, serum leptin levels depend heavily on the quantity and quality of food intake. High carbohydrate meals increase leptin levels compared to low carbohydrate food. Therefore, we should know what kind of food and how much the included FM patients consumed while starting the 12-h fasting period before blood collection. Serum leptin also increases with the amount of adipose tissue[2]. Therefore, we should know whether all patients had the same amount of body fat and whether leptin levels were correlated with the amount of body fat. Did all patients have the same body mass index? Leptin is an adipokine that regulates appetite and body mass and has many other pleiotropic functions[2]. Leptin acts as a signal to the brain to inhibit food intake and allows excess calories to be stored in fat cells[2].

In addition to FM, IGF-1 is also low when growth hormone, parathyroid, and oestrogen levels are low. Therefore, we should know whether IGF-1 levels correlated with growth hormone, parathyroid, and oestrogen levels. Again, since high-protein diets can increase IGF-1[3], it is important to know what type of food the 30 FM patients included consumed before the 12-h fasting period. It is also important to know whether the 30 FM patients consumed a high-fat diet, particularly whether the proportion of unsaturated fat was high. A diet high in unsaturated fatty acids is known to lower IGF-1[3]. Fasting is also known to reduce IGF-1 levels[3].

Based on these considerations, we do not believe that elevated leptin or low IGF-1 is involved in the pathogenesis of FM. High serum leptin and low IGF-1 are multicausal and, before considering a causal relationship between high leptin/low IFG-1 and FM, more plausible causes of high leptin/low IGF-1 must be excluded.

In summary, the excellent study has limitations that should be addressed before drawing final conclusions. Clarifying the weaknesses would strengthen the conclusions and could improve the study. Before determining that serum leptin is high and serum IGF-1 is low in FM, all alternative causes of low IGF-1 and high leptin must be considered and ruled out.

AUTHORS' CONTRIBUTIONS

JF: Conceptualisation, Data curation, Formal Analysis, Investigation, Validation, Writing – original draft. **FAS:** Formal Analysis, Validation, Writing – review & editing.

[1]Neurology and Neurophysiology Center – Vienna, Austria.
[2]Universidade Federal de São Paulo/Escola Paulista de Medicinal, Neuroscience Discipline – São Paulo (SP), Brazil.
*Corresponding author: fifigs1@yahoo.de

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 15, 2024. Accepted on January 19, 2024.

REFERENCES

1. Atamer Y, Şahbaz T, Aşık HK, Saraç S, Atamer A. The relationship between serum leptin, insulin-like growth factor-1, and insulin-like growth factor binding protein-3 levels and clinical parameters in primary fibromyalgia patients. Rev Assoc Med Bras (1992). 2023;69(10):e20230240. https://doi.org/10.1590/1806-9282.20230240
2. Korczynska J, Czumaj A, Chmielewski M, Swierczynski J, Sledzinski T. The causes and potential injurious effects of elevated serum leptin levels in chronic kidney disease patients. Int J Mol Sci. 2021;22(9):4685. https://doi.org/10.3390/ijms22094685
3. Jafari E, Gheysarzadeh A, Mahnam K, Shahmohammadi R, Ansari A, Bakhtyari H, et al. In silico interaction of insulin-like growth factor binding protein 3 with insulin-like growth factor 1. Res Pharm Sci. 2018;13(4):332-42. https://doi.org/10.4103/1735-5362.235160

LETTER TO THE EDITOR

https://doi.org/10.1590/1806-9282.20240153

Comments on "Investigations of the effects of upper extremity home exercises on grip strength, range of motion, activity performance, and functionality in individuals with systemic sclerosis: a randomized controlled trial"

Pooja Sunil Patil[1], Amit Kumar[1*], Shashi Prakash Sharma[1], Mahima Guleria[1]

Dear Editor,

First and foremost, we extend our sincerest gratitude to the authors for their remarkable ability to articulate their thoughts with utmost clarity and conciseness on the published article entitled "Investigations of the effects of upper extremity home exercises on grip strength, range of motion, activity performance, and functionality in individuals with systemic sclerosis: a randomized controlled trial"[1]. This study has discovered that the implementation of upper extremity home exercises results in an augmentation of grip strength, range of motion, activity performance, and overall functionality among patients diagnosed with systemic sclerosis. We aim to draw attention to several methodological and statistical concerns pertaining to the study, with the purpose of enhancing the utilization of the study findings among healthcare practitioners who handle systemic sclerosis, ultimately leading to improvements in prognosis.

First, as indicated by the title and objective of the abstract, it is not evident that the study was conducted to determine the comparative effects of home exercises versus patients' education on patients with systemic sclerosis (Ssc). However, in the hypothesis section, the authors have discussed a comparison that may cause confusion among the readers. In the participants section, the authors have utilized the 2013 ACR/EULAR criteria for participant selection, but they have not clearly mentioned the scoring system. According to this criterion, a score of ≥9 is classified as definite Ssc[2]. The authors should have carefully specified the criteria to prevent negligence in future studies. Second, in the protocols section, specifically the upper extremity home exercises sub-section, the intervention group comprised patients performing home exercises. However, in the outcome measures section, grip strength and active/passive range of motion were measured using handheld dynamometer and goniometer, respectively. This may be perplexing for readers, as the authors have not mentioned the procedure for measuring these outcomes throughout the entire article. To our understanding, patients either visited the clinical setting or therapists visited the patients' homes for data collection. This crucial information is missing in the article.

Third, in the statistical analysis section, authors have not mentioned clearly about the statistical tests. According to normality, if data follow normal distribution, they should be interpreted in mean and standard deviation with parametric tests. If data do not follow normal distribution, they should be interpreted in median and interquartile range with non-parametric tests[3]. But in this study, authors have mentioned both types of tests (parametric and non-parametric), which may misinterpret the results. Intention-to-treat analysis could have been used by the authors as nine patients lost to follow-up[4]. The results would be incomplete without finding effect size and power analysis. Authors should have focused on this as this is the randomized controlled trial. From the aforementioned valid discussion, we advise the readers to proceed with caution in interpreting the results.

AUTHORS' CONTRIBUTIONS

PSP: Conceptualization. **AK:** Writing – review & editing. **SPS:** Data curation. **MG:** Data curation.

[1]Lovely Professional University, School of Allied Medical Sciences – Phagwara, India.
*Corresponding author: narwalamit336@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 31, 2024. Accepted on February 10, 2024.

REFERENCES

1. Sahin EI, Cetin SY, Ayan A. Investigation of the effects of upper extremity home exercises on grip strength, range of motion, activity performance, and functionality in individuals with systemic sclerosis: a randomized controlled trial. Rev Assoc Med Bras (1992). 2023;69(9):e20230252. https://doi.org/10.1590/1806-9282.20230252
2. Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72(11):1747-55. https://doi.org/10.1136/annrheumdis-2013-204424
3. Cessie S, Goeman JJ, Dekkers OM. Who is afraid of non-normal data? Choosing between parametric and non-parametric tests. Eur J Endocrinol. 2020;182(2):E1-3. https://doi.org/10.1530/EJE-19-0922
4. McCoy CE. Understanding the intention-to-treat principle in randomized controlled trials. West J Emerg Med. 2017;18(6):1075-8. https://doi.org/10.5811/westjem.2017.8.35985

https://doi.org/10.1590/1806-9282.20240183

Digital therapeutics and its role in cancer treatment management: current development and future scope

Akhilesh Vikram Singh[1*]

The progressing era has stepped toward the modernized techniques needed in health development by applying information technology to deliver a better therapeutic intervention. This is similar to other digital apps, which connect patients' clinical reports to software that scrutinizes the patient's required improvements to prevent diseases. Therefore, this is efficiently possible through introducing and implementing digital therapeutics (DTx)[1]. Unlike other diseases/disorders, cancer cells modulate the host zone and suddenly invade other regions, irrespective of therapy. Recently, the global statistical analysis revealed that a 50% increase in prevalence is expected in the forthcoming 20 years, and this would be seen as high in female breast cancer[2]. Therefore, it is essential to choose DTx, a steadily growing interest among patients in which the device principle actively responds to patients' queries based on evidence-based clinical reports built into algorithm/software (Software as a Medical Device – SaMD that empowers timely knowledge, monitoring the symptoms and related stress behavior, its management to improve the quality of life of cancer patient and to keep track on the adverse events of anti-cancer treatments, and reduces hospitalization budget[3]).

Cancer treatment has been the world's highest-expenditure treatment strategy, which remains a challenge due to the unavailability of specific diagnostic biomarkers and effective chemotherapy drugs, showing poor solubility, stability, and limited bio-distribution due to inter-individual ethnicity variations, leading to toxic side effects, and misleading both medical practitioners and patients; however, diverse minority nations attain the least benefit of the treatment or left untreated. In several cases, cancer cells are resistant to anti-cancer drugs, and to overcome this, a scarcity of immunomodulatory drug development is observed, which might benefit patients[4,5]. To overcome the challenges and to keep a self-check, patients must be aware of using the currently available approaches like DTx, which can drive a better outcome through acting like a rehabilitation center under the guidance of a physician, providing counselling sessions to bring changes in lifestyle activities like diet, regular exercise, awareness on the risk factors associated with cancer. The DTx sets some activities and some tasks to attempt and reach the goal of achieving control of mood swings, recovering from depression, and the capability to self-reduce the side effects through yoga and meditation. The DTx gives daily feedback to patients attending the automated medication management combined with a multidisciplinary remote clinical-care team[6].

With a rapid application of cloud computing technologies and artificial intelligence (AI) embedded as sensors in mobiles and wearable gadgets like smartwatches with inbuilt measurable digital biomarkers that touch skin through which they detect and track the changes occurring in patients and transmit the vital signs as biofeedback to the patient, they have been a very useful tool in oncology[7]. A similar trend has been observed in the case of patients with breast cancer and lung cancer. The Sidekick smartphone app was designed with inbuilt information from oncologists and consisted of daily tasks regarding patient's sleep patterns, nutrition intake, psychological mood swings, depression, stress, and meditation. The outcome was recorded for 1 month as increased knowledge, self-awareness, and post-study QoL questionnaires. Finally, high acceptability, retention, and engagement were found in cancer patients who were comfortable using DTx. A global pharmaceutical organization has partnered with the Sidekick Health app to benefit breast cancer patients to confer potential therapeutic plans[8]. Similarly, Moovcare is another DTx that focuses on a questionnaire set every week to detect relapse or complications in lung cancer patients[9].

Although the DTx apps have significantly occupied a place in routine checkups, they will be an added advantage in the future. Still, ethically, DTx is experiencing complex issues like security, regulation, and adoption of this app in all medical sectors. It is a known fact that the FDA, HIPAA, and HITECH are regulatory departments that assure the safety of patients while applying any novel app application as digital medicine to provide reliability and effectiveness, as the DTx is still evolving in

[1]Graphic Era Deemed to be University, Department of Biotechnology – Dehradun, India.
*Corresponding author: akhileshvikram@outlook.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 31, 2024. Accepted on February 16, 2024.

medical treatment; there could be skepticism in adopting DTx and probability of lacking clarity on its consistent requirement. Crucially, with DTx, cybersecurity issues are raised as hackers obtain patients' personal medical information that is secured; therefore, DTx organizations must secure patients' data with appropriate algorithms[1].

With the emerging DTx, in the future, there is scope for collaboration of pharma and DTx organizations to benefit the patient community. The future pipeline involves nine leading DTx companies to bring therapeutics in other therapeutic areas with major applications in neurosciences, cardiovascular, and oncology.

REFERENCES

1. Fürstenau D, Gersch M, Schreiter S. Digital therapeutics (DTx). Bus Inf Syst Eng. 2023;65:349-60. https://doi.org/10.1007/s12599-023-00804-z
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. https://doi.org/10.3322/caac.21660
3. Gussoni G, Ravot E, Zecchina M, Recchia G, Santoro E, Ascione R, et al. Digital therapeutics in oncology: findings, barriers and prospects. A narrative review. Ann Res Oncol. 2022;2:55-69.
4. Chakraborty S, Rahman T. The difficulties in cancer treatment. Ecancermedicalscience. 2012;6:ed16. https://doi.org/10.3332/ecancer.2012.ed16
5. Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. Nano Converg. 2019;6(1):23. https://doi.org/10.1186/s40580-019-0193-2
6. Adatia C, Dreischmeier R, Shah S, Sharma K. The health benefits and business potential of digital therapeutics. 2023. [cited on 2023 Nov 12]. Available from: https://www.mckinsey.com/industries/life-sciences/our-insights/the-health-benefits-and-business-potential-of-digital-therapeutics
7. Gudmundsson GH, Mészáros J, Björnsdóttir ÁE, Ámundadóttir ML, Thorvardardottir GE, Magnusdottir E, et al. evaluating the feasibility of a digital therapeutic program for patients with cancer during active treatment: pre-post interventional study. JMIR Form Res. 2022;6(10):e39764. https://doi.org/10.2196/39764
8. Fiercebiotech.com. n.d. [cited on 2023 Nov 11]. Available from: https://www.fiercebiotech.com/medtech/eli-lilly-taps-sidekick-health-tailor-smartphone-apps-breast-cancer-patients.
9. Lath G, Rhee R, Nikam K. Digital therapeutics in oncology: a better outlook for cancer patients in the future. J Art Intell Big Data. 2022;2:1-8. https://doi.org/10.31586/jaibd.2022.347

https://doi.org/10.1590/1806-9282.20240215

MTHFR genetic testing: is there a clinical utility?

Aline Cristiane Planello[1,2,3], Darine Villela[1*], Thereza Loureiro[1*]

OVERVIEW

The *MTHFR* genetic testing investigates two common variants, 677C>T and 1298A>C, and is frequently ordered by several providers due to the association of those variants with several multifactorial diseases, including cancer[1], autism[2], mental disorders[3], cardiovascular diseases[4], and congenital malformations[5]. The *MTHFR* gene encodes the enzyme methylenetetrahydrofolate reductase (*MTFHR*), which is a crucial component in the one-carbon metabolism pathway that involves folate and homocysteine. Genetic variants in the *MTFHR* may result in decreased enzyme activity, leading to alterations in homocysteine levels. However, it is not clear whether common variants are a major risk factor for diseases because their association with health conditions varies among different populations[5,6]. Notably, some medical companies are currently offering routine *MTHFR* investigation as direct-to-consumer genetic testing. Although this type of testing is becoming increasingly popular due to its accessibility and affordability, it raises concerns about the accuracy of the results and the lack of proper genetic interpretation and counseling for individuals who receive positive results[7]. Several studies have demonstrated the complexity of translating research findings from genetic association studies of common variants in the clinical setting. The relationship between polymorphisms (i.e., common variants) and the risk for multifactorial diseases is complex and influenced by numerous aspects, including ancestry[8]. Furthermore, interpreting polymorphisms, especially in admixed populations, as is the case of the Brazilian population, poses a significant challenge. In this article, we discuss why testing *MTHFR* polymorphisms needs caution, considering that it may not provide significant benefit to patients until more association studies are conducted in diverse populations and the effect of these variants on diseases is fully understood.

MTFHR POLYMORPHISMS: CHALLENGES IN INTERPRETATION

Understanding the impact of DNA sequence variants on the *MTHFR* gene and one-carbon metabolism is crucial given the challenge in interpreting genetic variants. Besides environmental factors such as dietary nutrients, the one-carbon metabolism can be affected by either common or rare variants in the *MTHFR*, each presenting different levels of effect[6]. It is expected that most disease-causing variants are rare in the population, with a low allele frequency (AF) of less than 0.1%. The rarer a variant is, the higher the probability of its pathogenicity. The Online Catalog of Human Genes and Genetic (OMIM) database (https://www.omim.org/) describes 10 rare variants in the *MTHFR* gene associated with enzyme deficiency and severe homocysteinemia. In the context of multifactorial diseases, the genetic background that gives rise to an individual's allelic architecture of the disease reflects the contribution of several variants, which individually or in combination contribute to small increments in risk[8,9]. The AF of those small-effect variants is typically higher in the population than the large-effect variants and are often referred to as polymorphisms (AF>1%). Importantly, the effect of a variant will depend on its type, location, and whether the gene is dosage-sensitive or not. Also, the level of effect or penetrance differs among individuals, which can be explained by interactions between different genetic backgrounds and the individual's exposure to environmental factors[8,9].

The C677T and A1298C represent the two polymorphisms in *MTHFR* which are most frequently investigated in clinical practice. Nonetheless, the results of studies showing the association between *MTHFR* polymorphisms and diseases are mixed, and the strength of the association varies depending on the specific polymorphism and population studied. The global AF of the

[1]Diagnósticos da América S.A., DASA – São Paulo (SP), Brazil.
[2]Faculty of Medicine of Jundiaí, Jundiaí Medical School, Department of Morphology and Pathology – Jundiaí (SP), Brazil.
[3]Universidade Estadual de Campinas, Piracicaba Dental School, Department of Bioscience – Campinas (SP), Brazil.
*Corresponding author: darinevillela@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 05, 2024. Accepted on March 18, 2024.

C677T in the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/) is 30% and reaches 50% in Latino/Admixed American population, in the Brazilian Genomic Variants database (ABraOM) (https://abraom.ib.usp.br/). However, the AF is 33%. In the case of A1298C, the AF is 28 and 24% in gnomAD and ABraOM, respectively (Figure 1). Both variants present an excess of homozygous carriers in those databases. It is worth mentioning that the *MTHFR* polymorphisms in the homozygous form only reduce enzyme production mildly, which has limited pathogenicity. In addition, studies demonstrate that the individual's ancestry can influence the association of common variants with multifactorial diseases[10-14]. Ancestry-specific variants in combination with environmental factors affect gene–gene and gene–environment interactions, differently from rare variants that lead to Mendelian diseases regardless of the population where they occur. In multifactorial diseases, the variants are pathogenic when combined with other variants and environmental factors, in an additive polygenic model. It is important to acknowledge that most genetic studies have primarily focused on individuals with European ancestry, not capturing the degree of diversity that exists in the global population. As a result, the accuracy of risk estimation of a particular variant for non-European populations can be compromised. Not surprisingly, researchers have demonstrated an association between variants in the *MTHFR* and elevated homocysteine levels only in specific populations. We may then argue that such evidence raises concerns about the clinical utility of *MTHFR* genetic testing.

In particular, the Brazilian population is highly admixed and has one of the most heterogeneous genetic compositions in the world, consisting of three ancestral populations: Native Americans, Europeans, and Africans[15]. A high degree of genetic admixture from these three ancestral populations in Brazil was demonstrated. Indeed, in the ABraOM database, 75% of the studied cohort showed admixture from two or more ancestral populations[16]. Therefore, this level of admixture demands caution when applying findings from polymorphism studies to the Brazilian population. This is especially relevant for *MTHFR*, which could confer susceptibility to various diseases via a polygenic model that can be heterogeneous between ethnic groups. For example, a meta-analysis of 40,173 individuals explored the association between C677T and hypertension risk and showed that the T allele was associated with an increased risk of hypertension in individuals carrying the homozygous TT genotype[17]. However, stratification by ethnicity revealed that the association only existed in Asians and Europeans, but not

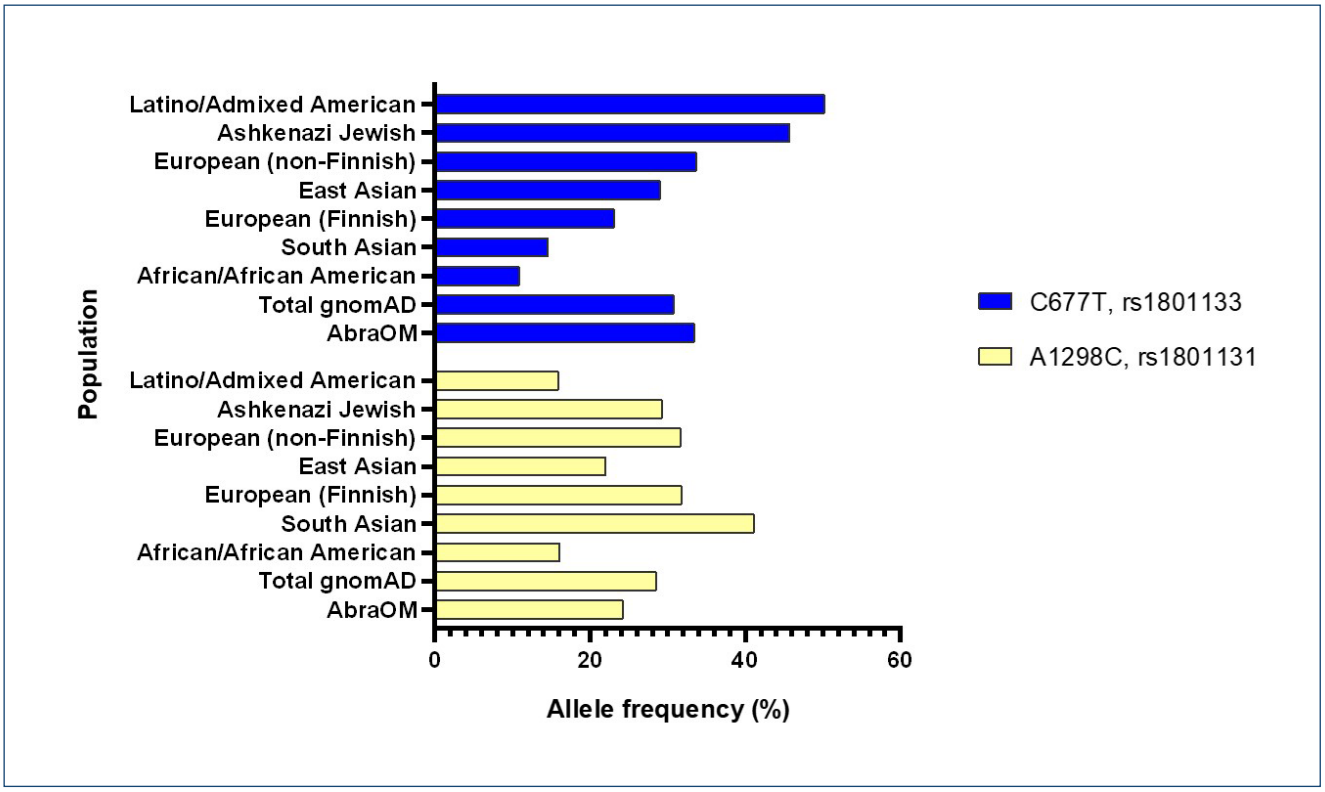

Figure 1. C677T and A1298C allele frequencies from the gnomAD v2 and AbraOM databases. The histogram shows the distribution of the allele frequency of the two most frequent polymorphisms in the methylene tetrahydrofolate reductase gene across different populations.

in Americans and Africans. Another systematic review, which comprised 20 case-control studies, investigated the association between *MTHFR* polymorphisms and the risk of bladder cancer, revealing no overall association, but when data were stratified by ethnicity, both C677T and A1298C were associated with the risk of bladder cancer only in Asians and not Europeans[18].

Even though meta-analyses can provide a more comprehensive understanding of the association between *MTHFR* polymorphisms and various phenotypes across populations, these studies are not without limitations. The limitations include differences in phenotype definitions and insufficient information on environmental covariates that could affect multifactorial disease models. It is relevant to mention that the evidence and recommendations for *MTHFR* genetic testing have changed over the past years. Currently, the consensus is that, in the absence of elevated homocysteine levels, *MTHFR* variants alone are not a risk factor for any disease. In 2013, the American College of Medical Genetics published a practical guideline advising against routine *MTHFR* genetic testing[19]. Also, many other prominent medical associations discourage the use of the test, including the American College of Obstetrics and Gynecology, the College of American Pathologists, the American Academy of Family Physicians, and the American Heart Association[20]. Despite the lack of evidence for clinical utility, testing for MTHFR polymorphisms remains widespread and providers continue to order this unwarranted test. Moreover, the application of direct-to-consumer genetic testing for *MTHFR* complicates the translation of variant associations, as there is no consideration for the population where those tests are being applied and no health professionals to elucidate the complexity of any variant association. Thus, these limitations raise concerns about the potential harm that may result from individuals making uninformed decisions about their health, being crucial to carefully consider them when utilizing direct-to-consumer genetic testing for clinical purposes.

CONCLUSIONS AND RECOMMENDATIONS

The *MTHFR* polymorphisms, C677T and A1298C, may or may not be associated with elevated homocysteine levels and the risk for multifactorial diseases. *MTHFR* genetic testing is not likely to provide accurate estimates of disease risk, particularly in highly admixed populations such as the Brazilian population. These variants should be considered part of an additive polygenic model of genes and environment, rather than high penetrant variants because the relationship between *MTHFR* polymorphisms and homocysteine levels or risk for diseases may depend on the presence of other genetic variants in the same individual. Accordingly, genotyping of these two *MTHFR* polymorphisms may not provide significant benefit to patients until further association studies are conducted in diverse populations. If the goal is to correct elevated homocysteine levels through supplementation, it may be more reasonable to test homocysteine levels directly before performing a genetic test for *MTHFR*.

ETHICS APPROVAL

This study was approved by the Ethics Committee of Hospital 9 de Julho (CAAE: 55817821.3.0000.5455).

AUTHORS' CONTRIBUTIONS

ACP: Conceptualization, Data curation, Methodology. **DV**: Writing – review and editing. **TL**: Supervision.

REFERENCES

1. Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, Smith GD, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2010;19(6):1632-42. https://doi.org/10.1158/1055-9965.EPI-10-0180
2. Guo BQ, Li HB, Ding SB. Blood homocysteine levels in children with autism spectrum disorder: an updated systematic review and meta-analysis. Psychiatry Res. 2020;291:113283.
3. Moradi F, Lotfi K, Armin M, Clark CCT, Askari G, Rouhani MH. The association between serum homocysteine and depression: a systematic review and meta-analysis of observational studies. Eur J Clin Invest. 2021;51(5):e13486. https://doi.org/10.1111/eci.13486
4. Chen A, Wu W, Gong J, Han Y, Xu G, Xie L. Association of homocysteine with carotid atherosclerosis in hypertension. J Hum Hypertens. 2023;37(3):227-34. https://doi.org/10.1038/s41371-022-00665-1
5. Froese DS, Fowler B, Baumgartner MR. Vitamin B12, folate, and the methionine remethylation cycle—biochemistry, pathways, and regulation. J Inherited Metab Dis. 2019;42:673-85. https://doi.org/10.1002/jimd.12009
6. Weile J, Kishore N, Sun S, Maaieh R, Verby M, Li R, et al. Shifting landscapes of human MTHFR missense-variant effects. Am J Hum Genet. 2021;108(7):1283-300. https://doi.org/10.1016/j.ajhg.2021.05.009
7. Martins MF, Murry LT, Telford L, Moriarty F. Direct-to-consumer genetic testing: an updated systematic review of healthcare professionals' knowledge and views, and ethical and legal concerns. Eur J Hum Genet. 2022;30(12):1331-43. https://doi.org/10.1038/s41431-022-01205-8
8. Crouch DJM, Bodmer WF. Polygenic inheritance, GWAS, polygenic risk scores, and the search for functional variants. Proc Natl Acad Sci USA. 2020;117:18924-33. https://doi.org/10.1073/pnas.2005634117
9. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53. https://doi.org/10.1038/nature08494

10. Sadeghiyeh T, Dastgheib SA, Mirzaee-Khoramabadi K, Morovati-Sharifabad M, Akbarian-Bafghi MJ, Poursharif Z, et al. Association of MTHFR 677C>T and 1298A>C polymorphisms with susceptibility to autism: a systematic review and meta-analysis. Asian J Psychiatr. 2019;46:54-61. https://doi.org/10.1016/j.ajp.2019.09.016
11. Zhang YX, Yang LP, Gai C, Cheng CC, Guo ZY, Sun HM, et al. Association between variants of MTHFR genes and psychiatric disorders: a meta-analysis. Front Psychiatry. 2022;13:976428. https://doi.org/10.3389/fpsyt.2022.976428
12. Chen Y, Lu M, Nie J, Liu J, Liu Y, Meng Y, et al. Increasing prevalence of gestational diabetes mellitus when carrying the T variant allele of the MTHFR gene C677T polymorphism: a systematic review and meta-analysis. Arch Gynecol Obstet. 2022;305(5):1193-202. https://doi.org/10.1007/s00404-021-06303-4
13. du Plessis JP, Lammertyn L, Schutte AE, Nienaber-Rousseau C. H-type hypertension among black South Africans and the relationship between homocysteine, its genetic determinants and estimates of vascular function. J Cardiovasc Dev Dis. 2022;9(12):447. https://doi.org/10.3390/jcdd9120447
14. Liao S, Guo S, Ma R, He J, Yan Y, Zhang X, et al. Association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and H-type hypertension: a systematic review and meta-analysis. Ann Hum Genet. 2022;86(5):278-89. https://doi.org/10.1111/ahg.12468
15. Pena SDJ, Santos FR, Tarazona‐Santos E. Genetic admixture in Brazil. Am J Med Genet C Semin Med Genet. 2020;184:928-38.
16. Naslavsky MS, Scliar MO, Yamamoto GL, Wang JYT, Zverinova S, Karp T, et al. Whole-genome sequencing of 1,171 elderly admixed individuals from São Paulo, Brazil. Nat Commun. 2022;13(1):1004. https://doi.org/10.1038/s41467-022-28648-3
17. Fu L, Li YN, Luo D, Deng S, Wu B, Hu YQ. Evidence on the causal link between homocysteine and hypertension from a meta-analysis of 40 173 individuals implementing Mendelian randomization. J Clin Hypertens (Greenwich). 2019;21(12):1879-94. https://doi.org/10.1111/jch.13737
18. Farshid S, Alijanpour A, Barahman M, Dastgheib SA, Narimani N, Shirinzadeh-Dastgiri Z, et al. Associations of MTHFR rs1801133 (677C>T) and rs180113 (1298A>C) polymorphisms with susceptibility to bladder cancer: a systematic review and meta-analysis. Asian Pac J Cancer Prev. 2022;23(5):1465-82. https://doi.org/10.31557/APJCP.2022.23.5.1465
19. Hickey SE, Curry CJ, Toriello HV. ACMG practice guideline: lack of evidence for MTHFR polymorphism testing. Genet Med. 2013;15:153-6.
20. Parkhurst E, Calonico E, Noh G. Medical decision support to reduce unwarranted methylene tetrahydrofolate reductase (MTHFR) genetic testing. J Med Syst. 2020;44:152.

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231020

Genetic association of the BsmI variant of vitamin D receptor gene with risk of morbid obesity

Seyma Ozsoy[1*], Serbulent Yigit[2], Ayse Feyda Nursal[3], Zeki Ozsoy[4], Mehmet Fatih Dasiran[5], Emin Daldal[6], Akin Tekcan[7]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the vitamin D receptor (*VDR*) BsmI variant in morbidly obese patients compared with healthy normal controls.

METHODS: The study included 103 patients with morbid obesity and 120 healthy individuals serving as normal controls. The DNA samples obtained from blood were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The gender, age, smoking status, triglycerides, total cholesterol, insulin, mean body mass index, and frequency of allele and genotype of the BsmI variant in the *VDR* gene in morbidly obese patients were evaluated.

RESULTS: The body mass index of the patients was 47.14 ± 7.19. The *VDR* B/B, B/b, and b/b genotype frequencies were 27.2% versus 28.3%; 54.4% versus 50%; and 18.4% versus 21.7% in the morbidly obese patients and the control group, respectively. There was no statistically significant difference between patients and control subjects in the genotype and allele distribution of the *VDR* BsmI variant ($p>0.05$). Both patients and control genotype frequencies are consistent with Hardy-Weinberg equilibrium.

CONCLUSION: The BsmI variant in the *VDR* gene may not seem to predispose to morbid obesity in our study population. Further studies with a larger number of subjects are needed to make a more precise evaluation of this relationship.

KEYWORDS: Morbid obesity. Vitamin D receptor. BsmI. Variant.

INTRODUCTION

The escalating prevalence of obesity worldwide is a matter of significant concern[1]. The body mass index (BMI) is used to assess the severity of obesity. BMI is determined by taking a person's body weight in kilograms and dividing it by the square of their height in meters. A BMI ≥30 is defined as obesity, and a BMI value exceeding 40 is defined as morbid obesity (obesity class III)[2]. Numerous chronic diseases, including diabetes, gallstones, hypertension, nonalcoholic fatty liver disease, metabolic syndrome, and cardiovascular disorders, have been linked to obesity, according to reports[2]. The etiology of obesity is multifactorial, exhibiting a complex interplay between genes and the environment.

Vitamin D (VitD) is necessary for the homeostasis of bone tissue as well as the minerals such as calcium and phosphorus. Serum 25-hydroxyvitamin D (25(OH)D) is often used as an index of VitD nutritional status. Circulating 25(OH)D concentrations of obese individuals were found to be lower than their race, socioeconomic status, and age-matched peers[3]. Once synthesized, VitD exerts its biological effects primarily through the VitD receptor (VDR, NR1I1). VDR belongs to the nuclear hormone receptor superfamily and forms a heterodimer with the retinoid X receptor. Together, they regulate the activity of target genes containing VitD response elements[4]. VDR expression is present in all essential VitD target tissues, including the intestine, kidney, bone, and parathyroid gland. In these tissues, VDR plays a vital role in maintaining calcium and phosphorus homeostasis, ensuring proper mineral balance within the body. VDR knockout mice have been shown to have reduced body weight and are resistant to high-fat diet-induced obesity[5].

[1]Gaziosmanpasa University, Faculty of Medicine, Department of Physiology – Tokat, Turkey.
[2]Ondokuz Mayıs University, Faculty of Veterinary, Department of Genetics – Samsun, Turkey.
[3]Hitit University, Faculty of Medicine, Department of Medical Genetics – Çorum, Turkey.
[4]Medical Park Hospital, Department of General Surgery – Tokat, Turkey.
[5]Gaziosmanpasa University, Faculty of Medicine, Department of General Surgery – Tokat, Turkey.
[6]Samsun University, Faculty of Medicine, Department of General Surgery – Samsun, Turkey.
[7]Amasya University, Faculty of Medicine, Department of Medical Biology – Amasya, Turkey.
*Corresponding author: seyma.ozsoy@hotmail.com, seyma.ozsoy@gop.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 29, 2024. Accepted on March 17, 2024.

The *VDR* gene is located on chromosome 12q13 and spans 75 kb of genomic DNA, consisting of 14 exons. Within the *VDR* gene, specific single-nucleotide polymorphisms (SNPs) have been identified, one of which is the rs1544410 SNP located in the intronic region (intron 8 near the 3′ end). This SNP, also known as the BsmI variant, represents a restriction fragment length polymorphism (RFLP) of the restriction endonuclease BsmI[6]. Apart from its association with VitD levels, the BsmI polymorphism has been linked to obesity, insulin resistance, and type 2 diabetes in some study cohorts. This suggests that genetic variations in the *VDR* gene may play a role in these conditions, possibly influencing VitD metabolism and related physiological pathways[7,8].

Based on this information, we aimed to examine whether there is a relationship between the VDR BsmI variant and morbid obesity in this study.

METHODS

Study population

A total of 103 morbidly obese patients (30 males and 73 females; mean age: 39.47 ± 12.60 standard deviation [SD] years) who underwent bariatric surgery in the Department of General Surgery, Gaziosmanpaşa University Research Hospital, Tokat, Turkey, were included in this study. An accurately measured BMI is obtained at the first visit to the clinic. All participants were unrelated, with no consanguinity at all, and no spouses were included. BMI was higher than 40 in all patients. Gender and age compatibility of 120 healthy volunteers (55 males and 65 females; mean age: 41.51 ± 12.11 SD years) with a normal BMI were recruited as the control group. Prior to the study, informed consent was obtained from each participant to ensure their understanding and voluntary participation. The study's protocol was reviewed and confirmed by the institutional ethics committee at Ahi Evran University (approval number: 2017-05/36). The research adhered to the principles and guidelines set forth in the Helsinki Declaration to ensure the ethical treatment of the participants and protect their rights and welfare throughout the study.

Genotyping analysis

In this study, approximately 5 mL of peripheral blood was collected from each participant through venipuncture using Vacutainer tubes containing EDTA as an anticoagulant. DNA was removed from leukocytes using a DNA extraction kit (Sigma-Aldrich, Germany) following the manufacturer's instructions. The extracted DNA samples were stored at -20°C until analysis. To genotype the *VDR* BsmI variant, the polymerase chain reaction-RFLP (PCR-RFLP) method was employed, as previously described[9]. After PCR amplification, the products were subjected to digestion overnight at 37°C using the BsmI restriction enzyme. After the digestion process, the resulting products were separated on a 2% agarose gel, which was stained with ethidium bromide. The visualization of the separated fragments was achieved using ultraviolet transillumination. The BsmI restriction enzyme digestion yields three genotypes: B/B (825 bp), B/b (825, 650, 175 bp), and b/b (650, 175 bp).

Statistical analysis

The data obtained from the study were analyzed using the SPSS version 22.0 software by Windows (SPSS Inc., Chicago, IL, USA). The number of sample groups in the research was determined using the G power Package program. According to the results of the analysis, the total sample size was determined to be 214 with a 95% confidence interval (CI), an alpha error rate of 0.05, and an effect size of 0.5.

To assess the statistical significance of differences between the patient and control groups, a logistic regression analysis was conducted. Odds ratios (OR) and their corresponding 95%CI were computed to quantify the associations. To compare the genotype and allele differences in the *VDR* BsmI between the patient and control groups, the chi-square test was used. In cases where sample sizes were small, Fisher's exact test was applied. The chi-square test was used to assess Hardy-Weinberg equilibrium (HWE). In all statistical analyses, two-tailed tests were used, and differences were considered statistically significant when the p-value was below 0.05 ($p<0.05$).

RESULTS

In the present study, a total of 223 participants were included, comprising 103 individuals diagnosed with morbid obesity and 120 healthy adult controls. The genotyping for the *VDR* BsmI variant was performed on all subjects. Table 1 presents the characteristics of the participants in both the patient and control groups. Notably, in both study groups, there were more female participants than male participants. While there were 73 (70.9%) female and 30 (29.1%) male patients, the healthy control group consisted of 65 (54.2%) women and 55 (45.8%) men. The mean ages of morbidly obese patients and healthy individuals were 39.47 ± 12.60 and 41.51 ± 12.11, respectively. The mean BMI of patients was 47.14 ± 7.19.

The study results, as shown in Table 2, indicate the genotype distributions and allele frequencies of the *VDR* gene BsmI variant in all participants. However, upon analysis, no significant

Table 1. Baseline clinical and demographic features of the patient and control groups.

Characteristics	Control group (n = 120) (%)	Patient group (n = 103) (%)
Gender, females/males, n (%)	65/55 (54.2/45.8)	73/30 (70.9/29.1)
Age, mean ± SD, years	41.51 ± 12.11	39.47 ± 12.60
Smoking status, no/yes, n (%)		74/25 (74.7/25.3)
BMI, mean ± SD	–	47.14 ± 7.19
Triglycerides, mean ± SD, years	–	110.66 ± 107.98
Total cholesterol, mean ± SD years	–	130.33 ± 88.94
Insulin, mean ± SD years	–	26.80 ± 52.37

SD: Standard deviation; BMI: body mass index; n: number of samples.

Table 2. Genotype and allele frequencies of the *VDR BsmI* gene variant in the patient and control groups.

VDR *BsmI*	Patient group n = 103 (%)	Control group n = 120 (%)	X^2	p-value	OR (95%CI)
Genotypes					
B/B	28 (27.2)	34 (28.3)	1.62	>0.05	0.855 (0.40–1.78)
B/b	56 (54.4)	60 (50.0)		>0.05	0.864 (0.50–1.47)
b/b	19 (18.4)	26 (21.7)		>0.05	0.854 (0.35–2.04)
B/B+B/b:b/b	84:19	94:26		>0.05	1.222 (0.63–2.39)
B/B:B/b+b/b	28:75	34:86		>0.05	0.944 (0.52–1.70)
HWE	0.33	0.96			
Alleles					
B	112 (19.6)	128 (24)		>0.05	1.042 (0.71–1.51)
b	94 (80.3)	112 (76)			

n: number of samples; p-value: the statistical significance; HWE: Hardy-Weinberg equilibrium.

differences were observed between morbidly obese patients and healthy individuals concerning the *VDR* BsmI genotype and allele frequencies (p>0.05). This implies that the presence of the *VDR* BsmI variant does not appear to be associated with morbid obesity in this study population. Both patients and control genotype frequencies are consistent with HWE (p>0.05).

DISCUSSION

Obesity has emerged as a pressing global health concern, affecting a growing number of countries due to its high prevalence, substantial economic costs, and significant health implications[2]. The excess of macronutrients in fatty tissues stimulates the release of inflammatory mediators such as TNFα and IL-6[10]. VitD deficiency is positively associated with serum levels of inflammatory markers such as IL-6, TNFα, and C-reactive protein in obese individuals[11]. VitD may have immunomodulatory effects with its anti-adipogenic properties. This may help reduce inflammation in fatty tissues[12]. It has been reported that there is a relationship between VitD deficiency during pregnancy and gestational diabetes[13].

Metabolic regulation of VitD in adipose tissue is dependent on VDR. VDR belongs to the steroid hormone receptor superfamily, and it has been found to play a significant role in the development of obesity. Data show a widespread expression of VitD-related metabolic enzymes and VDR in human adipose tissue[14]. Since VDRs are present in various body tissues, their gene polymorphisms may influence the risk of VitD-related metabolic disorders and regulate the receptor's effectiveness based on VitD status[15].

Genetic changes in the *VDR* gene can cause defects in gene activation. But they also support cell proliferation, differentiation, calcium metabolism, immune function, etc[16]. Among the various polymorphisms identified in the *VDR* gene to date, BsmI, ApaI, TaqI, and FokI have been studied the most[17]. Various *VDR* polymorphisms have been associated with type 2 diabetes mellitus, insulin release[18], and metabolic parameters related to obesity[19]. The *VDR* BsmI variant is believed to affect the gene's mRNA stability and gene transcription, potentially altering the expression of other genes. Studies conducted on different populations have reported varying associations between the BsmI variant and obesity. In a study conducted

in France, it was shown that the *VDR* BsmI b/b genotype is predisposed to obesity compared to B/B and B/b genotypes[20]. Another study reported an association between body weight and BsmI variant in men in age-adjusted analysis[21]. In the study conducted with Saudi men, it was found that the BsmI b/b genotype was higher in the obese group compared to the lean group[22]. Additionally, those with the b/b genotype had higher BMI and HOMA-IR than those with the B/B and B/b genotypes. A study in obese children in Turkey found that BsmI polymorphism had a positive effect on the formation of obesity, metabolic syndrome, and hepatosteatosis[23]. However, some studies have not observed significant relationships between the *VDR* BsmI variant and obesity-related parameters. For example, a Polish cohort of postmenopausal women did not find any association of the *VDR* BsmI variant with BMI, total fat volume, or visceral fat[24]. Similarly, a study on obese and non-obese women with polycystic ovary syndrome did not show a significant association with the *VDR* BsmI variant[25].

In the current study, we investigated the genetic association between the *VDR* BsmI variant and morbid obesity. The genotype and allele frequencies of this variant showed no statistically significant difference between the morbidly obese patients and the healthy controls (Table 2). These findings were consistent with some previous studies.

This study has some limitations. The first limitation is that it evaluates only one variant of the *VDR* gene. Other variants of this gene may also affect the development of morbid obesity. Additionally, gene–environment and gene–gene interactions were not evaluated. The final limitation is that the VDR blood level was not assessed. However, the strength of our study is that only patients from a single region were included.

CONCLUSION

Identifying the relationship between genetic variants and obesity is crucial to understanding pathogenesis. This study represents a valuable step in investigating the potential link between *VDR* BsmI and obesity susceptibility. Large samples and replication in different ethnic groups are required to evaluate the results. The results may help develop personalized therapeutic approaches.

AUTHORS' CONTRIBUTIONS

SO: Conceptualization, Formal Analysis, Supervision, Project administration, Writing – original draft, Writing – review & editing. **SY:** Conceptualization, Data curation, Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **AFN:** Conceptualization, Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **ZO:** Conceptualization, Data curation, Supervision, Writing – review & editing. **MFD:** Conceptualization, Supervision, Writing – review & editing. **ED:** Conceptualization, Supervision, Writing – review & editing. **AT:** Conceptualization, Data curationm, Supervision, Writing – review & editing.

REFERENCES

1. Khan SM, Hajj Chehadeh S, Abdulrahman M, Osman W, Safar H. Establishing a genetic link between FTO and VDR gene polymorphisms and obesity in the Emirati population. BMC Med Genet. 2018;19(1):11. https://doi.org/10.1186/s12881-018-0522-z
2. Lin X, Li H. Obesity: epidemiology, pathophysiology, and therapeutics. Front Endocrinol (Lausanne). 2021;12:706978. https://doi.org/10.3389/fendo.2021.706978
3. Vranić L, Mikolašević I, Milić S. Vitamin D deficiency: consequence or cause of obesity? Medicina (Kaunas). 2019;55(9):541. https://doi.org/10.3390/medicina55090541
4. Martínez-Sena T, Soluyanova P, Guzmán C, Valdivielso JM, Castell JV, Jover R. The vitamin D receptor regulates glycerolipid and phospholipid metabolism in human hepatocytes. Biomolecules. 2020;10(3):493. https://doi.org/10.3390/biom10030493
5. Wang PQ, Pan DX, Hu CQ, Zhu YL, Liu XJ. Vitamin D-vitamin D receptor system down-regulates expression of uncoupling proteins in brown adipocyte through interaction with Hairless protein. Biosci Rep. 2020;40(6):BSR20194294. https://doi.org/10.1042/BSR20194294
6. El-Beshbishy HA, Tawfeek MA, Taha IM, FadulElahi T, Shaheen AY, Bardi FA, et al. Association of vitamin D receptor gene BsmI (A>G) and FokI (C>T) polymorphism in gestational diabetes among Saudi Women. Pak J Med Sci. 2015;31(6):1328-33. https://doi.org/10.12669/pjms.316.7525
7. Akter R, Afrose A, Sharmin S, Rezwan R, Rahman MR, Neelotpol S. A comprehensive look into the association of vitamin D levels and vitamin D receptor gene polymorphism with obesity in children. Biomed Pharmacother. 2022;153:113285. https://doi.org/10.1016/j.biopha.2022.113285
8. Nam SW, Choi J, Jeon HJ, Oh TK, Lee DH. The associations between vitamin D receptor BsmI and ApaI polymorphisms and obesity in korean patients with type 2 diabetes mellitus. Diabetes Metab Syndr Obes. 2021;14:557-64. https://doi.org/10.2147/DMSO.S293032
9. Yildiz S, Tumer MK, Yigit S, Nursal AF, Rustemoglu A, Balel Y. Relation of vitamin D and BsmI variant with temporomandibular diseases in the Turkish population. Br J Oral Maxillofac Surg. 2021;59(5):555-60. https://doi.org/10.1016/j.bjoms.2020.08.101
10. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13(4):851-63. https://doi.org/10.5114/aoms.2016.58928

11. Rodríguez-Rodríguez E, Aparicio A, Andrés P, Ortega RM. Moderate vitamin D deficiency and inflammation related markers in overweight/obese schoolchildren. Int J Vitam Nutr Res. 2014;84(1-2):98-107. https://doi.org/10.1024/0300-9831/a000197
12. Athanassiou L, Mavragani CP, Koutsilieris M. The immunomodulatory properties of vitamin D. Mediterr J Rheumatol. 2022;33(1):7-13. https://doi.org/10.31138/mjr.33.1.7
13. Eller ABP, Ejzenberg D, Monteleone PAA, Soares JM, Baracat EC. Vitamin D and in vitro fertilization: a systematic review. J Assist Reprod Genet. 2023;40(4):735-43. https://doi.org/10.1007/s10815-023-02767-2
14. Lu S, Cao ZB. Interplay between vitamin D and adipose tissue: implications for adipogenesis and adipose tissue function. Nutrients. 2023;15(22):4832. https://doi.org/10.3390/nu15224832
15. Benito LAO, Kogawa EM, Silva CMS, Melo FF, Sales-Peres SHC, Silva ICRD, et al. Bariatric surgery and vitamin D: trends in older women and association with clinical features and VDR gene polymorphisms. Nutrients. 2023;15(4):799. https://doi.org/10.3390/nu15040799
16. Akter R, Afrose A, Sharmin S, Rezwan R, Rahman MR, Neelotpol S. A comprehensive look into the association of vitamin D levels and vitamin D receptor gene polymorphism with obesity in children. Biomed Pharmacother. 2022;153:113285. https://doi.org/10.1016/j.biopha.2022.113285
17. Saad M, Ahmad EA. Vitamin D receptor gene polymorphism among egyptian obese children. Asian J Clin Nutr. 2017;9:24-9. https://doi.org/10.3923/ajcn.2017.24.29
18. Aravindhan S, Almasoody MFM, Selman NA, Andreevna AN, Ravali S, Mohammadi P, et al. Vitamin D receptor gene polymorphisms and susceptibility to type 2 diabetes: evidence from a meta-regression and meta-analysis based on 47 studies. J Diabetes Metab Disord. 2021;20(1):845-67. https://doi.org/10.1007/s40200-020-00704-z
19. Karonova T, Grineva E, Belyaeva O, Bystrova A, Jude EB, Andreeva A, et al. Relationship between vitamin D status and vitamin D receptor gene polymorphisms with markers of metabolic syndrome among adults. Front Endocrinol (Lausanne). 2018;9:448. https://doi.org/10.3389/fendo.2018.00448
20. Ye WZ, Reis AF, Dubois-Laforgue D, Bellanné-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. Eur J Endocrinol. 2001;145(2):181-6. https://doi.org/10.1530/eje.0.1450181
21. Correa-Rodríguez M, Carrillo-Ávila JA, Schmidt-RioValle J, González-Jiménez E, Vargas S, Martín J, et al. Genetic association analysis of vitamin D receptor gene polymorphisms and obesity-related phenotypes. Gene. 2018;640:51-6. https://doi.org/10.1016/j.gene.2017.10.029
22. Al-Hazmi AS, Al-Mehmadi MM, Al-Bogami SM, Shami AA, Al-Askary AA, Alomery AM, et al. Vitamin D receptor gene polymorphisms as a risk factor for obesity in Saudi men. Electron Physician. 2017;9(10):5427-33. https://doi.org/10.19082/5427
23. Özhan B, Bilgihan E, Çetin O, Ağladıoğlu K. The relation of vitamin D receptor gene polymorphisms with risk of obesity, metabolic syndrome, hepatostetosis in Turkish children. Pamukkale Med J. 2023;16(2):274-80. https://doi.org/10.31362/patd.1249471
24. Tworowska-Bardzińska U, Lwow F, Kubicka E, Łaczmański Ł, Jedzrzejuk D, Dunajska K, et al. The vitamin D receptor gene BsmI polymorphism is not associated with anthropometric and biochemical parameters describing metabolic syndrome in postmenopausal women. Gynecol Endocrinol. 2008;24(9):514-8. https://doi.org/10.1080/09513590802302985
25. Kumar AN, Satyanarayana U, Naidu JN, Ramalingam K, Anitha M. Vitamin D receptor gene polymorphism in obese and non obese Indian women with polycystic ovary syndrome. Int J Curr Res. 2015;7(8):19686-91.

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231270

The pregnant women's perception of risks and pregnancy stress levels: a cross-sectional study from Turkey

Aytan Maharramova[1], Elif Yagmur Gur[2*]

SUMMARY

OBJECTIVE: The aim of this study was to compare pregnant women's perceptions of risk and pregnancy-specific stress levels.

METHODS: This cross-sectional descriptive study was conducted with 410 healthy pregnant women at the city hospital located in the east of Turkey. Data were collected via Personal Information Form, Perception of Pregnancy Risk Questionnaire, and Pregnancy Stress Rating Scale.

RESULTS: The pregnancy risk perception mean score was 2.43±1.82, and the pregnancy-specific stress mean score was 22.27±12.67. There is a statistically significant and strong positive correlation between the perception of pregnancy risk and pregnancy-specific stress level ($p<0.01$). Pregnant women's pregnancy risk perception decreased as the duration of marriage and the number of living children increased, and it increased as the gestational week increased ($p<0.05$). Pregnancy-specific stress decreased as the duration of marriage ($p<0.001$), the age of the spouse, the number of pregnancies, and the number of living children increased ($p<0.01$), and it increased as the gestational week increased ($p<0.01$).

CONCLUSION: The pregnant women's perceptions of pregnancy risks and pregnancy-specific stress were low, but pregnancy-specific stresses increased as their perceptions of pregnancy risks increased.

KEYWORDS: Pregnancy. Stress.

INTRODUCTION

The concept of pregnancy-related risk perception can affect women's emotional state and decision-making about pregnancy and childbirth process[1]. Also, pregnancy-specific stress is briefly defined as a woman's concerns, anxiety, and fear about pregnancy[2]. Pregnancy-related stress and its adverse effects[3-6] are considered to be one of the leading causes of maternal perinatal deaths[3]. In addition, pregnancy-specific stress may be creating adverse effects on the fetus regardless of obstetric risks[5], such as risks for low birth weight, premature birth[7,8], and fetal developmental disorders[6]. Therefore, it is important to identify women who suffer from psychological stress during pregnancy. The American Society of Gynecology and Obstetrics recommends prenatal screening and intervention for psychosocial risk factors, including stress, in all pregnant women[4]. Health care professionals could collaborate in determining the perception of pregnancy risks and pregnancy-specific stress and identifying and implementing effective strategies to manage this process[9]. Considering the importance of the topic, it aimed to determine the correlation between the women's perceptions of pregnancy risk and pregnancy-specific stress levels in this study.

METHODS

Study sample and design

This descriptive cross-sectional study was carried out according to STROBE guidelines. It was conducted in a city hospital in a province in eastern Turkey between December 2021 and March 2022. The study involved 11,623 women, who came to the hospital for routine pregnancy check-ups in 2021. Using the known sampling formula, the study sample was calculated as 372, and it was completed with 410 pregnant women.

Inclusion criteria

Inclusion criteria were as follows: healthy pregnant women, aged between 18 and 35 years, could speak Turkish language, were Turkish citizens, and did not have any physical/mental health problems/pregnancy risks.

Data collection

The data were collected using the Personal Information Form, Perception of Pregnancy Risk Questionnaire, and Pregnancy Stress Rating Scale. The interviews were held face to face, and the duration was about 10–15 min.

[1]Liv Bona Dea Hospital – Baku, Azerbaijan.
[2]Eskisehir Osmangazi University, Health Science Faculty, Department of Midwifery – Eskişehir, Turkey.
*Corresponding author: eyagmur.gur@ogu.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 26, 2024. Accepted on February 04, 2024.

Data collection tools

Personal Information Form: This form consisted of 17 questions about sociodemographic and obstetric characteristics.

Perception of Pregnancy Risk Questionnaire (PPRQ): The questionnaire consists of nine items and two factors. Each item on the scale has a 0–10-cm long line (0–100 mm), with the left extreme reading "no risk" and the other extreme "extremely high risk." The total score of the scale is obtained by summing the scores of the items and dividing the result by nine. The scale has no cutoff point. High total scores on the scale indicate that the risk perception of the pregnant woman about herself and her child increases[10,11]. In the current study, Cronbach's alpha value was found to be 0.80.

Pregnancy Stress Rating Scale (PSRS): This scale has 36 items and a 5-point Likert type. The sum of item scores gives the prenatal stress score. The score obtained from the scale is between 0 and 144. High total scores indicate an increase in perceived prenatal stress[12,13]. In the current study, Cronbach's alpha value was found to be 0.83.

Data analysis

The study was analyzed using the SPSS program (Statistics Package for Social Sciences for Windows, Version 21.0, IBM Corp., NY) using the counts, percentages, mean scores, one-way ANOVA, Kruskal-Wallis, Mann-Whitney U test, independent samples t-test, and Pearson correlation test. A p-value of <0.05 was considered significant for all statistical tests.

Ethical approval

The approval of the Ethics Committee of University Faculty of Medicine and the written permission of the hospital were obtained. It was conducted in compliance with the principles of the Declaration of Helsinki.

RESULTS

The mean age of the pregnant women was 25.75±4.05 years, the mean age of the spouses was 30.18±4.45 years, the mean duration of marriage was 4.09±3.40 years, and the mean gestational week was 28.49±7.05. Number of pregnancy was 1.85±1.13 and number of living children was 0.62±0.87 (data not shown).

A statistically significant difference was found between the descriptive characteristics of the pregnant women, namely, duration of the marriage, education level of the spouse, and parity and their mean scores on the total PPRQ and PSRS (p<0.05, Table 1).

Findings about the relationships between some descriptive variables of the study and PPRQ and PSRS are given in Table 2. There was a negative correlation between the mean PPRQ score of the pregnant women, duration of the marriage, and the number of living children (r=-0.125, p=0.011; r=-0.169, p=0.001, respectively) and a statistically significant positive correlation with the gestational week (r=0.126, p=0.011). It was determined that there was a statistically significant difference between pregnant women's age groups, perception of social support, and their mean PSRS scores (p<0.05). There was a negative correlation between pregnant women's characteristics, namely, the age of the spouse, duration of the marriage, parity, the number of living children, and their mean score on the total PSRS (r=-0.129, p=0.009; r=-0.185, p=0.000; r=-0.143, p=0.004; r=-0.192, p=0.000, respectively) and a statistically significant positive correlation with the gestational week (r=0.153, p=0.002).

It was determined that the PPRQ mean score of the pregnant women was 2.43±1.82 and 22.27±12.67 on the total PSRS. There was a statistically significant positive correlation between the mean scores of the pregnant women on the total PPRQ and the PSRS (p<0.01, Table 3).

DISCUSSION

The study indicated that both the pregnancy risk perception and the pregnancy-specific stress score averages of pregnant women were low. The fact that the majority of women had social support and had a planned pregnancy may have affected this result. Also, some studies report similar findings as the current study[14,15].

In this study, as the risk perception of pregnant women increased, their pregnancy-specific stresses increased as well. Similar to this finding, some studies in the literature show that stress had a significant effect on the perception of pregnancy risk[14,16]. It is thought that women's thoughts about possible harm to themselves and their babies could have increased the perception of pregnancy risk.

In this study, women's perceptions of pregnancy risk and pregnancy-related stress were highest in the 24–29 age group, and it was also observed that pregnancy-specific stress decreased significantly as the age of the pregnant women's spouses increased. The age of the spouse can also influence pregnancy-specific stress levels. However, an adult and experienced partner could help reduce/control pregnancy stress by positively affecting the psychological adjustment of the pregnant woman[17]. In the current study, as the duration of the marriage of pregnant women decreased, perceptions of pregnancy risks increased. Pregnant women who are newly married or have a short marriage period may not be able to adapt psychologically to pregnancy, which is an important period of life, as they may

Table 1. Comparison of participants' Perception of Pregnancy Risk Questionnaire and Pregnancy Stress Rating Scale mean scores with their descriptive characteristics.

Variables	n	%	PPRQ		PSRS	
			$\bar{x} \pm SD$	Test and p-value	$\bar{x} \pm SD$	Test and p-value
Age						
18–23	130	31.7	2.39±1.77	F=0.56 p=0.57	22.32±12.51	F=3.72 **p=0.02**
24–29	189	46.1	2.53±1.87		23.67±13.45	
30–35	91	22.2	2.29±1.80		19.27±10.68	
Education						
Primary education	116	28.3	2.17±1.83	F=2.77 p=0.06	20.92±12.55	F=2.54 p=0.08
High school	165	40.2	2.40±1.68		21.63±12.21	
University	129	31.5	2.71±1.96		24.30±13.19	
Marriage duration						
1–5 years	306	74.6	2.61±1.83	KW=13.57 **p=0.001**	23.62±12.66	KW=17.56 **p=0.0001**
6–10years	79	19.3	1.93±1.79		19.50±12.58	
≥11 years	25	6.1	1.84±1.46		14.40±8.30	
Partner's education						
Primary education	84	20.5	2.06±1.79	F=3.95 **p=0.02**	19.71±13.01	F=4.64 **p=0.01**
High school	170	41.5	2.35±1.70		21.45±11.37	
University	156	38.0	2.72±1.93		24.53±13.50	
Family type						
Nucleus	346	84.4	2.45±1.78	t=0.61 p=0.54	22.52±12.62	t=0.92 p=0.35
Large	64	15.6	2.30±2.06		20.92±12.94	
Social support						
Yes	348	84.9	2.38±1.82	t=1.27 p=0.20	21.56±12.59	t=2.69 **p=0.007**
No	62	15.1	2.70±1.85		26.24±12.45	
Planned pregnancy						
Yes	343	83.7	2.39±1.84	t=1.03 p=0.30	21.86±12.64	t=1.48 p=0.13
No	67	16.3	2.64±1.70		24.37±12.69	
Parity						
Primiparous	218	53.2	2.62±1.83	t=2.29 **p=0.02**	23.44±12.82	t=1.99 **p=0.04**
Multiparous	192	46.8	2.21±1.79		20.94±12.38	

not have had enough time to adapt to their spouse, family, and new living environment[18]. In addition, as the duration of the marriage increased in this study, their pregnancy-specific stress decreased. Similarly, one study reported that pregnancy-specific stress decreased as the duration of the marriage increased[4]. The increase in the duration of the marriage may pave the way for the formation of planned pregnancies by boosting the harmony between couples, marital harmony, and social support. In this study, the majority of pregnant women had a planned pregnancy, which may have facilitated their adaptation to pregnancy and may have reduced pregnancy-specific stress.

The current study has shown that as the education level of the spouses of pregnant women increased, the perceptions of pregnancy risks and pregnancy-related stress increased as well. Increasing the level of consciousness may cause excessive focus on the healthy process of pregnancy in spouses. Another reason that increases pregnancy stress may be the increase in the control of the spouse over the pregnancy and the decrease in the self-control of the pregnant woman, which is supported by a previous study[19]. In this study, it was seen that the pregnancy-specific stress of pregnant women receiving social support was statistically significantly lower than the stress of those

with no social support. Staneva et al.[20] reported similar findings as that of the current study. It has been emphasized that a decrease in pregnant woman's personal–social factors increases her stress level[21].

In the current study, the perceptions of pregnancy risks and pregnancy-specific stress of multiparous women were less than those of primiparous. This result can be interpreted as follows. Multiparous pregnant women have more experience with pregnancy than primiparous women. Having experience with an event means that perceived risk will be generally lower when encountering the same event. Low levels of pregnancy-specific stress can be explained by previous pregnancy experience and adaptation to stressors. Similar to the findings of this study, some studies have shown that multiparous pregnant women's stress is significantly lower than that of the primiparous[17,19]. Another remarkable parameter in the study was the gestational week. As the gestational week progressed, pregnant women's risk perception and pregnancy-specific stress increased. The progressing gestational week or the upcoming delivery may cause an increase in women's concerns about delivering their child healthily and providing a good future for it. It has been reported that such concerns are effective in the perception of pregnancy risks[14]. In the study, as having living children increased, perceptions of pregnancy risks and pregnancy-specific stress decreased. As the survival rate of children born by a woman increases, the experience can shape her psychological state positively. It is thought that the finding of this study may be related to this situation.

Table 2. Correlation of some descriptive variables with Perception of Pregnancy Risk Questionnaire and Pregnancy Stress Rating Scale.

		PPRQ	PSRS
Age	r	-0.038	-0.072
	p	0.440	0.146
Partner age	r	-0.059	-0.129**
	p	.234	**0.009**
Marriage duration	r	-0.125*	-0.185**
	p	**0.011**	**0.000**
Gestational weeks	r	0.126*	0.153**
	p	**0.011**	**0.002**
Number of pregnancy	r	-0.056	-0.143**
	p	0.261	**0.004**
Number of living children	r	-0.169**	-0.192**
	p	**0.001**	**0.000**

*p<0.05. **p<0.01.

CONCLUSION

In this study, it was found that pregnant women had a low perception of pregnancy risks and pregnancy-specific stress. It was also observed that pregnancy-specific stress increased as their perceptions of pregnancy risks increased. Further research, including prospective studies with different sample groups and influencing factors, is needed to elucidate the relationship between pregnancy risk perception and pregnancy-specific stress.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript.

Table 3. The relationship between the Perception of Pregnancy Risk Questionnaire and Pregnancy Stress Rating Scale mean scores of study participants.

Scales	Theoretical Min–Max value	Received Min–Max value	$\bar{x} \pm SD$	r	p
PPRQ	0–10	0–9.44	2.43±1.82	0.662**	0.000
PSRS	0–144	0–62.00	22.27±12.67		

**p<0.01.

REFERENCES

1. Lennon SL. Risk perception in pregnancy: a concept analysis. J Adv Nurs. 2016;72(9):2016-29. https://doi.org/10.1111/jan.13007
2. Faramarzi M, Amiri FN, Rezaee R. Relationship of coping ways and anxiety with Pregnancy Specific-stress. Pak J Med Sci. 2016;32(6):1364-9. https://doi.org/10.12669/pjms.326.10892
3. Kaboli KS, Mahmoodi Z, Tourzani ZM, Tehranizadeh M, Kabir K, Dolatian M. The effect of group counseling based on cognitive-behavioral approach on pregnancy-specific stress and anxiety. Shiraz E-Med J. 2017;18(5):e13183. https://doi.org/10.5812/semj.45231
4. Pakzad M, Dolatian M, Jahangiri Y, Nasiri M, Dargah FA. The correlation between Islamic lifestyle and pregnancy-specific stress: a cross-sectional, correlational study. Open Access Maced J Med Sci. 2018;6(6):1163-7. https://doi.org/10.3889/oamjms.2018.104
5. Penengo C, Colli C, Garzitto M, Driul L, Cesco M, Balestrieri M. Validation of the Italian version of the revised prenatal coping inventory (NuPCI) and its correlations with pregnancy-specific stress. BMC Pregnancy Childbirth. 2020;20(1):466. https://doi.org/10.1186/s12884-020-03159-5
6. Ibrahim SM, Lobel M. Conceptualization, measurement, and effects of pregnancy-specific stress: review of research using the

original and revised prenatal distress questionnaire. J Behav Med. 2020;43(1):16-33. https://doi.org/10.1007/s10865-019-00068-7

7. Levine TA, Grunau RE, Segurado R, Daly S, Geary MP, Kennelly MM, et al. Pregnancy-specific stress, fetoplacental haemodynamics, and neonatal outcomes in women with small for gestational age pregnancies: a secondary analysis of the multicentre prospective observational trial to optimise paediatric health in intrauterine growth restriction. BMJ Open. 2017;7(6):e015326. https://doi.org/10.1136/bmjopen-2016-015326
8. Esfandiari M, Faramarzi M, Nasiri-Amiri F, Parsian H, Chehrazi M, Pasha H, et al. Effect of supportive counseling on pregnancy-specific stress, general stress, and prenatal health behaviors: a multicenter randomized controlled trial. Patient Educ Couns. 2020;103(11):2297-304. https://doi.org/10.1016/j.pec.2020.04.024
9. Caparros-Gonzalez RA, Romero-Gonzalez B, Puertas-Gonzalez JA, Quirós-Fernández S, Coca-Guzmán B, Peralta-Ramirez MI. Midwives and psychologists as profesionals to screen and prevent pregnancy-specific stress. Rev Esp Salud Publica. 2021;95:95:e202104060. PMID: 33896933
10. Heaman MI, Gupton AL. Psychometric testing of the perception of pregnancy risk questionnaire. Res Nurs Health. 2009;32(5):493-503. https://doi.org/10.1002/nur.20342
11. Evcili F, Dağlar G. Perception of pregnancy risk scale: Turkish validity and reliability study. Cukurova Med J. 2019;44:211-22. https://doi.org/10.17826/cumj.554151
12. Chen CH. Revision and validation of a scale to assess pregnancy stress. J Nurs Res. 2015;23(1):25-32. https://doi.org/10.1097/jnr.0000000000000047
13. Aksoy SD, Dutucu N, Özdilek R. Adaption of "Pregnancy Stress Rating Scale" to Turkish language and its factor analysis. Kocaeli Üniversitesi Sağlık Bilimleri Dergisi. 2019;5(1):10-4. https://doi.org/10.30934/kusbed.467716
14. Bayrampour H, Heaman M, Duncan KA, Tough S. Predictors of perception of pregnancy risk among nulliparous women. J Obstet Gynecol Neonatal Nurs. 2013;42(4):416-27. https://doi.org/10.1111/1552-6909.12215
15. Entringer S, Epel ES, Lin J, Buss C, Shahbaba B, Blackburn EH, et al. Maternal psychosocial stress during pregnancy is associated with newborn leukocyte telomere length. Am J Obstet Gynecol. 2013;208(2):134.e1-7. https://doi.org/10.1016/j.ajog.2012.11.033
16. Pimsuwan P, Boaoung C, Sansiriphun N. Pregnancy-related anxiety, risk knowledge, and pregnancy risk perception among advanced age pregnant women. Nurs J. 2020;47(3):50-60.
17. Genç Koyucu R, Ülkar D, Erdem B. Comparison of pregnancy stresses in primiparous and multiparous pregnants. J Inonu Univ Health Serv Vocational School. 2020;8(3):652-63. https://doi.org/10.33715/inonusaglik.745511
18. Yalçın H. 2014. Relationship between adaptation of the marriage and demographic characteristics. J Res Educ Teach. 2014;3(1):250-61.
19. Sis Çelik A, Atasever İ. Determination of the level of perceived stress and affecting factors in pregnant women. J Anatolia Nurs Health Sci. 2020;23(2):267-76. https://doi.org/10.17049/ataunihem.622304
20. Staneva AA, Morawska A, Bogossian F, Wittkowski A. Maternal psychological distress during pregnancy does not increase the risk for adverse birth outcomes. Women Health. 2018;58(1):92-111. https://doi.org/10.1080/03630242.2017.1282395
21. Tang X, Lu Z, Hu D, Zhong X. Influencing factors for prenatal Stress, anxiety and depression in early pregnancy among women in Chongqing, China. J Affect Disord. 2019;253:292-302. https://doi.org/10.1016/j.jad.2019.05.003

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231321

Metabolic dysfunction-associated steatotic liver disease prevalence and risk factors in inflammatory bowel disease in tertiary center

Lívia dos Remédios Pamplona de Oliveira[1*], Tarsila Campanha da Rocha Ribeiro[1], Carlos Alberto Mourao-Junior[2], Maria Antônia de Lima Barra[1], Mariana Hippert Gonçalves Silva[1], Luis Pordeus Shafee[1], Sarah Mendes Zacarias[1], Lenilton da Costa Campos[1], Helena Maria Giordano Valério[1], Júlio Maria Fonseca Chebli[1]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the prevalence and risk factors related to metabolic dysfunction-associated steatotic liver disease in inflammatory bowel disease patients.

METHODS: This is a cross-sectional study conducted on adults with inflammatory bowel disease from 2019 to 2021. Metabolic dysfunction-associated steatotic liver disease encompasses patients with steatosis and at least one cardiometabolic risk factor. Patients with alcohol consumption ≥20 g/day, chronic liver diseases, or methotrexate use were excluded.

RESULTS: Almost 140 patients were included: 67.1% were female, with a mean age of 49.7±13.7 years, and 63.6% had Crohn's disease. The mean duration of inflammatory bowel disease was 9.7±7.9 years. Metabolic dysfunction-associated steatotic liver disease was observed in 44.3% and advanced liver fibrosis was excluded in 63.5% by Fibrosis-4. Patients with metabolic dysfunction-associated steatotic liver disease were older ($p = 0.003$) and had a higher number of metabolic syndrome components (2.9±1.1 versus 1.6±1.0; $p<0.001$), greater abdominal circumference ($p<0.001$), and body mass index ($p<0.001$). The only factor related to inflammatory bowel disease associated with metabolic dysfunction-associated steatotic liver disease was disease duration (11.6±9.5 versus 8.3±6.2; $p = 0.017$). A higher number of metabolic syndrome components and obesity increase by 2.2 times and an altered waist circumference by 2.6 times the occurrence of metabolic dysfunction-associated steatotic liver disease.

CONCLUSION: A high prevalence of metabolic dysfunction-associated steatotic liver disease was observed in patients with inflammatory bowel disease, with the main risk factors being associated with metabolic syndrome predicting it, but not with inflammatory bowel disease features and/or its treatment.

KEYWORDS: Inflammatory bowel diseases. Crohn's disease. Ulcerative colitis. Nonalcoholic fatty liver disease. Metabolic syndrome. Liver fibrosis.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease, mainly related to insulin resistance and metabolic syndrome (MS), after the exclusion of secondary causes[1]. Its spectrum ranges from simple steatosis to advanced fibrosis (AF) and hepatocellular carcinoma[2]. Recently, a new NAFLD nomenclature was proposed to contemplate its physiopathology and reduce the impact of subject stigmatization based mainly on exclusion criteria. The new term "metabolic dysfunction-associated steatotic liver disease (MASLD)" comprises hepatic steatosis (HS) associated with one out of five cardiometabolic risk criteria in individuals without significant alcohol consumption[3].

The prevalence of MASLD is increasing worldwide, in parallel with the obesity epidemic and the expansion of cardiovascular diseases, being estimated at 25–30% of the general population[4-6]. Despite the scant data regarding the prevalence of MASLD in Brazil, it is known that in Latin America, the disease is highly prevalent, occurring in 24% of the population[7]. Recent studies indicate that the prevalence of NAFLD in IBD patients varies from 1.5 to 55%[8,9]. Both diseases are multifactorial, involving environmental, genetic, and immunological determinants[10].

In most cases, MASLD is linked to insulin resistance and is considered the hepatic manifestation of MS[2]. However, the pathogenesis of MASLD in the IBD population may involve specific risk factors, such as chronic inflammatory response, drug hepatotoxicity, frequent steroid use, malnutrition, previous intestinal resection, and intestinal dysbiosis[8]. It is postulated that MASLD in IBD patients may occur through two

[1]Universidade Federal de Juiz de Fora, Hospital Universitário – Juiz de Fora (MG), Brazil.
[2]Universidade Federal de Juiz de Fora, Department of Physiology – Juiz de Fora (MG), Brazil.
*Corresponding author: lviapamplona@yahoo.com.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 09, 2024. Accepted on March 02, 2024.

distinct phenotypes, one triggered by factors directly related to IBD and the other associated with the components of MS[10,11].

Earlier studies have found an association between MASLD and features of IBD, such as disease severity and duration, previous intestinal resection, and drugs used in the treatment[10,11]. Conversely, anti-tumor necrosis factor (anti-TNF) agents may have a protective role against the occurrence of MASLD[12,13]. However, more recent studies have pointed out that components of MS are more critical to the occurrence of MASLD in IBD.

With advancing knowledge of IBD and its therapeutic arsenal, the disease phenotype has changed in recent years, with steatosis and liver disease increasingly reported in patients with IBD. However, data regarding predisposing factors for the occurrence of steatosis are still conflicting. With the implementation of new nomenclature and the absence of studies that evaluate the prevalence of MASLD and its risk factors in our country, the present study was proposed.

METHODS

This cross-sectional study included patients over 18 years old followed up at the IBD Reference Center of the University Hospital of the Federal University of Juiz de Fora (HU-UFJF) from January 2019 to December 2021. The diagnosis of IBD was established by clinical, endoscopic/histological, and/or imaging criteria. Patients with alcohol consumption >20 g/day, chronic liver disease, or use of methotrexate were excluded. This study was approved by the Human Research Ethics Committee of HU-UFJF (CAAE 06129419.0.0000.5133) and participants signed an informed consent form before inclusion.

Data on clinical–demographic and IBD features (disease type and location, age at diagnosis and disease length, current and previous treatment) were collected. Disease activity was defined by a colonoscopy showing the presence of ulcers in CD or a Mayo score ≥2 in UC[14] and/or compatible imaging and/or biochemical findings (C-reactive protein>6 mg/dL).

The diagnosis of metabolic syndrome was established according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) criteria: elevated waist circumference (≥94 cm in males or ≥80 cm in females); triglycerides ≥150 mg/dL; HDL cholesterol <40 mg/dL in males or <50 mg/dL in females; elevated blood pressure (systolic ≥130 and/or diastolic ≥85 mm Hg); or fasting glucose ≥100 mg/dL[15]. Biochemical assessment included metabolic, liver, and inflammatory profiles, as well as viral and autoimmune markers.

The HS diagnosis was established using an imaging technique (ultrasonography, tomography, or magnetic resonance) at the time of inclusion or in the previous 6 months, if available, and carried out in our service. The diagnosis of MASLD was defined by steatosis and at least one cardiovascular risk factor, as recently proposed by the American and European Association of Liver Diseases.[3]

For assessing advanced liver fibrosis, the Index for Liver Fibrosis-4 (FIB-4) was used as a noninvasive test, widely validated, and recommended for screening in a low prevalence population of liver fibrosis. Values lower than 1.3 or higher than 2.67 excluded or confirmed the diagnosis of AF. Intermediate values were considered indeterminate and nondiagnostic[16,17].

Continuous variables were described as mean and standard deviation and categorical variables were described as frequency and percentage. Comparison between continuous variables was established using the Student's t-test, while the chi-square test or Fisher's exact test was used to evaluate categorical variables. All tests were two-tailed and adopted a significance level of 5%. For MASLD prediction models, binary logistic regression models were performed. The choice of independent variables was based on previous univariate analyses and clinical criteria previously established in the literature. The goodness of fit of the regression model was verified by the Omnibus test and the respective ROC area under the curve (AUC). Inferential and modeling analyses were carried out using the Jamovi version 2.3 application.

RESULTS

A total of 217 patients were evaluated, of which 77 were excluded (63 lost to follow-up, 5 had alcohol use disorder, 5 had chronic liver disease, and 4 were using methotrexate). Of the 140 patients included, 67.1% were female, with a mean age of 49.7±13.7 years, and the majority had Crohn's disease (63.6%). HS was evident in 45% of the sample. The diagnosis of MASLD was established in 44.3%. Only 20% of patients had elevated alanine aminotransferase, while advanced liver fibrosis was present in 6.5% of cases and could be excluded using the noninvasive FIB-4 score in 63.5% of patients. The clinical–demographic characteristics of the studied population are described in Table 1.

Patients with MASLD had a higher frequency of MS, diabetes, hypertension, altered waist circumference (CW), and obesity (Figure 1). Furthermore, the MASLD patients had more ATP III metabolic risk factors, were older, and had a higher length of disease than those without MASLD. Conversely, data related to IBD (type, extent, phenotype, treatment, disease activity) were unrelated to the presence of MASLD (Table 1).

Table 1. Clinical–demographic characteristics of inflammatory bowel disease patients with steatotic liver disease associated with metabolic dysfunction.

	Total (n = 140)	Without MASLD (n = 78)	MASLD (n = 62)	p-value
Women % (n)	67.1 (94)	52.1 (49)	47.9 (45)	0.222
Age (years)	49.7 ± 13.7	46.6 ± 14.3	53.5 ± 11.9	0.003
Age at diagnosis (years)	39.9 ± 13.1	38.3 ± 13.5	41.9 ± 12.3	0.102
Illness duration (years)	9.7 ± 7.9	8.3 ± 6.2	11.6± 9.5	0.017
Type of IBD				
CD/UC % (n)	63.6 (89)/36.4 (51)	55.1 (49)/56.9 (29)	44.9 (49)/43.1 (22)	0.836
Location of CD				
L1/L2/L3/L4 % (n)	31.5 (28)/20.2 (18)/ 46.1 (41)/2.2 (2)	30.6 (15)/14.3 (7)/55.1 (27) -	32.5 (13)/27.5 (11)/ 35 (14)/5 (2)	0.091
CD phenotype				
B1/B2/B3 % (n)	31.5 (28)/36 (32)/32.6 (29)	30.6 (15)/38.8 (19)/30.6 (15)	32.5 (13)/32.5 (13)/35 (14)	0.862
Perianal disease % (n)	18.1 (21)	57.1 (12)	42.9 (9)	0.708
Location of UC				
E1/E2/E3 % (n)	9.8 (5)/45.1 (23)/45.1 (23)	6.9 (2)/41.4 (12)/51.7 (15)	13.6 (3)/50 (11)/36.4 (8)	0.556
Previous surgery % (n)	21 (29)	58.6 (17)	41.4 (12)	0.730
Treatment				
Anti-TNF therapy % (n)	35.7 (50)	56 (28)	44 (22)	0.960
Steroid use % (n)	28.6 (40)	55 (22)	45 (18)	0.914
Active disease % (n)	60 (84)	51.2 (43)	48.8 (62)	0.187
Number of SM components	2.1 ± 1.3	1.6 ± 1.0	2.9 ± 1.1	<0.001
Blood glucose	100.2 ± 29.8	92.3 ± 12.4	110 ± 40.6	<0.001
Glycated hemoglobin	5.5 ± 1.4	5.2 ± 0.5	5.9 ± 1.9	0.003
Insulin	10 ± 5.4	8.5 ± 5.0	11.9 ± 5.4	<0.001
HOMA-IR	2.5 ± 1.8	2.0 ±1.4	3.3 ± 2.1	<0.001
Triglycerides	146.7 ± 82.2	131.3 ± 67.1	166.2 ± 95	0.012
Total cholesterol	187.7 ± 42.2	185.2 ± 43	190.9 ± 41.6	0.427
HDL	49 ±12.7	50.5 ± 13.8	47 ± 11	0.117
LDL	109.6 ± 35.8	108.4 ± 37.7	111.1 ± 33.5	0.665
AST	23.5 ± 9.6	23.1 ± 7.6	24.1 ±11.7	0.582
ALT	21.3 ± 11.2	20.1 ± 11.2	22.8 ±11.2	0.168
Albumin	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	0.291
Creatinine	0.8 ± 0.2	0.8 ± 0.2	0.7 ±0.2	0.221
CRP	7.4 ± 14.1	7.6 ± 16.8	7.2 ± 9.3	0.921
ESR	21.8 ± 19.8	21.7 ± 23	22.1± 15.1	0.930

MASLD: metabolic dysfunction-associated steatotic liver disease; IBD: inflammatory bowel disease; CD: Crohn's disease; UC: ulcerative colitis; L1: ileal; L2: colonic; L3: ileocolonic; L4: upper gastrointestinal tract; B1: non-stenosing, nonpenetrating; B2: stenosing; B3: penetrating; E1 proctitis; E2: left colitis; E3: pancolitis; SM: metabolic syndrome; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Multivariate analysis showed that MASLD was independently associated with a higher number of ATP III metabolic risk factors, obesity, and an altered CW, with an AUC of 0.85 (Table 2).

DISCUSSION

The present study showed a high prevalence of MASLD (44.3%) in IBD patients, with the main associated predictors related to MS risk factors. Although most individuals do not

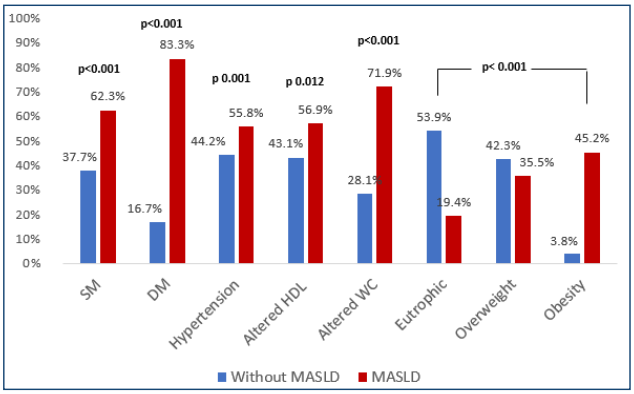

Figure 1. Correlation between components of the metabolic syndrome and the presence of steatotic liver disease associated with metabolic dysfunction. SM: metabolic syndrome; DM: diabetes mellitus; HDL: high-density lipoprotein; WC: waist circumference; MASLD: metabolic dysfunction-associated steatotic liver disease.

Table 2. Predictive factors associated with the presence of steatotic liver disease in patients with inflammatory bowel disease.

Variables	OR (95%CI)	p-value
Number of SM components	2.20 (1.47–3.29)	<0.001
Presence of obesity	2.29 (1.18–4.42)	0.014
Altered WC*	2.98 (0.97–9.12)	0.056

SM: metabolic syndrome. *Waist circumference greater than 94 cm in males and 80 cm in females.

have advanced liver fibrosis, it was predicted in 6.5% of our casuistic, even though they were asymptomatic, showing the importance of screening for MASLD in this population, given its silent progressive behavior.

With the global obesity epidemic and the increasing prevalence of cardiovascular events, HS has been reported in 25% of the world's population. Greater awareness of IBD and an improved therapeutic arsenal of the disease have influenced the change in IBD patients' phenotype, with a more significant occurrence of overweight or obesity. A growing interest in the prevalence of HS in IBD patients has occurred in recent years, reported between 8.6 and 54%, which may vary according to the method used for the diagnosis[8,18]. The presence of AF or liver cirrhosis is reported in 18.3%[10,12]. The prevalence of HS in our study was similar to that reported in previous studies.

The pathophysiology of HS in IBD patients still needs to be well established. Bessissow et al. identified the activity and duration of the disease, as well as previous intestinal resection, as independent risk factors for HS[19]. Some of these results were replicated in subsequent studies that observed a correlation with corticosteroids or methotrexate use[10,20]. Conversely, therapy with anti-TNF could have a protective effect[12,20]. In our study, only the disease duration was longer in those with MASLD compared to patients without MASLD; however, it did not remain significant in the multivariate analysis. Data inherent to the type, extent, phenotype, or previous treatment, including intestinal resection, were not associated with MASLD.

More recent studies, such as the one by Palumbo et al., showed older age, higher BMI, and higher triglyceride levels as independent risk factors for HS[10]. Regarding the AF presence, Palumbo et al. found age and BMI predictors of its occurrence. In our series, older age, duration of the disease, and several MS diagnostic criteria (DM, hypertension, obesity, low HDL, and increased WC), as well as a higher number of ATP III MS components, were associated with MASLD. However, only a higher number of ATP III MS components, obesity, and an altered WC were independently associated with MASLD in the multivariate analysis. All these data are in line with more recent studies[2,9].

Noninvasive diagnosis of liver fibrosis through tests such as FIB-4 is increasingly being recommended, especially in populations at low risk of AF, to exclude it and early referral to a specialist for those "at risk" of AF[18,21,22]. In our casuistic, we could exclude the presence of AF in 63.5% of cases and diagnose it in 6.5%, despite awareness of FIB-4 positive predictive value limitations. However, with this strategy, only 20 out of the 62 patients with MASLD would need to continue diagnostic investigation using more advanced methods. Our results agree with those of Trifan et al. that most patients undergoing a more sensitive method for AF diagnosis (liver transient elastography) did not present it[23].

The main drawback of our study was that the methods used for HS diagnosis were heterogeneous and, in most cases, established by ultrasonography (US) findings. It is known that the diagnostic accuracy of the US may be inadequate for mild steatosis recognition, as it is also an operator-dependent technique, with MRI being the most accurate method, despite the cost that often limits its use[20]. Furthermore, as ours is a tertiary hospital with reference services in IBD and hepatology, there may have been some selection bias, with more severe patients being included in the study, which should not reflect the national scenario.

To the best of our knowledge, this is the first study that used the new nomenclature established for steatotic liver disease, which consists of better-established criteria that consider the metabolic nature of the physiopathology. The high prevalence of MASLD in patients with IBD makes it necessary to have a plan to prevent progression to more severe forms of disease. It is mandatory for the professional responsible for managing

these patients to be aware of the interaction between IBD and MASLD to adopt screening measures and refer "at-risk MASLD" to a hepatologist.

CONCLUSION

Our findings supported the reported high prevalence of MASLD in IBD patients and its close relationship with MS risk factors, highlighting the importance of careful screening and management of MASLD in this scenario.

AUTHORS' CONTRIBUTIONS

LRPO: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **TCRR:** Data curation, Formal Analysis, Software, Supervision, Writing – original draft, Writing – review & editing. **CAMJ:** Formal Analysis, Software. **MALB:** Data curation, Investigation. **MHGS:** Data curation, Investigation. **LPS:** Data curation, Investigation. **SMZ:** Investigation. **LCC:** Investigation. **HMGV:** Conceptualization, Writing – original draft. **JMFC:** Conceptualization, Project administration, Supervision, Writing – review & editing.

REFERENCES

1. Principi M, Iannone A, Losurdo G, Mangia M, Shahini E, Albano F, et al. Nonalcoholic fatty liver disease in inflammatory bowel disease: prevalence and risk factors. Inflamm Bowel Dis. 2018;24(7):1589-96. https://doi.org/10.1093/ibd/izy051
2. Karaivazoglou K, Konstantakis C, Tourkochristou E, Assimakopoulos SF, Triantos C. Non-alcoholic fatty liver disease in inflammatory bowel disease patients. Eur J Gastroenterol Hepatol. 2020;32(8):903-6. https://doi.org/10.1097/MEG.0000000000001679
3. Lazarus JV, Newsome PN, Francque SM, Kanwal F, Terrault NA, Rinella ME. Reply: a multi-society Delphi consensus statement on new fatty liver disease nomenclature. Hepatology. 2024;79(3):E93-4. https://doi.org/10.1097/HEP.0000000000000696
4. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004;40(6):1387-95. https://doi.org/10.1002/hep.20466
5. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84. https://doi.org/10.1002/hep.28431
6. Le MH, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, et al. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One. 2017;12(3):e0173499. https://doi.org/10.1371/journal.pone.0173499
7. Rojas YAO, Cuellar CLV, Barrón KMA, Arab JP, Miranda AL. Non-alcoholic fatty liver disease prevalence in Latin America: a systematic review and meta-analysis. Ann Hepatol. 2022;27(6):100706. https://doi.org/10.1016/j.aohep.2022.100706
8. Chao CY, Battat R, Khoury A, Restellini S, Sebastiani G, Bessissow T. Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: a review article. World J Gastroenterol. 2016;22(34):7727-34. https://doi.org/10.3748/wjg.v22.i34.7727
9. Lin A, Roth H, Anyane-Yeboa A, Rubin DT, Paul S. Prevalence of nonalcoholic fatty liver disease in patients with inflammatory bowel disease: a systematic review and meta-analysis. Inflamm Bowel Dis. 2021;27(6):947-55. https://doi.org/10.1093/ibd/izaa189
10. Saroli Palumbo C, Restellini S, Chao CY, Aruljothy A, Lemieux C, Wild G, et al. Screening for nonalcoholic fatty liver disease in inflammatory bowel diseases: a cohort study using transient elastography. Inflamm Bowel Dis. 2019;25(1):124-33. https://doi.org/10.1093/ibd/izy200
11. Papaefthymiou A, Potamianos S, Goulas A, Doulberis M, Kountouras J, Polyzos SA. Inflammatory bowel disease-associated fatty liver disease: the potential effect of biologic agents. J Crohns Colitis. 2022;16(5):852-62. https://doi.org/10.1093/ecco-jcc/jjab212
12. Sourianarayanane A, Garg G, Smith TH, Butt MI, McCullough AJ, Shen B. Risk factors of non-alcoholic fatty liver disease in patients with inflammatory bowel disease. J Crohns Colitis. 2013;7(8):e279-85. https://doi.org/10.1016/j.crohns.2012.10.015
13. Likhitsup A, Dundulis J, Ansari S, Patibandla S, Hutton C, Kennedy K, et al. High prevalence of non-alcoholic fatty liver disease in patients with inflammatory bowel disease receiving anti-tumor necrosis factor therapy. Ann Gastroenterol. 2019;32(5):463-8. https://doi.org/10.20524/aog.2019.0405
14. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353(23):2462-76. https://doi.org/10.1056/NEJMoa050516
15. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5. https://doi.org/10.1161/CIRCULATIONAHA.109.192644
16. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77(5):1797-835. https://doi.org/10.1097/HEP.0000000000000323
17. Zamani M, Alizadeh-Tabari S, Singh S, Loomba R. Meta-analysis: prevalence of, and risk factors for, non-alcoholic fatty liver disease in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2022;55(8):894-907. https://doi.org/10.1111/apt.16879
18. Schreiner AD, Livingston S, Zhang J, Gebregziabher M, Marsden J, Koch DG, et al. Identifying patients at risk for fibrosis in a primary care NAFLD cohort. J Clin Gastroenterol. 2023;57(1):89-96. https://doi.org/10.1097/MCG.0000000000001585
19. Bessissow T, Le NH, Rollet K, Afif W, Bitton A, Sebastiani G. Incidence and predictors of nonalcoholic fatty liver disease by serum biomarkers in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2016;22(8):1937-44. https://doi.org/10.1097/MIB.0000000000000832
20. Hoffmann P, Jung V, Behnisch R, Gauss A. Prevalence and risk factors of nonalcoholic fatty liver disease in patients with inflammatory bowel diseases: a cross-sectional and longitudinal analysis. World J Gastroenterol. 2020;26(46):7367-81. https://doi.org/10.3748/wjg.v26.i46.7367

21. Archer AJ, Belfield KJ, Orr JG, Gordon FH, Abeysekera KW. EASL clinical practice guidelines: non-invasive liver tests for evaluation of liver disease severity and prognosis. Frontline Gastroenterol. 2022;13(5):436-9. https://doi.org/10.1136/flgastro-2021-102064

22. Cusi K, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, et al. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). Endocr Pract. 2022;28(5):528-62. https://doi.org/10.1016/j.eprac.2022.03.010

23. Trifan A, Stafie R, Rotaru A, Stratina E, Zenovia S, Nastasa R, et al. Screening for liver steatosis and fibrosis in patients with inflammatory bowel disease using vibration controlled transient elastography with controlled attenuation parameter. J Clin Med. 2022;11(19):5959. https://doi.org/10.3390/jcm11195959

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231327

Analysis of long-term family dynamics in mothers who have undergone fetal myelomeningocele surgery using telemedicine: a pilot study

Tatiane Santos Nunes[1], Edward Araujo Júnior[1], Liliam Cristine Rolo[1], Adriana Sañudo[2], Sergio Cavalheiro[3], Antonio Fernandes Moron[1]

SUMMARY

OBJECTIVE: The aim of this study was to understand the dynamics of families with children with myelomeningocele undergoing intrauterine fetal surgery.

METHODS: A retrospective cohort pilot study was carried out with 11 mothers of children who had undergone intrauterine myelomeningocele repair. Participants in this study responded to an electronic questionnaire (via Google Forms), developed by the study authors, that consisted of 22 multiple-choice questions, of which 17 were closed-ended and 5 had a standardized format.

RESULTS: The mean (± standard deviation) of the mothers' age was 37.6 (± 3.5) years. The median of gestational age at delivery and birthweight were 34.9 (range, 33 to 36.1) weeks and 2,300 (range, 1,950 to 2,763) g, respectively. The majority of mothers were white (81.8%), had university degree (81.8%), were Catholic (63.6%), and were married (100%). The majority of mothers rated their relationship with their husband, family, and friends as excellent (54.5, 72.7, and 54.5%, respectively). All 11 mothers reported that the newborn with myelomeningocele was born <37 weeks gestation and the birthweight most often<2,500 g. Approximately 64% of the mothers reported that their child required adaptations or had special needs, of which walking aids (50%) and bladder control (50%) were the most common ones.

CONCLUSION: Telemedicine proved to be a useful tool in the long-term follow-up of children who underwent intrauterine surgery to correct myelomeningocele.

KEYWORDS: Spina bifida. Surgery. Follow-up study. Family dynamics. Telemedicine.

INTRODUCTION

Fetal myelomeningocele is considered a severe form of neural tube closure disorder, resulting from a failure of the primary neurulation process, with closure normally occurring in the fourth week of gestation[1]. It affects approximately 2,000 live births worldwide[2], with an incidence of approximately 3.4–6 per 10,000 newborns in the United States[3]. The main clinical alterations of myelomeningocele are motor and cognitive deficits, hydrocephalus, varying degrees of skeletal delay and motor deformities, and bladder and bowel dysfunction throughout life, requiring prolonged assistance from the child's caregiver to perform basic activities of daily living[4].

Myelomeningocele is one of the most serious congenital defects and is considered a nonlethal fetal anomaly for which there is no satisfactory postnatal treatment, with significant morbidities, including dysfunction of the bowel, bladder, and reproductive organs, requiring a careful surgical procedure and careful analysis of risks and benefits[5]. Studies have shown improved quality of life for fetuses subjected to intrauterine surgery, minimized exposure of neural elements to amniotic fluid, improved cerebellar herniation, reduced hydrocephalus, as well as a significant contribution to prognosis and reduced economic costs of treating the disease in the postnatal period[6]. Other benefits of intrauterine surgery include a reduction in ventriculo-peritoneal shunt rates by 12 months of age and the ability to walk by 30 months of age[7].

Currently, most developed countries offer telehealth services. In developing countries, on the other hand, telemedicine faces a major health challenge in terms of expanding access to specialized medical services in places where the quality of health care has not improved[8]. It should be noted that the care of a child with myelomeningocele does not end in the postnatal period, but throughout life, this care will be continuous, actively requiring the involvement of the family. In this scenario, the

[1]Universidade Federal de São Paulo, Escola Paulista de Medicina, Department of Obstetrics – São Paulo (SP), Brazil.
[2]Universidade Federal de São Paulo, Escola Paulista de Medicina, Department of Preventive Medicine – São Paulo (SP), Brazil.
[3]Universidade Federal de São Paulo, Escola Paulista de Medicina, Department of Neurology and Neurosurgery – São Paulo (SP), Brazil.
*Corresponding author: araujojred@terra.com.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 11, 2024. Accepted on March 02, 2024.

use of the telemedicine tool becomes indispensable in accompanying families, so that it can help them care for their child who will need special care and teams trained to accompany them[9]. In addition, the multi-professional team should know the dynamics of each family, taking into account its individuality in order to recognize its different needs, personalize the practice of care, develop strategies that promote the child's autonomy, preserve its cognitive functions, and promote an improved quality of life[10].

Therefore, the aim of the present study was to identify the family dynamics of children diagnosed with fetal myelomeningocele who underwent intrauterine corrective surgery using the telemedicine tool.

METHODS

A retrospective cohort pilot study was carried out between 2018 and 2023 with 11 mothers of children who had undergone intrauterine myelomeningocele repair. This study was approved by the Ethics Committee of the Federal University of São Paulo (UNIFESP) and the mothers signed a consent form. To participate in the study, mothers received an invitation letter via email and WhatsApp. Patients with severe cognitive impairment and those who refused to participate at any point in the study were excluded.

Participants in this study responded to an electronic questionnaire (via Google Forms), developed by the study authors, that consisted of 22 multiple-choice questions, of which 17 were closed-ended and 5 had a standardized format. The first part of the questionnaire was designed to assess the socio-demographic profile of the participants and, through closed-ended questions, to understand the ongoing family and psychological impact of the diagnosis of myelomeningocele and its impact on pregnancy. The second part was designed to understand the family dynamics after the intrauterine correction surgery. The estimated time to complete the questionnaire was approximately 25 min, but as much time as necessary could be used. The questionnaire could not be completed until the last question had been answered.

To gain a better understanding of daily routines and family dynamics, a telemedicine interview was scheduled, with a time and date based on the participant's availability. On the agreed day and time, a telemedicine consultation was scheduled through a secure commercial platform (Versatilis^{O}), and the participant received an electronic access link.

In the present study, telemedicine was used as a tool to facilitate virtual interviews and complement the research, allowing the researchers (nurse and physician) to compare the participants' responses and the level of satisfaction expressed by the families. In addition, participants were able to share their experiences through the interactive audiovisual communication method offered by the telemedicine tool, which was crucial given the expertise and guidance provided by health professionals. Another factor observed by the researchers was the convenience and flexibility of choosing the best day and time for the interview, regardless of the distance, as participants expressed satisfaction, affection, and confidence during the interview.

The data was transferred to an Excel 2010 spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed using the STATA/SE 15.1 (Stata Corp., College Station, TX, USA) statistical program. Qualitative variables were described as number (N) and percentage (%), while quantitative variables were described as mean (standard deviation—SD), minimum and maximum values, or as median, 25th and 75th percentile, and minimum and maximum values.

RESULTS

We selected 13 mothers, but 2 refused to participate in the study, leaving 11 mothers in the final sample. All of them completed the questionnaire in full, with a 100% success rate. Only 2 (18.2%) of the mothers interviewed had tried to get pregnant again, and most of them reported fear and insecurity about being diagnosed with fetal myelomeningocele again. The mean (± SD) mothers' age was 37.6 (± 3.5) years. The majority of mothers were white (81.8%), had university degree (81.8%), were Catholic (63.6%), and were married (100%). The majority of mothers rated their relationship with their husband, family, and friends as excellent (54.5, 72.7, and 54.5%, respectively). Of the six mothers who have other children, about 83% rated their relationship with them as excellent. Table 1 shows the description of the socioeconomic and demographic data as well as the relationships with their husband, children, and friends.

Table 2 showed that most of the mothers reported 2 (45.4%) previous pregnancies, the most frequent number of deliveries was 1 (45.4%), approximately 91% of the women reported not having had abortions, and all of them reported no stillbirths or neonatal deaths. As for the number of children, the most frequent answer was 1 (45.4%) child. When asked about the number of preterm births, 9 (81.8%) of them reported 1. All 11 mothers reported that the newborn with myelomeningocele was delivered with a median of 34.9 (range, 33 to 36.1) weeks' gestation and a birthweight median of 2,300 (range, 1,950 to 2,763) g. Only one woman reported

a history of fetal malformation, which was a neural tube malformation. Notably, 2 (18.2%) mothers reported a family

Table 1. Description of socioeconomic and demographic data and relationship with husband/children/friends.

Variables	N (%)
Age, years	
Mean (standard deviation)	37.6 (3.5)
Minimum–maximum	32–44
Gestational age at delivery, weeks	
Median	34.9
Minimum–maximum	33–36.1
Birthweight, g	
Median	2,300
Minimum–maximum	1,950–2,763
Race	
White	9 (81.8%)
No-white	2 (18.2%)
Schooling	
Incomplete university degree	2 (18.2%)
University degree	9 (81.8%)
Religion	
Catholic	7 (63.6%)
Spiritist	2 (18.2%)
Evangelica	1 (9.1%)
Marital status, married	11 (100%)
Lives with partner, yes	11 (100%)
Relationship with the partner	
Great	6 (54.5%)
Good	2 (18.2%)
Regular	2 (18.2%)
Not applicable	1 (9.1%)
Relationship with the family	
Great	8 (72.7%)
Good	2 (18.2%)
Regular	1 (9.1%)
Do you have other children?	
No	5 (45.5%)
Yes	6 (54.5%)
Relationship with other children	(n = 6)
Great	5 (83.3%)
Good	1 (16.7%)
Relationship with friends	
Great	6 (54.5%)
Good	5 (45.5%)

Table 2. Maternal history and perinatal outcomes.

Variables	N (%)
Number of previous pregnancies	
1	4 (36.4%)
2	5 (45.4%)
3	2 (18.2%)
Number of deliveries	
1	5 (45.4%)
2	4 (36.4%)
3	2 (18.2%)
Number of abortions	
None	10 (90.9%)
1	1 (9.1%)
Number of live children	
1	5 (45.4%)
2	4 (36.4%)
3	2 (18.2%)
Stillbirth, none	11 (100%)
Stillborn, none	11 (100%)
Number of preterm births (<37 weeks)	
1	9 (81.8%)
2	2 (18.2%)
Number of post-term births (≥42 weeks)	
0	10 (90.9%)
1	1 (9.1%)
Birthweight of newborn with myelomeningocele	
<2,500 g	8 (72.7%)
2,500–4,000 g	3 (27.3%)
Gestational age at delivery of newborn with myelomeningocele	
<37 weeks	11 (100%)
History of fetal malformation	
No	10 (90.9%)
Yes	1 (9.1%)
What? Neural tube defect	
Family history of fetal malformation	
No	9 (81,8%)
Yes	2 (18,2%)
What?	
No definitive diagnosis	1 (50%)
Myelomeningocele	1 (50%)
Do you intend to get pregnant again?	
No	9 (81.8%)
Yes	2 (18.2%)
If you want to get pregnant, do you plan it?	
Yes	2 (100%)

Table 3. Description of current activities with your child.

Variables	N (%)
Do you have any kind of specialist follow-up?	
Yes	11 (100%)
Type of follow-up	(n = 11)
Physiotherapy	10 (90.9%)
Neurology	10 (90.9%)
Urology	9 (81.8%)
Orthopedics	9 (81.8%)
Occupational therapy	4 (36.4%)
Psychology	3 (27.3%)
Speech therapy	2 (18.2%)
Orthodontists	1 (9.1%)
Other specialists	4 (36.4%)
How often?	
One a weak	1 (9.1%)
Three times a weak	2 (18.2%)
Others	8 (72.7%)
Does your child need adaptations or special needs?	
No	4 (36.4%)
Yes	7 (63.6%)
Use of a wheelchair	
No	9 (81.8%)
Yes	2 (18.2%)
Use of a walker or crutches	
No	8 (72.7%)
Yes	3 (27.3%)
Orthopedic shoes or special orthoses	
No	3 (27.3%)
Yes	8 (72.7%)
Have you had to make any changes or adaptations to your home to better accommodate your child?	
No	8 (72.7%)
Yes	3 (27.3%)
Changing or adapting your car	
No	10 (90.9%)
Yes	1 (9.1%)
Does your child need any daily procedures?	
No	3 (27.3%)
Yes	8 (72.7%)
What are the daily procedures?	(n = 8)
Use of disposable diapers	6 (75%)
Intermittent bladder catheterization	4 (50%)
Other products/materials	1 (12.5%)
Special needs	(n = 8)
Walking aid	4 (50%)
Fecal elimination	4 (50%)
Bladder elimination	3 (37.5%)
Supporting oneself	2 (25%)
Feeding	1 (12.5%)
Speech difficulties	1 (12.5%)
Holding an object	1 (12.5%)
Difficulty interacting with other children	1 (12.5%)

Continue...

Table 3. Continuation..

history of fetal malformations, and 1 of them was myelomeningocele. Only two mothers reported that they intended to get pregnant again. Most of the mothers (n = 7, 63.6%) had undergone previous surgical procedures, and the most frequent procedure was intrauterine surgery for myelomeningocele (n = 4, 57.1%).

Table 3 shows the description of the activities currently carried out with the child, being that all the children have some kind of follow-up with a specialist, with physiotherapy and neurology the most frequently reported (91%). Approximately 64% of the mothers reported that their child required adaptations or had special needs. Most of the children did not use wheelchairs, walkers, or crutches (81.8 and 72.7%, respectively). However, the majority of children, approximately 73%, used orthopedic shoes or special orthoses. Regarding the need for adaptations, the most common response was that there was no need for adaptations, both at home and in the car (72.7 and 90.9%, respectively). More than 70% of the children required some type of daily procedure, the most common being the use of disposable diapers (75%) and intermittent catheterization (50%). The special needs most often reported by mothers were walking aids (50%) and bladder control (50%).

DISCUSSION

The active participation of the family is essential for the child's development and autonomy. Parents, for their part, try to learn how to adapt their routine and meet their child's needs in order to overcome the greatest challenges and become as independent as possible, especially in the adult phase. From this perspective, it is necessary for the professional responsible for the rehabilitation of children with myelomeningocele to know the dynamics of each family and to develop strategies for personalized

care, especially those related to family-centered care and not just that of the child[10].

After intrauterine surgery for myelomeningocele, women are advised not to become pregnant for the first 2 years. Of the mothers, 2 (18.8%) did not try to become pregnant because they feared complications in subsequent pregnancies. Goodnight et al.[11] assessed the obstetric risk in subsequent pregnancies after intrauterine repair of myelomeningocele. From 693 cases of intrauterine surgeries, 77 subsequent pregnancies in 60 women were observed. The uterine rupture rate was 9.6% (n = 5), resulting in 2 fetal deaths, and maternal transfusion was required in 4 (7.7%) patients.

In our study, 7 (63.6%) of the children required special care, including 8 (72.7%) with orthopedic shoes or special orthoses. In a pioneer study developed in our service after the first six intrauterine repairs for myelomeningocele at the age of 3.5 years, two children had normal leg movements, sacral functional level, and were community ambulators. One child with a lesion at the L1–L2 anatomical level was nonambulatory and completely dependent on a wheelchair for mobility[12].

This study showed that most of the children have special needs, but there was no need to adapt the family home or car. Buoro and Nogueira[13] searched to identify the main challenges facing the family of a child with meningomyelocele. They observed that the quality of life of mothers and caregivers of children with meningomyelocele was affected regarding functional capacity, emotional aspects, and mental health. The most common difficulties faced by caregivers were performing bladder catheterization, providing general care, financial burden, and accessibility.

Intrauterine surgery for myelomeningocele is associated with a higher risk of adverse outcomes for the maternal–fetal binomial. In our study, all deliveries were <37 weeks, and the birthweight<2,500 g occurred in 8 (72.3%) cases. Moron et al.[14] assessed the perinatal outcomes of 237 intrauterine surgeries for myelomeningocele. The mean gestational age at delivery and birthweight were 33.6 ± 2.4 weeks and 2,186 ± 506 g, respectively, and 86.9% of deliveries were <37 weeks.

A comparative multicenter study assessed the long-term impact on families and caregivers of children with myelomeningocele who underwent intrauterine surgery in the pre- and postnatal periods. They found that the group who underwent intrauterine surgery had a lower social impact on the family when compared to the group who underwent postnatal surgical correction[15].

This study showed that the active participation of the family was essential for the development and autonomy of the children. Parents try to learn the necessary care to adapt to the routine and meet the needs of their children so that they can overcome their major challenges and become increasingly independent. In this scenario, professionals working to rehabilitate these children must know the dynamics of each family and develop personalized care strategies that focus on the families, not just the children.

Some studies describe the benefits and ease of use of telemedicine in health services, highlighting the use of technology for interventions in maternal and child health. The main objective is to improve not only care, but also prenatal care, postnatal follow-up, and child care, and to reduce the risk of maternal and neonatal mortality or morbidity[16,17]. In Arkansas, telemedicine is commonly used in obstetrics through the Antenatal and Neonatal Guidelines, Education and Learning System (ANGELS), the statewide telemedicine network. This network is primarily used for teleultrasound consultations and maternal–fetal medicine[18].

Obviously, the frequency of telemedicine encounters has increased significantly in recent years. We can still emphasize the safety and quality of the services provided by this tool in the field of obstetrics and fetal medicine. These include prenatal care, postpartum care, diabetes mellitus management, medication abortion, lactation support, hypertension control, genetic counseling, ultrasound, contraception, and mental health services. For many users of these services, telemedicine has several potential or proven benefits, including expanded patient access, increased patient satisfaction, and reduced disparities in care and health outcomes compared to in-person encounters[19].

In the current study, most of the participants used the telemedicine tool successfully, showing trust and satisfaction to the researchers, i.e., the remote format did not impede the effectiveness of the care. The knowledge generated by this study will contribute to the support of different educational and professional actions in the care of pregnant women, from the prenatal period to the postnatal period. Such knowledge will strengthen the professional–patient bond, with benefits such as improving access to postnatal care, promoting better integration, and improving clinical outcomes, even for those who live in places with difficult access. In addition, the new knowledge will provide continuity of care, bringing patients closer to the professional and allowing them "autonomy" and security. We believe that the results of this study will contribute to the expansion of primary care, extending to health reference services, especially those of high complexity and high-risk pregnancies, as well as those of fetal medicine throughout the country.

CONCLUSION

Telemedicine proved to be a useful tool in the long-term follow-up of children who underwent intrauterine surgery to correct myelomeningocele. The majority of children required special care, with walking aids and bladder control being the most common. Also, this study showed that the use of technological tools in monitoring families is fundamental and serves as a strategic support model. We emphasize that the use of these tools in long-term monitoring can provide a better quality of life for children and support for their families, strengthening the bond and promoting a closer relationship between professionals and their clients/patients.

AUTHORS' CONTRIBUTIONS

TSN: Data curation, Investigation. **EAJ:** Writing – original draft. **LCR:** Methodology. AS: Formal Analysis. **SC:** Writing – review & editing. **AFM:** Supervision.

REFERENCES

1. Boulet SL, Yang Q, Mai C, Kirby RS, Collins JS, Robbins JM, et al. Trends in the postfortification prevalence of spina bifida and anencephaly in the United States. Birth Defects Res A Clin Mol Teratol. 2008;82(7):527-32. https://doi.org/10.1002/bdra.20468
2. Leidinger A, Piquer J, Kim EE, Nahonda H, Qureshi MM, Young PH. Experience in the early surgical management of myelomeningocele in Zanzibar. World Neurosurg. 2019;121:e493-9. https://doi.org/10.1016/j.wneu.2018.09.145
3. Saadai P, Farmer DL. Fetal surgery for myelomeningocele. Clin Perinatol. 2012;39(2):279-88. https://doi.org/10.1016/j.clp.2012.04.003
4. Copp AJ, Adzick NS, Chitty LS, Fletcher JM, Holmbeck GN, Shaw GM. Spina bifida. Nat Rev Dis Primers. 2015;1:15007. https://doi.org/10.1038/nrdp.2015.7
5. Adzick NS, Thom EA, Spong CY, Brock JW, Burrows PK, Johnson MP, et al. A randomized trial of prenatal versus postnatal repair of myelomeningocele. N Engl J Med. 2011;364(11):993-1004. https://doi.org/10.1056/NEJMoa1014379
6. Danzer E, Johnson MP, Adzick NS. Fetal surgery for myelomeningocele: progress and perspectives. Dev Med Child Neurol. 2012;54(1):8-14. https://doi.org/10.1111/j.1469-8749.2011.04049.x
7. Flanders TM, Heuer GG, Madsen PJ, Buch VP, Mackell CM, Alexander EE, et al. Detailed analysis of hydrocephalus and hindbrain herniation after prenatal and postnatal myelomeningocele closure: report from a single institution. Neurosurgery. 2020;86(5):637-45. https://doi.org/10.1093/neuros/nyz302
8. Keri VC, Brunda RL, Sinha TP, Wig N, Bhoi S. Tele-healthcare to combat COVID-19 pandemic in developing countries: a proposed single centre and integrated national level model. Int J Health Plann Manage. 2020;35(6):1617-9. https://doi.org/10.1002/hpm.3036
9. Kritikos TK, Holmbeck GN. Family functioning guidelines for the care of people with spina bifida. J Pediatr Rehabil Med. 2020;13(4):535-42. https://doi.org/10.3233/PRM-200720
10. Bonelli MA, Borges AA, Souza ROD, Castro GVZB, Oliveira GBS, Dupas G. Seeking tirelessly for better health and life conditions for the child with myelomeningocele. Rev Lat Am Enfermagem. 2021;29:e3428. https://doi.org/10.1590/1518-8345.3957.3428
11. Goodnight WH, Bahtiyar O, Bennett KA, Emery SP, Lillegard JB, Fisher A, et al. Subsequent pregnancy outcomes after open maternal-fetal surgery for myelomeningocele. Am J Obstet Gynecol. 2019;220(5):494.e1-7. https://doi.org/10.1016/j.ajog.2019.03.008
12. Hisaba WJ, Cavalheiro S, Almodim CG, Borges CP, Faria TC, Araujo Júnior E, et al. Intrauterine myelomeningocele repair postnatal results and follow-up at 3.5 years of age--initial experience from a single reference service in Brazil. Childs Nerv Syst. 2012;28(3):461-7. https://doi.org/10.1007/s00381-011-1662-z
13. Buoro RS, Nogueira MP. Quality of life and challenges of family members of children with meningomyelocele. Acta Ortop Bras. 2020;28(6):291-5. https://doi.org/10.1590/1413-785220202806237160
14. Moron AF, Barbosa MM, Milani H, Sarmento SG, Santana E, Suriano IC, et al. Perinatal outcomes after open fetal surgery for myelomeningocele repair: a retrospective cohort study. BJOG. 2018;125(10):1280-6. https://doi.org/10.1111/1471-0528.15312
15. Antiel RM, Adzick NS, Thom EA, Burrows PK, Farmer DL, Brock JW, et al. Impact on family and parental stress of prenatal vs postnatal repair of myelomeningocele. Am J Obstet Gynecol. 2016;215(4):522.e1-6. https://doi.org/10.1016/j.ajog.2016.05.045
16. Nuhu AGK, Dwomoh D, Amuasi SA, Dotse-Gborgbortsi W, Kubio C, Apraku EA, et al. Impact of mobile health on maternal and child health service utilization and continuum of care in Northern Ghana. Sci Rep. 2023;13(1):3004. https://doi.org/10.1038/s41598-023-29683-w
17. Brown HL, DeNicola N. Telehealth in maternity care. Obstet Gynecol Clin North Am. 2020;47(3):497-502. https://doi.org/10.1016/j.ogc.2020.05.003
18. Rabie NZ, Canon S, Patel A, Zamilpa I, Magann EF, Higley J. Prenatal diagnosis and telemedicine consultation of fetal urologic disorders. J Telemed Telecare. 2016;22(4):234-7. https://doi.org/10.1177/1357633X15595556
19. Healy A, Davidson C, Allbert J, Bauer S, Toner L, et al. Society for maternal-fetal medicine special statement: telemedicine in obstetrics-quality and safety considerations. Am J Obstet Gynecol. 2023;228(3):B8-17. https://doi.org/10.1016/j.ajog.2022.12.002

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231390

Isolated polyhydramnios in the third trimester or polyhydramnios secondary to late-onset gestational diabetes: is it worth distinguishing?

Sadullah Özkan[1*], Murat Levent Dereli[1], Sadun Sucu[1], Erol Nadi Varlı[1], Arife Akay[1], Safiye Elif Uzlu[2], Ali Turhan Çağlar[1], Yaprak Engin-Ustun[1]

SUMMARY

OBJECTIVE: The aim of this study was to compare pregnancy outcomes of patients with polyhydramnios due to late-onset gestational diabetes mellitus and patients with isolated polyhydramnios.

METHODS: Of the women who fully participated in prenatal examinations at Etlik Lady Zübeyde Hospital between January 1, 2018, and December 31, 2019, women with polyhydramnios of nonfetal–placental origin manifesting in the third trimester were retrospectively reviewed. Women with normal 75-g oral glucose tolerance test results between 24 and 28 weeks gestation who met the inclusion criteria were enrolled in the study and divided into two groups based on the results of rescreening with the 75-g oral glucose tolerance test for polyhydramnios in the third trimester: women with isolated polyhydramnios (group 1) and women with late-onset polyhydramnios due to gestational diabetes mellitus (group 2).

RESULTS: There were a total of 295 participants, of whom 35 (11.8%) were diagnosed with polyhydramnios due to late-onset gestational diabetes mellitus. There were no differences in the main outcomes. Birthweight and gestational age at birth were identified as independent risk factors for predicting composite maternal outcome {[odds ratio (OR)=1.273, 95% confidence interval (CI) 1.063–1.524, p=0.009]} and composite neonatal outcome (OR=0.606, CI 0.494–0.744, p<0.001), respectively.

CONCLUSION: Polyhydramnios in late pregnancy without evidence of pregnancy-related causes leading to polyhydramnios may be a sign of late-onset gestational diabetes mellitus in women with a normal prior oral glucose tolerance test. As pregnancy outcomes and management were indifferent, it does not seem necessary or useful to diagnose whether or not late-onset gestational diabetes mellitus is present.

KEYWORDS: Gestational diabetes mellitus. Polyhydramnios. Late-onset. Outcome. Oral glucose tolerance test.

INTRODUCTION

Polyhydramnios, defined as an increase in amniotic fluid volume, occurs in 0.4–2.0% of all pregnancies[1]. While about 60% of cases are idiopathic, known causes include fetal anomalies leading to fetal dysphagia or increased fetal urination, fetal–placental disorders causing fetal anemia, and maternal diabetes[2,3]. Polyhydramnios in which no causes can be identified are called isolated polyhydramnios[2].

Gestational diabetes mellitus (GDM) is one of the most common complications of pregnancy (4–16%), which is defined as a disorder of glucose metabolism that occurs or is first recognized during pregnancy[4]. A 75-g oral glucose tolerance test (OGTT) is the most widely used method for diagnosing GDM, although there are new promising methods such as measuring the fetal thymic–thoracic ratio or fetal thymus transverse diameter[5]. GDM increases the risk of severe pregnancy complications such as macrosomia, shoulder dystocia, cesarean section, wound infection, polyhydramnios, and neonatal hypoglycemia[6]. The underlying mechanism by which polyhydramnios develop in maternal diabetes is not fully understood. The highlighted pathophysiology is fetal polyuria caused by increased osmotic diuresis due to fetal hyperglycemia and increased accumulation of fluid from the chorioamniotic membranes due to higher glucose concentrations in the amniotic fluid[4].

Despite normal screening tests at 24–28 weeks of gestation (WG), some physicians recommend retesting for late-onset GDM in the third trimester if macrosomia is suspected or polyhydramnios develops[7]. In addition, there are still many questions about the side effects and benefits of antihyperglycemic medications in pregnancy that need to be clarified. Besides, many studies have demonstrated the association between cause-specific polyhydramnios and adverse perinatal outcomes, whereas the association between isolated polyhydramnios and adverse maternal–neonatal outcomes is contradictory[8,9]. As there is a

[1]Etlik Lady Zübeyde Maternity and Women's Health Teaching and Research Hospital, Department of Obstetrics and Gynaecology – Ankara, Turkey.
[2]Etlik Lady Zübeyde Maternity and Women's Health Teaching and Research Hospital, Department of Neonatology – Ankara, Turkey.
*Corresponding author: sadullahozkan@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 05, 2024. Accepted on March 03, 2024.

need for studies that bridge the gap between theory and practice regarding perinatal and maternal outcomes in such pregnancy populations, we conducted this study.

METHODS

Study design

Medical records of all women diagnosed with third-trimester polyhydramnios between January 1, 2018, and December 31, 2019, in the high-risk pregnancy department of Etlik Lady Zübeyde Maternity and Women's Health Education and Research Hospital, Ankara, Turkey, were retrospectively reviewed after the hospital's local medical research ethics committee approved the conduct, protocol, and procedures of the study (09/29-2020-15/08).

Study population characteristics, patient selection, and definitions

Pregnant women with an abnormal 75-g OGTT performed at 24–28 WG of current pregnancy, metabolic disorders other than late-onset GDM, multiple pregnancies, and patients with missing data and pregnancies with fetal features such as congenital infections, malformations, neuromuscular disorders, anemia, and genetic abnormalities were excluded. During the study period, there were a total of 29,238 live singleton births. Of these, 562 (1.9%) were diagnosed with third trimester polyhydramnios. Finally, 295 women without fetal–placental pathology who met the study criteria were included. Of the 295 (100%) participants, 258 (88.2%) were diagnosed with isolated polyhydramnios (group 1), whereas 35 (11.8%) were diagnosed with polyhydramnios due to late-onset GDM (group 2).

The diagnosis of GDM was made using the International Association of Diabetes and Pregnancy Study Groups consensus panel criteria[10]. Gestational age was calculated from the first day of the last menstrual period and confirmed by sonographic dating. Polyhydramnios was identified with the same sonography system [GE Voluson 730 Expert System (General Electric Medical Systems, Milwaukee, WI, USA) with a 2–7-MHz abdominal transducer] and classified as mild, moderate, or severe based on sonographic measurement of the maximum vertical pocket (MVP) of the amniotic fluid of 8–11, 12–15, and ≥16 cm, respectively[8].

Data collection

All data were obtained from the hospital database and medical records and compared between the two groups.

No serious neonatal adverse events occurred in either group, including neonatal death, sepsis, meconium aspiration syndrome, intraventricular hemorrhage, and necrotizing enterocolitis. Due to low incidence of some neonatal and maternal complications, which led to underestimation of the difference between the two groups, we combined various components of neonatal and maternal outcomes into composite outcome measures to draw conclusions. In the present study, the composite neonatal outcome (CNO) consisted of at least one or more of the following: neonatal intensive care unit (NICU) admission, shoulder dystocia, birth asphyxia, APGAR scores (appearance, pulse, grimace, activity, and respiration) of less than 7 at 5 min, transient tachypnea of newborn, respiratory distress syndrome (RDS) need for mechanical ventilation and continuous positive airway pressure (CPAP) therapy, hypoglycemia, hyperbilirubinemia, premature rupture of membranes (PROM), cord prolapse, and abruptio placentae. On the other hand, the composite maternal outcome (CMO) consisted of at least one or more of the following adverse maternal complications, including third- or fourth-degree vaginal tears, postpartum hemorrhage (PPH) due to uterine atony, hypertensive disorders of pregnancy (HDP), postpartum endometritis, and wound infection.

Statistical analysis

All statistical analyses were performed using the RStudio program (version 2021.09.4+403.pro3). Kolmogorov-Smirnov or Shapiro-Wilk tests were used to assess normality. Levene's test was used to assess homogeneity of variance. Descriptive analyses for normally distributed variables were presented using means and standard deviation. The independent-samples t-test was used to compare these parameters between groups. Descriptive analyses for the numerical data that were not normally distributed were performed using medians and quartiles (Q1–Q3). Mann-Whitney U tests were used to compare these parameters between groups. Descriptive analyses for the categorical variables were performed using frequency and percentage. Relationships between categorical variables were analyzed using the chi-square test or Fisher's exact test when expected cell counts were low. For multivariate analysis, the possible factors identified in the univariate analyses were entered into a binary logistic regression analysis to identify additional independent predictors of CMO and CNO. The Hosmer-Lemeshow goodness-of-fit statistic was used to assess model fit. A type I error level of 5% overall was used to derive statistical significance. A p-value of <0.05 was considered a statistically significant result.

RESULTS

Demographic and clinical characteristics at the time of diagnosis are described in Table 1. Mean number of previous cesarean deliveries, previous miscarriages, amniotic fluid measurements, and mean fasting blood glucose levels were indifferent between

the two groups, while mean values for age, body mass index (BMI), gravidity, and parity were significantly higher in group 2 (p=0.001, 0.005, 0.012, and 0.022, respectively). Moreover, there was no difference in the severity of polyhydramnios.

An analysis of birth characteristics and early neonatal and maternal outcomes of the study groups is shown in Table 2. The rate of cesarean delivery was high in both groups, with no statistical difference between the two groups, and the most

Table 1. Demographic and clinical characteristics of patients in the study groups at the time of diagnosis.

Variables	Group 1 (n=258)	Group 2 (n=35)	Total (n=293)	p-value
Age (years)	28 (24–32)	34 (26–37)	28 (24–33)	**0.001**
BMI (kg/m²)	29 (26–32)	32 (28–35)	29 (26–33)	**0.005**
Gravidity (number)	3 (2–3)	3 (2–4)	3 (2–4)	**0.012**
Parity (number)	1 (0–2)	2 (1–3)	1 (1–2)	**0.022**
Previous cesarean delivery	0 (0–0)	0 (0–1)	0 (0–0)	0.073
Miscarriage (number)	0 (0–0)	0 (0–1)	0 (0–0)	0.734
MVP of amniotic fluid (mm)	95 (87–109.2)	97 (85–110)	95 (87–110)	0.857
Mild	227 (88%)	31 (88.6%)	258 (88.1%)	0.322
Moderate	25 (9.7%)	2 (5.7%)	27 (9.2%)	
Severe	6 (2.3%)	2 (5.7%)	8 (2.7%)	
Fasting blood glucose (g/dL)	80 (75–88)	79 (71–98)	79.5 (75–88.2)	0.931
Postprandial blood glucose (g/dL)	113.2±22.2	116.1±34.3	113.9±24.9	**0.013**
HbA1c (%)	5.1±0.4	5.9±0.8	5.3±0.6	**<0.001**

BMI: body mass index; HbA1c: glycated hemoglobin; MVP: maximum vertical pocket; PHA: polyhydramnios. Data are expressed as median (Q1–Q3), mean±standard deviation or number (percentage) where appropriate. A p<0.05 indicates a significant difference. Statistically significant p-values are indicated in bold.

Table 2. Birth characteristics and early neonatal and maternal outcomes of the study groups.

Variables	Group 1 (n=258)	Group 2 (n=35)	Total (n=293)	p-value
Cesarean delivery	175 (67.8)	27 (77.1)	202 (68.9)	0.356
GAB (weeks)	36 (34–38)	34 (31.25–35)	36 (33–38)	**<0.001**
PTB	32 (12.4%)	7 (20%)	39 (13.3%)	0.285
<34 weeks	4 (12.5%)	1 (14.3%)	5 (12.8%)	>0.05
≥34 weeks	28 (87.5%)	6 (85.7%)	34 (87.2%)	
Birthweight (g)	3,465 (3,205–3,755)	3,605 (3,300–3,960)	3,490 (3,212.5–3,790)	0.085
APGAR 1	9 (9–9)	9 (9–9)	9 (9–9)	0.325
APGAR 5	10 (10–10)	10 (10–10)	10 (10–10)	0.363
Female gender	106 (41.1)	15 (42.9)	121 (42.3)	0.987
NICU admission	21 (8.1)	5 (14.3)	26 (8.9)	0.215
Hypoglycemia	0 (0)	2 (5.7)	2 (0.7)	**0.014**
Hyperbilirubinemia	4 (1.6)	1 (2.9)	5 (1.7)	0.473
RDS	9 (3.5)	2 (5.7)	11 (3.8)	0.627
CNO	22 (8.5%)	5 (14.3%)	27 (9.2%)	0.344
PROM	16 (6%)	1 (2.9%)	17 (5.6%)	0.704
HDP	3 (1.2%)	1 (2.9%)	4 (1.4%)	0.400
PPH	4 (1.6%)	1 (2.9%)	5 (1.7)	0.473
CMO	21 (8.1%)	2 (5.7%)	23 (7.8%)	1.000

APGAR: appearance, pulse, grimace, activity, and respiration score; CMO: composite maternal outcome; CNO: composite neonatal outcome; GAB: gestational age at birth; HDP: hypertensive disorders in pregnancy; NICU: neonatal intensive care unit; PHA: polyhydramnios; PPH: postpartum hemorrhage; PROM: premature rupture of membranes; PTB: preterm birth; RDS: respiratory distress syndrome. Data are expressed as median (Q1–Q3) or number (percentage) where appropriate. A p<0.05 indicates a significant difference. Statistically significant p-values are indicated in bold.

common indication was labor with a previous cesarean scar. Birth characteristics, including mean number of preterm deliveries, birthweight, APGAR scores at the first and fifth minutes, female gender, NICU admission, hyperbilirubinemia, RDS, PROM, HDP, and PPH, were indifferent. Gestational age at birth (GAB) was significantly higher in group 1, while hypoglycemia was more frequent in group 2 (p<0.001 and p=0.014, respectively). No significant differences were found between the two groups in CNO and CMO, which were defined as the main outcomes. Birthweight and GAB were identified as independent risk factors for predicting CMO {[odds ratio (OR)=1.273, 95% confidence interval (CI) 1.063–1.524, p=0.009]} and CNO (OR=0.606, CI 0.494–0.744, p<0.001) (Table 3). In this context, each 100-g increase in birthweight contributed to a 1.273-fold increase in the adverse consequences of CMO, whereas each 1-week increase in GAB contributed to a 1.650-fold protection against the adverse consequences of CNO.

DISCUSSION

The main findings were as follows. (1) The rates of adverse CNO, CMO, or any of the individual maternal and neonatal complications were similar in both groups, whereas neonatal hypoglycemia was more common in the polyhydramnios due to late-onset GDM group. (2) GAB was significantly lower in the polyhydramnios due to late-onset GDM group. Therefore, the higher rates of neonatal hypoglycemia (0 versus 2 neonates) could be related to the higher rate of preterm birth in this group. (3) Cesarean delivery rates were high in both groups compared with the general population, but no significant difference was observed between the two groups. (4) Birthweight and GAB were identified as independent protective factors predicting CMO and CNO, respectively.

In a retrospective study, abnormal results were obtained after rescreening with a glucose challenge test (GCT) in 165 of 513 women with previously normal GCT results who had at least one of the risk factors for GDM, such as obesity, hypertension, and family history of diabetes. Of these women, 154 underwent OGTT, and 20 of them were eventually diagnosed with late-onset GDM. Among the risk factors for GDM, only age greater than 30 years was significantly associated with the diagnosis of late-onset GDM, while there were no analyses of pregnancy outcomes, perinatal outcomes, and neonatal outcomes. Consequently, rescreening for GDM was recommended in advanced maternal age and in pregnancies with macrosomia, even if previous screening tests were negative[11].

In a recent study by Parveen et al, 71 women diagnosed with early-onset GDM by OGTT performed before 24 WG due to the presence of risk factors for GDM were compared with 90 women diagnosed with GDM at or after 24 WG. A history of GDM, macrosomia, and stillbirths in previous pregnancies were found to be significant risk factors for predicting early-onset GDM. Early-onset GDM significantly increased rates of recurrent urinary tract infection, polyhydramnios, intrauterine fetal loss, macrosomia, fetal birth trauma and related conditions, low APGAR scores, and NICU admission, and it was significantly related to decreased fetal movements, possibly due to polyhydramnios[12]. These results were confirmed in other studies[13,14]. Therefore, in women with risk factors for developing GDM, diagnosis by OGTT in early pregnancy can lead to a significant reduction in poor pregnancy outcomes for both the mother and the fetus.

In another recent retrospective study by Cauldwell et al, pregnant women diagnosed with late-onset GDM by home glucose monitoring at or after 33 WG had higher rates of macrosomia and associated shoulder dystocia, PPH, third- and fourth-degree vaginal injuries, and NICU admissions than pregnant women without GDM and/or with early-onset GDM diagnosed by OGTT before 33 WG[15]. This study concluded that active GDM management can improve perinatal outcomes in all pregnant women with risk factors for late-onset GDM, regardless of specific glucose thresholds. However, in contrast to our study, women with late-onset GDM were not screened for GDM at or before 24–28 WG. Therefore, the high complication rates in these pregnancies are likely due to overlooked

Table 3. Multivariate logistic regression analysis of risk factors for composite maternal and composite neonatal outcomes.

Variables	Composite maternal outcome		Composite neonatal outcome	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	0.954 (0.815–1.118)	0.563	1.057 (0.983–1.137)	0.132
BMI	0.801 (0.641–1.001)	0.051	1.041 (0.948–1.143)	0.400
Birthweight (100 g)	1.273 (1.063–1.524)	**0.009**	0.991 (0.905–1.084)	0.837
GAB	0.934 (0.548–1.593)	0.803	0.606 (0.494–0.744)	**<0.001**

BMI: body mass index; CI: confidence interval; GAB: gestational age at birth; OR: odds ratio. Data are expressed as median (minimum–maximum). A p<0.05 indicates a significant difference. Statistically significant p-values are in bold.

diagnosis of GDM (delayed diagnosis of GDM) in the early stages of pregnancy and prolonged exposure of the fetuses to a hyperglycemic environment. Furthermore, a 75-g OGTT performed between 24 and 28 weeks were normal in all participants in our study, so all patients diagnosed with late-onset GDM had actual late-onset GDM. Therefore, the duration of fetal exposure to a hyperglycemic environment is limited in this group, explaining that late development of GDM has less impact on fetal weight gain and that resulting macrosomia is not common. Accordingly, there were no significant differences in most pregnancy outcomes between patients with isolated polyhydramnios and patients with polyhydramnios due to late-onset GDM. In parallel with our study, Sohn et al found no difference in perinatal outcomes between patients without GDM and those diagnosed with late-onset GDM after an OGTT performed for suspected large-for-gestational-age fetus at an advanced stage of their pregnancy, despite normal GDM screening results in their early pregnancy[16].

Among the recommended laboratory tests to be performed when investigating the etiology of polyhydramnios, a 75-g OGTT ranks first to rule out GDM[7]. However, pregnancy outcomes are similar in women with isolated polyhydramnios and with polyhydramnios due to late-onset GDM, identifying GDM as the cause of third-trimester polyhydramnios that is not of fetal–placental origin and repeating an OGTT despite a previous negative OGTT not only has no impact on pregnancy management but also may result in additional health care costs and unnecessary parental worry.

Our study has some limitations, mainly owing to its retrospective design and relatively small sample. The major strength of this study is the ability to analyze a specific population that has not been thoroughly studied on this topic. In addition, the study was conducted in a single tertiary medical center with a high volume of patients, where the standardized algorithms for diagnosis, treatment, and follow-up of polyhydramnios and GDM were applied.

CONCLUSION

Polyhydramnios after the second trimester without evidence of fetal–placental causes leading to polyhydramnios may be a sign of late-onset GDM in women with previously normal OGTT results. As our study showed no difference in pregnancy outcomes and management was indifferent between isolated polyhydramnios and polyhydramnios due to late-onset GDM, it does not seem necessary or useful to identify late-onset GDM in patients with polyhydramnios in the third trimester. We think our study will guide further prospective randomized controlled trials to draw more definitive conclusions on this topic.

AUTHORS' CONTRIBUTIONS

SÖ: Conceptualization. **MLD:** Writing – original draft. **SS:** Formal Analysis. **ENV:** Data curation, Writing – original draft. **AA:** Data curation. **SEU:** Data curation. **YEÜ:** Writing – review & editing.

REFERENCES

1. Brace RA, Cheung CY, Anderson DF. Regulation of amniotic fluid volume: insights derived from amniotic fluid volume function curves. Am J Physiol Regul Integr Comp Physiol. 2018;315(4):R777-89. https://doi.org/10.1152/ajpregu.00175.2018
2. Wiegand SL, Beamon CJ, Chescheir NC, Stamilio D. Idiopathic polyhydramnios: severity and perinatal morbidity. Am J Perinatol. 2016;33(7):658-64. https://doi.org/10.1055/s-0036-1571320
3. Rodríguez-Ayala G, Vega A, Correa-Rivas M, Jímenez A. Chorioangioma: an uncommon cause of hydramnios and consequent preterm labor in second trimester of pregnancy. Bol Asoc Med P R. 2013;105(1):36-9. PMID: 23767383
4. Crimmins S, Mo C, Nassar Y, Kopelman JN, Turan OM. Polyhydramnios or excessive fetal growth are markers for abnormal perinatal outcome in euglycemic pregnancies. Am J Perinatol. 2018;35(2):140-5. https://doi.org/10.1055/s-0037-1606186
5. Gök K, Özden S. Finding the best method for screening for gestational diabetes mellitus: fetal thymic-thoracic ratio or fetal thymus transverse diameter. Rev Assoc Med Bras (1992). 2023;69(2):303-7. https://doi.org/10.1590/1806-9282.20221012
6. Metzger BE, Coustan DR, Trimble ER. Hyperglycemia and adverse pregnancy outcomes. Clin Chem. 2019;65(7):937-8. https://doi.org/10.1373/clinchem.2019.303990
7. Hamza A, Herr D, Solomayer EF, Meyberg-Solomayer G. Polyhydramnios: causes, diagnosis and therapy. Geburtshilfe Frauenheilkd. 2013;73(12):1241-6. https://doi.org/10.1055/s-0033-1360163
8. Pri-Paz S, Khalek N, Fuchs KM, Simpson LL. Maximal amniotic fluid index as a prognostic factor in pregnancies complicated by polyhydramnios. Ultrasound Obstet Gynecol. 2012;39(6):648-53. https://doi.org/10.1002/uog.10093
9. Wax JR, Cartin A, Craig WY, Pinette MG. Transient idiopathic polyhydramnios: maternal and perinatal outcomes: maternal and perinatal outcomes. J Ultrasound Med. 2022;41(11):2859-66. https://doi.org/10.1002/jum.15974
10. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676-82. https://doi.org/10.2337/dc09-1848

11. Boriboonhirunsarn D, Chutimongkonkul W. Rate of abnormal results from repeated screening tests for gestational diabetes mellitus after normal initial tests. J Med Assoc Thai. 2007;90(7):1269-73. PMID: 17710963
12. Parveen N, Hassan SU, Zahra A, Iqbal N, Batool A. Early-onset of gestational diabetes vs. late-onset: can we revamp pregnancy outcomes? Iran J Public Health. 2022;51(5):1030-9. https://doi.org/10.18502/ijph.v51i5.9418
13. Muche AA, Olayemi OO, Gete YK. Effects of gestational diabetes mellitus on risk of adverse maternal outcomes: a prospective cohort study in Northwest Ethiopia. BMC Pregnancy Childbirth. 2020;20(1):73. https://doi.org/10.1186/s12884-020-2759-8
14. Weschenfelder F, Hein F, Lehmann T, Schleußner E, Groten T. Contributing factors to perinatal outcome in pregnancies with gestational diabetes-what matters most? A retrospective analysis. J Clin Med. 2021;10(2):348. https://doi.org/10.3390/jcm10020348
15. Cauldwell M, Chmielewska B, Kaur K, l'Isle Y, Sherry A, Watt Coote I, et al. Screening for late-onset gestational diabetes: are there any clinical benefits? BJOG. 2022;129(13):2176-83. https://doi.org/10.1111/1471-0528.17154
16. Sohn J, Lim HJ, Kim S, Kim TH, Kim BJ, Hwang KR, et al. Delayed diagnosis of gestational diabetes mellitus and perinatal outcomes in women with large for gestational age fetuses during the third trimester. Obstet Gynecol Sci. 2020;63(5):615-22. https://doi.org/10.5468/ogs.20007

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231421

Prospective monitoring of patients undergoing radiotherapy during COVID-19

Denise Ferreira Silva Alves[1*], Daniela Dornelles Rosa[2], Luciane Borelli Finatto[3], Brenda Rigatti[2], Pedro Tofani Sant' Anna[2]

SUMMARY

OBJECTIVE: The objective of this study was to evaluate the quality of life of consecutive patients undergoing radiotherapy during the coronavirus disease 2019 pandemic at a private hospital in Southern Brazil from September 2020 to September 2021.

METHODS: This study was approved by the Research Ethics Board under project number 112 on April 17, 2020, and it was a prospective descriptive cohort study conducted in a Brazilian radiotherapy department from September 2020 to September 2021. It involved the weekly administration of the European Organisation for Research and Treatment of Cancer Questionnaire Core 30 questionnaires via telephone to consecutively assess patients with pathology-proven cancer diagnoses. These questionnaires captured both demographic data and patients' concerns related to the pandemic, providing a comprehensive overview of their quality of life during radiotherapy treatment.

RESULTS: In this study, 141 patients were analyzed, predominantly female (69.5%) with an average age of 61 years. Breast and prostate were the most treated sites, accounting for 51 and 19% of cases, respectively. The majority of treatments lasted between 3 and 5 weeks (73.77%). A small fraction (4.26%) tested positive for coronavirus disease 2019. The findings also highlighted a relatively high quality of life, with mean global scores of 77.95 and emotional functioning scores of 87.53, indicating maintained well-being during treatment.

CONCLUSIONS: Oncological patients continuing radiotherapy at our center during the pandemic experienced a low coronavirus disease 2019 infection rate and maintained a high quality of life with minimal emotional distress throughout their treatment period.

KEYWORDS: COVID-19. SARS-CoV-2. Quality of life. Radiotherapy. Therapeutics.

INTRODUCTION

In December 2019, the emergence of atypical pneumonia in Wuhan, Hubei Province, China, led to the identification of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), by the World Health Organization (WHO). This virus quickly escalated into a global health crisis, with the WHO declaring it an international public health emergency on January 30, 2020, and a pandemic by March 11, 2020[1,2].

The elderly and those with comorbidities, notably cancer patients, were found to be at increased risk of severe outcomes due to immunosuppression from the disease and treatments. Notably, a study of 2007 coronavirus disease 2019 (COVID-19) cases across China indicated a higher incidence among cancer patients (0.9%) compared with the general population (0.29%)[3]. Further research highlighted variable infection rates among different cancer types, with lung cancer showing the highest COVID-19 incidence[4-6]. Another significant finding was the elevated risk of severe infection in cancer patients compared with the non-cancer population[3,7].

Few studies have specifically addressed the impact of COVID-19 on patients undergoing radiotherapy (RT), with one indicating no significant rise in severe events[8]. Despite the global shift toward social isolation or "Lockdown" to curb virus spread, cancer patients continued their treatments, thereby possibly heightening their exposure risk.

The dual threat of cancer and COVID-19 significantly affected these patients' fears, compounded by the challenges of isolation, information scarcity, and financial strains associated with ongoing cancer care. Amid such unprecedented times, the resultant impact on their quality of life (QoL) underscores the necessity of integrating patient experiences into clinical research. Yet, literature focusing on the QoL among RT patients during the pandemic remains sparse, highlighting the need for focused studies to understand the unique challenges faced by this demographic[9].

[1]Hospital Moinhos de Vento, Department of Radiation Oncology – Porto Alegre (RS), Brazil.
[2]Hospital Moinhos de Vento – Porto Alegre (RS), Brazil.
[3]Hospital Moinhos de Vento, Department of Radiation Therapy – Porto Alegre (RS), Brazil.
*Corresponding author: denise.com@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: None.
Received on February 19, 2024. Accepted on March 02, 2024.

This study aimed to assess the QoL of consecutive patients receiving RT during the COVID-19 pandemic at a private hospital in Southern Brazil, from September 2020 to September 2021. It seeks to gain insights into how cancer treatment intersects with pandemic-induced isolation, affecting patient well-being.

METHODS

We conducted a Research Ethics Board-approved investigation within a single Brazilian RT department, prospectively assessing consecutive patients with pathologically proven cancer diagnoses treated from September 2020 to September 2021. This timeframe delineates the period for follow-up and data collection activities. The study was approved by the local ethics committee (under project number 112 on April 17, 2020) and ensured that all participants provided informed consent. We assessed all consecutive cancer patients treated with RT in our department from September 2020 to September 2021. Patients with a pathologically proven diagnosis of cancer receiving RT treatment at any site during the study period were included. Patients who were unable to complete the questionnaires, including those who were illiterate, were excluded from the study.

This prospective descriptive cohort study involved the weekly administration of the European Organisation for Research and Treatment of Cancer (EORTC) QoL questionnaires [Questionnaire Core 30 (QLQ-C30)], incorporating demographic data and pandemic-related concerns through telephone communication. This approach allowed for a comprehensive evaluation of patient well-being during their RT treatment amid the COVID-19 pandemic.

The EORTC QLQ-C30 consists of 30 items that assess five functions (physical, functional, emotional, cognitive, and social), nine symptom subscales/items (fatigue, nausea/vomiting, pain, dyspnea, insomnia, loss of appetite, constipation, diarrhea, and financial difficulties), and subscale overall health/QoL[9]. The questionnaire uses a 4-point Likert scale, ranging from "not at all" to "a lot," for items 1–28, and a 7-point scale for items 29 and 30, ranging from 1 (very poor) to 7 (excellent). Scores are linearly transformed on a scale from 0 to 100. A higher score on the functional scale and on global QoL indicates better functioning, while a higher score on the symptom scales indicates worse functioning[10,11].

Patient recruitment, exposure to treatment, and baseline data collection on QoL and demographics occurred from September 2020 to September 2021. No follow-up was required, as the study focused solely on collecting baseline QoL information and demographic data, without endpoints such as survival or local control.

This study utilized convenience sampling by including consecutive patients undergoing RT treatment in our department. Given the descriptive nature of this research, calculating a precise sample size was not deemed necessary, as the primary objective was not to test a specific hypothesis but rather to provide a comprehensive overview of the QoL and demographic profiles of our patient cohort during the study period.

STATISTICAL ANALYSIS

To characterize the study population, we employed descriptive analysis. Categorical variables were summarized using absolute frequencies (n) and percentages (%), and continuous variables were presented as means±standard deviation (SD) or medians with interquartile ranges (IQR: Q1–Q3), depending on data distribution. The Shapiro-Wilk test was utilized to assess data asymmetry.

Quality of life scores were derived according to the guidelines in the EORTC QLQ-C30 Scoring Manual. The EORTC QLQ-C30, which is an internationally recognized tool for assessing the health-related QoL of cancer patients, includes five functional scales, three symptom scales, and a global health status, all scored from 0 to 100, with higher scores indicating better functioning or more severe symptoms. For this study, we used version 3.0 of the QLQ-C30[11]. Scoring followed a two-step process:

Calculation of the raw score as the average of items contributing to the scale: Raw Score (RS)=∑i=1nIinRaw Score (RS)=$n\sum i=1nIi$

1. Linear transformation of the raw score to a 0–100 scale:
2. For functional scales and global health status/QoL: S=(1-RS-1range)×100S=(1-rangeRS-1)×100
3. For symptom scales/items: S=(RS-1range)×100S=(rangeRS-1)×100

The "range" represents the difference between the maximum and minimum possible RS values, typically 3 for most items (scored 1–4), except for global health status/QoL items (scored on a 7-point scale, range=6). Our presentation focused on specific scales identified by the two authors as most representative. Any conflicts were resolved by consulting a third, expert author. Scoring procedures are detailed in the EORTC QLQ-C30 Scoring Manual, which provides coding for the statistical analysis[11]. Statistical analysis was performed using the SAS software (Statistical Analysis System, SAS Institute Inc., Cary, NC), version 9.4.

RESULTS

A total of 150 patients were consecutively evaluated for inclusion in the study and provided informed consent. However, nine were subsequently excluded for several reasons. Unfortunately, one patient, diagnosed with glioblastoma, died before completing the treatment. The other eight patients were excluded due to a lack of response to follow-up calls or their decision to withdraw from the study after initially agreeing to participate.

Among the 141 patients who participated in the study, a significant majority (69.5%) were women, with an average age of 61 years (SD±12.9). The predominant site of treatment was the breast (51.1%), followed by the prostate (19.9%). Consistent with global guidelines[12-14] advocating for hypofractionation when feasible, the vast majority of treatments (73.8%) were completed within 3–5 weeks. Notably, a small fraction of the cohort, representing 4.3% (6/141), tested positive for COVID-19 during the course of their treatment. Regarding transportation methods to the hospital, 73.4% of the patients opted for individual means, either driving themselves or walking. Furthermore, 57.9% of the study participants resided alone or with just one other person (Table 1).

In Table 2, we present patients' concerns during RT, showing varied levels of insecurity about treatment, with 46.8% reporting less concern. The majority (99.3%) felt no need for additional information about COVID-19. Confidence in the hospital's precautionary measures was high, with 78.0% expressing a great sense of security.

Table 3 shows a consistent QoL (EORTC QLQ-C30) and functional status among patients over the treatment period, with median global health status/QoL scores remaining stable across the initial weeks and slightly decreasing by the seventh week. Physical, role, emotional, cognitive, and social functioning scores were generally high, reflecting minimal impact on patients' overall well-being. The EORTC QLQ-C30 revealed a mean global QoL score of 77.9 and an emotional functioning score of 87.5, indicating that patients sustained high QoL and minimal emotional distress during the study period. Symptom subscale analysis highlighted fatigue, insomnia, pain, and appetite loss as the most significant issues, with scores ranging from 19.7 (fatigue) to 10.3 (appetite loss). Dyspnea, potentially indicative of COVID-19, scored the lowest at 3.4, aligning with the observed low infection rates within the sample.

Approximately 10–15 days post-treatment, a final survey was conducted, comprising questions about QoL, general health, and symptoms specifically related to the COVID-19 infection such as any kind of breathing difficulty, cough, sore throat, and fever. Some patients with typical symptoms of COVID-19 were instructed to perform the test, and the results revealed that three of them tested positive for the virus.

DISCUSSION

This study provides a comprehensive look at the experiences of patients receiving RT during the COVID-19 pandemic. It reveals a complex response marked by initial concern and strong trust in hospital protocols, evidenced by 78% of patients expressing

Table 1. Demographic and treatment characteristics of radiotherapy patients: a snapshot of gender distribution, age, treatment sites, duration, and coronavirus disease 2019 infection rates.

Characteristics	n (%) or mean (±SD)
Gender	
Female	98 (69.5)
Male	43 (30.5)
Age (years)	61.2 (12.9)
Site of treatment	
Breast	72 (51.1)
Prostate	28 (19.9)
Gynecological	10 (7.1)
Gastrointestinal	4 (2.8)
Thorax	2 (1.4)
Others	25 (17.7)
Duration of treatment (weeks)	
1	7 (5,0)
2	10 (7.1)
3	31 (22.0)
4	29 (20.6)
5	44 (31.2)
6	15 (10.6)
7	5 (3)
Means of transport to the hospital	
Public	37 (26.2)
Individual	93 (66.0)
Walking	11 (7.8)
Number of persons with whom the patient lives	
0	16 (11.4)
1	65 (46.4)
2	33 (23.6)
3	21 (15.0)
4	5 (3.6)
Positive test for COVID-19	6 (4.3)
During treatment	3 (2.1)
Right after	3 (2.1)

Demographic and treatment characteristics of 141 patients who underwent radiotherapy were included in our analysis.

Table 2. Patient concerns and perceptions during radiotherapy in the coronavirus disease 2019 pandemic: levels of insecurity, information sufficiency, and trust in hospital safety protocols.

Insecurity about the treatment during the pandemic (1—little to 7—a lot)	n (%) or mean
1	66 (46.8)
2	6 (4.3)
3	10 (7.1)
4	9 (6.4)
5	18 (12.8)
6	6 (4.3)
7	26 (18.4)
Need to receive more information about COVID-19 during the treatment	
No	140 (99.3)
Yes	1 (0.7)
Sense of security with the precautionary protocol instituted in the hospital (1—little to 7—a lot)	
1	5 (3.6)
2	1 (0.7)
3	1 (0.7)
4	2 (1.4)
5	7 (5.0)
6	15 (10.6)
7	110 (78.0)

This table reveals patients' responses regarding their concerns and perceptions during radiotherapy amid the COVID-19 pandemic. A significant portion (46.8%) reported less insecurity about treatment, while a substantial majority (99.3%) felt no need for additional COVID-19 information. Confidence in the hospital's precautionary measures was overwhelmingly high, with 78.0% expressing a great sense of security.

confidence, leading to stable or improved well-being. Despite fears of increased COVID-19 risk for cancer patients noted in other studies[3,4,6,7], our cohort showed low infection rates (5%), suggesting effective preventive measures. Contrary to expectations of heightened emotional distress[15], our findings indicate maintained QoL, underscoring the value of personalized care and robust safety protocols in ensuring patient well-being during challenging times.

In exploring the impact of COVID-19 on cancer patients' QoL, Ciążynska et al. identified significant declines in global QoL, particularly in cognitive and social functioning, while maintaining near-normal levels in physical and emotional aspects[16]. This contrasted with our findings, which did not investigate a population receiving RT in a department characterized by well-organized processes like ours. This difference in study settings may account for the variations in outcomes observed between the two studies.

Our study's low COVID-19 infection rates among RT patients contrast with broader data, indicating a heightened risk for cancer patients. Liang et al.[3] and Dai et al.[4] reported increased infection and serious complication rates in cancer patients, particularly those with lung, gastrointestinal, and breast cancers[5-7]. Despite the higher risk in general cancer populations, our focused study in a well-organized RT department showed a notably low infection rate of 4.2%. This suggests that rigorous hygiene protocols, team training, patient education, and enforced social distancing may have effectively mitigated the risk of COVID-19 transmission within this specific treatment setting.

While studies on RT patients during the pandemic are scarce, one notable finding is the lack of a significant increase in serious events, despite the high patient turnover in a closed

Table 3. Evolution of patient functional well-being over time: a week-by-week analysis of quality of life and functional scales during radiotherapy treatment.

	Week 1 (n=141)	Week 2 (n=134)	Week 3 (n=123)	Week 4 (n=94)	Week 5 (n=64)	Week 6 (n=21)	Week 7 (n=7)	Mean (±SD)
Global health status/QoL	83 (67–92)	83 (67–92)	83 (67–92)	83 (67–92)	83 (67–83)	83 (67–83)	67 (33–83)	77.9 (18.5)
Functional scales								
Physical functioning	93 (73–100)	93 (80–100)	93 (80–100)	93 (80–100)	93 (80–100)	100 (90–100)	100 (100–100)	88.3 (16.8)
Role functioning	100 (83–100)	100 (83–100)	100 (83–100)	100 (67–100)	100 (100–100)	100 (100–100)	100 (100–100)	88.1 (25.1)
Emotional functioning	92 (75–100)	92 (83–100)	92 (83–100)	92 (75–100)	100 (79–100)	100 (92–100)	100 (100–100)	87.5 (19.6)
Cognitive functioning	100 (83–100)	100 (83–100)	100 (83–100)	100 (83–100)	100 (83–100)	100 (83–100)	100 (83–100)	89.6 (17.6)
Social functioning	100 (67–100)	100 (83–100)	100 (67–100)	100 (67–100)	100 (67–100)	100 (67–100)	100 (100–100)	84.7 (31.2)

This table provides a comprehensive overview of the changes in patients' general functions over the course of their radiotherapy treatment, spanning from the first to the seventh week. It also documents median and mean scores for global health status/quality of life (QoL), along with functional scales, including physical, role, emotional, cognitive, and social functioning.

treatment environment. Our study reported a low COVID-19 infection rate of 4.2%, predominantly among patients receiving adjuvant treatment for breast tumors. Although it is challenging to definitively attribute this low rate to the stringent hygiene protocols, staff training, patient education, and enforced social isolation, these measures likely played a key role. Contrary to reports of emotional distress among quarantined individuals, our findings indicate maintained QoL and emotional functioning among our cohort, suggesting effective management of patient well-being during treatment.

Alongside personalized approaches such as weekly phone calls and daily screenings, the precautionary measures implemented to curb infection and enhance patient care during the pandemic likely played a pivotal role in our positive outcomes. These strategies suggest that patients felt comprehensively supported at our center during the COVID-19 crisis. This experience invites a broader contemplation on reorganizing healthcare services toward more personalized, patient-centric models, emphasizing the importance of humanized medicine in future healthcare delivery.

Finally, our study findings are significant yet underscored by limitations that warrant a nuanced understanding. Conducted at a single institution, the scope of this study may limit the generalizability of its results. The small sample size and limited participant number restrict our ability to perform robust statistical analyses and generalize findings. Moreover, the absence of baseline data before and during the COVID-19 pandemic, along with the use of the EORTC QLQ-C30—a globally validated tool, raises questions about its adequacy in fully capturing the pandemic's impact on patients' emotional and psychological well-being. Future research could benefit from developing tools specifically aimed at assessing the emotional well-being of cancer patients in pandemic scenarios, potentially offering more targeted and reliable insights.

CONCLUSION

During the COVID-19 pandemic, our study found that cancer patients undergoing RT maintained a high QoL with minimal emotional distress, alongside a low COVID-19 infection rate. These results highlight the effectiveness and safety of continuing RT treatments during such crises. The limited cases of COVID-19 in our cohort restrict a detailed analysis of long-term COVID implications. Nonetheless, our findings provide insights for managing oncological care in future pandemics, emphasizing the resilience and adaptability of healthcare delivery in challenging times.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study adhered to the principles outlined in the Helsinki Declaration. This study was approved by the Hospital Moinhos de Vento Research Ethics Committee under REDCap no. 112 on April 17, 2020, and authorization was granted to use the EORTC QLQ-30 questionnaire. Patients were enrolled from September 2020 to September 2021, and informed consent was obtained at the time of recruitment.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS

DFSA: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **DDR:** Conceptualization, Formal Analysis. **LBF:** Data curation. **BR:** Data curation. **PTSA:** Data curation.

REFERENCES

1. Madan A, Siglin J, Khan A. Comprehensive review of implications of COVID-19 on clinical outcomes of cancer patients and management of solid tumors during the pandemic. Cancer Med. 2020;9(24):9205-18. https://doi.org/10.1002/cam4.3534
2. Morens DM, Daszak P, Markel H, Taubenberger JK. Pandemic COVID-19 joins history's pandemic legion. mBio. 2020;11(3):e00812-20. https://doi.org/10.1128/mBio.00812-20
3. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. 2020;21(3):335-7. https://doi.org/10.1016/S1470-2045(20)30096-6
4. Dai M, Liu D, Liu M, Zhou F, Li G, Chen Z, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. Cancer Discov. 2020;10(6):783-91. https://doi.org/10.1158/2159-8290.CD-20-0422
5. Zhang L, Zhu F, Xie L, Wang C, Wang J, Chen R, et al. Clinical characteristics of COVID-19-infected cancer patients: a retrospective case study in three hospitals within Wuhan, China. Ann Oncol. 2020;31(7):894-901. https://doi.org/10.1016/j.annonc.2020.03.296
6. Liu C, Zhao Y, Okwan-Duodu D, Basho R, Cui X. COVID-19 in cancer patients: risk, clinical features, and management. Cancer Biol Med. 2020;17(3):519-27. https://doi.org/10.20892/j.issn.2095-3941.2020.0289

7. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. 2020;21(3):335-7. https://doi.org/10.1016/S1470-2045(20)30096-6
8. Garassino MC, Whisenant JG, Huang LC, Trama A, Torri V, Agustoni F, et al. COVID-19 in patients with thoracic malignancies (TERAVOLT): first results of an international, registry-based, cohort study. Lancet Oncol. 2020;21(7):914-22. https://doi.org/10.1016/S1470-2045(20)30314-4
9. Nolte S, Liegl G, Petersen MA, Aaronson NK, Costantini A, Fayers PM, et al. General population normative data for the EORTC QLQ-C30 health-related quality of life questionnaire based on 15,386 persons across 13 European countries, Canada and the Unites States. Eur J Cancer. 2019;107:153-63. https://doi.org/10.1016/j.ejca.2018.11.024
10. Jeppesen SS, Bentsen KK, Jørgensen TL, Holm HS, Holst-Christensen L, Tarpgaard LS, et al. Quality of life in patients with cancer during the COVID-19 pandemic - a Danish cross-sectional study (COPICADS). Acta Oncol. 2021;60(1):4-12. https://doi.org/10.1080/0284186X.2020.1830169
11. Fayers P, Aaronson NK, Bjordal K, Groenvold M, Curran D, Bottomley A. The EORTC QLQ-C30 scoring manual. 3rd ed. Brussels: European Organisation for Research and Treatment of Cancer; 2001.
12. Guckenberger M, Belka C, Bezjak A, Bradley J, Daly ME, DeRuysscher D, et al. Practice recommendations for lung cancer radiotherapy during the COVID-19 pandemic: an ESTRO-ASTRO consensus statement. Radiother Oncol. 2020;146:223-9. https://doi.org/10.1016/j.radonc.2020.04.001
13. Thomson DJ, Palma D, Guckenberger M, Balermpas P, Beitler JJ, Blanchard P, et al. Practice recommendations for risk-adapted head and neck cancer radiation therapy during the COVID-19 pandemic: an ASTRO-ESTRO consensus statement. Int J Radiat Oncol Biol Phys. 2020;107(4):618-27. https://doi.org/10.1016/j.ijrobp.2020.04.016
14. Thureau S, Faivre JC, Assaker R, Biver E, Confavreux CB, Debiais F, et al. Adapting palliative radiation therapy for bone metastases during the COVID-19 pandemic: GEMO position paper. J Bone Oncol. 2020;22:100291. https://doi.org/10.1016/j.jbo.2020.100291
15. Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet. 2020;395(10227):912-20. https://doi.org/10.1016/S0140-6736(20)30460-8
16. Ciążyńska M, Pabianek M, Szczepaniak K, Ułańska M, Skibińska M, Owczarek W, et al. Quality of life of cancer patients during coronavirus disease (COVID-19) pandemic. Psychooncology. 2020;29(9):1377-9. https://doi.org/10.1002/pon.5434

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231496

Evaluation of maternal serum fibroblast growth factor-23 levels in fetal growth restriction and gestational hypertensive disease

Erdener Karacan[1], Ümran Kılınçdemir Turgut[2*], Halil İbrahim Büyükbayram[3], Mehmet Güney[4]

SUMMARY

OBJECTIVE: The objective of this study was to determine serum fibroblast growth factor-23 levels in preeclampsia, eclampsia, gestational hypertension, and the presence of fetal growth restriction subgroups.

METHODS: A total of 55 pregnant women with planned cesarean section were included in this cross-sectional study. They were divided into two groups, namely, control (25) and gestational hypertensive disease (30). The gestational hypertensive disease group was evaluated by dividing it into three subgroups (preeclampsia, eclampsia, and gestational hypertension) according to the clinical and laboratory findings of the disease and two subgroups (presence of fetal growth restriction and absence of fetal growth restriction) according to the birth weight percentile. Demographic parameters, obstetric history, physical examination findings, and laboratory values were evaluated.

RESULTS: Demographic parameters and obstetric history were similar between the two groups, while gestational week of delivery was lower in the gestational hypertensive disease group (p=0.002). Laboratory parameters and serum fibroblast growth factor-23 (pg/mL) values were similar between the two groups. In the subgroup analysis for gestational hypertension, preeclampsia, and eclampsia, there was no statistically significant difference in serum fibroblast growth factor-23 levels between gestational hypertension, preeclampsia, eclampsia, and control groups. In the subgroup analysis based on the presence of fetal growth restriction, serum fibroblast growth factor-23 levels were similar to the control group in the gestational hypertensive disease absence of fetal growth restriction, while serum fibroblast growth factor-23 levels and serum calcium levels were statistically significantly lower in the gestational hypertensive disease with the presence of fetal growth restriction (p=0.044 and p<0.001, respectively).

Conclusion: Serum fibroblast growth factor-23 levels are similar between pregnancies complicated with gestational hypertensive disease and normotensive pregnancies. However, serum fibroblast growth factor-23 levels were found to be lower in pregnancies complicated with gestational hypertensive disease with the presence of fetal growth restriction.

KEYWORDS: Gestational hypertension. Fibroblast Growth Factor-23. Pregnancy. Fetal growth restriction.

INTRODUCTION

Gestational hypertensive disease (GHD), with or without proteinuria, is the onset of high blood pressure (systolic ≥ 140 mmHg; diastolic ≥ 90 mmHg) after 20 weeks of gestation[1], and it is one of the main causes of maternal mortality and morbidity. GHD is closely associated with poor perinatal outcomes, including fetal growth retardation and low birth weight (BW)[2]. Gestational hypertension (GHT) and preeclampsia/eclampsia are in the group of GHDs[3]. GHD/preeclampsia has been considered to have different pathophysiological pathways because of its different clinical courses and different fetal and maternal outcomes, according to clinical findings[4].

Recently, to understand the pathophysiology of GHD, the placental expression of the promoter region of Klotho in preeclamptic pregnant women was studied[5]. Three Klotho-related genes have been identified: α-Klotho, β-Klotho, and γ-Klotho[6]. Studies have shown that, in preeclamptic pregnancies, there is genotyping polymorphism and decreased expression of the Klotho gene[7]. In addition, maternal plasma α-Klotho elevation in preeclamptic pregnant women was associated with less placental villous maturation. Therefore, changes in the a-Klotho system are thought to be involved in the etiology of preeclampsia[8].

Fibroblast growth factor-23 (FGF23) is a member of the hormonal FGF subfamily that complexes with Klotho co-receptors[9]. FGF23 is likely to act on the FGFR1c receptor and, together with α-Klotho, has a 20-fold greater affinity for the FGFR receptor[10].

An increase in serum FGF23 levels results in hypophosphatemia, a decrease in 1–25 $(OH)_2$ D levels, and a decrease in bone mineral density, while a decrease results in hyperphosphatemia

[1]Süleyman Demirel University, Faculty of Medicine, Department of Obstetrics and Gynecology – Isparta, Turkey.
[2]Adana City Training and Research Hospital, Department of Obstetrics and Gynaecology – Adana, Turkey.
[3]Süleyman Demirel University, Faculty of Medicine, Department of Biochemistry – Isparta, Turkey.
[4]Dokuz Eylul University, Department of Obstetrics and Gynaecology – İzmir, Turkey.
*Corresponding author: umrankilincdemiritf@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 16, 2024. Accepted on February 16, 2024.

and an increase in 1–25 $(OH)_2$ D levels and soft tissue calcifications[11]. Part of the known physiological function of FGF23 in regulating mineral metabolism can also be explained by the effects of this hormone on the kidney. FGF23 is a regulator of the sodium chloride channel in the distal renal tubules, is known to cause renal sodium retention, plasma expansion, and increases in systolic and diastolic blood pressure[12].

The aim of this study was to determine the levels of serum FGF23 in pregnancies complicated with GHD and investigate FGF23 levels in preeclampsia, eclampsia, GHT, and the presence of fetal growth restriction (FGR) subgroups.

METHODS

The study design was approved by the local ethics committee (23.03.2021 8/152), and informed consent was obtained from all participants. Pregnant women for whom a cesarean delivery was planned were included in the study. The control group comprised pregnant women who had had a previous cesarean delivery, were normotensive, and had no maternal or fetal risks during their pregnancy.

The GHD group included pregnant women who were known to be normotensive before the 24th week of pregnancy and who were found to have systolic/diastolic blood pressures higher than 140/90 mmHg in two measurements measured at 4-h intervals. The GHD group was first divided into three subgroups according to GHD symptoms and laboratory findings as follows: (1) the preeclampsia subgroup had hypertension and proteinuria over 300 mg/dL in 24-h urine; (2) the GHT subgroup had hypertension without proteinuria; and (3) the eclampsia subgroup had convulsions in addition to the preeclampsia group. The GHD group was also divided into two subgroups according to BW. In the first subgroup, patients showed an absence of FGR (BW>10%), while in the second subgroup, patients showed a presence of FGR (BW<10%) (Figure 1).

The exclusion criteria included the following: multiple pregnancies, genetic or structural fetal anomaly, diabetes mellitus, gestational diabetes mellitus (GDM), chronic hypertensive disease, chronic kidney disease, autoimmune and rheumatic disease, the presence of thyroid disease (hypothyroidism or hyperthyroidism), delivery before the 32nd gestational week, smoking and alcohol use, and vaginal delivery.

Demographic parameters and obstetric/physical examination findings

The following variables were evaluated: demographic parameters and obstetric/physical examination findings, age, gravity, parity, abortion, number of fetuses, gestational week, estimated fetal weight, BW, amniotic fluid amount, and arterial blood pressure (systolic/diastolic).

Laboratory parameters and biochemical measurements

Maternal hemoglobin (Hgb) (g/dl) measurements were evaluated before delivery and at the sixth hour after delivery. Prenatal maternal venous blood samples were taken, and the blood serum was separated and analyzed within 12 h. Maternal serum calcium (Ca) (mg/dl), serum phosphate (Pi) (mg/dL), corrected Ca (mg/dl), and serum FGF23 (pg/mL) were measured. Serum FGF23 levels were studied following the ELISA kit procedure (Cat. No. E0059Hu, Lot No. 202109012, Shanghai Korain BT Laboratory, Jiaxing, China). The intra-assay CV

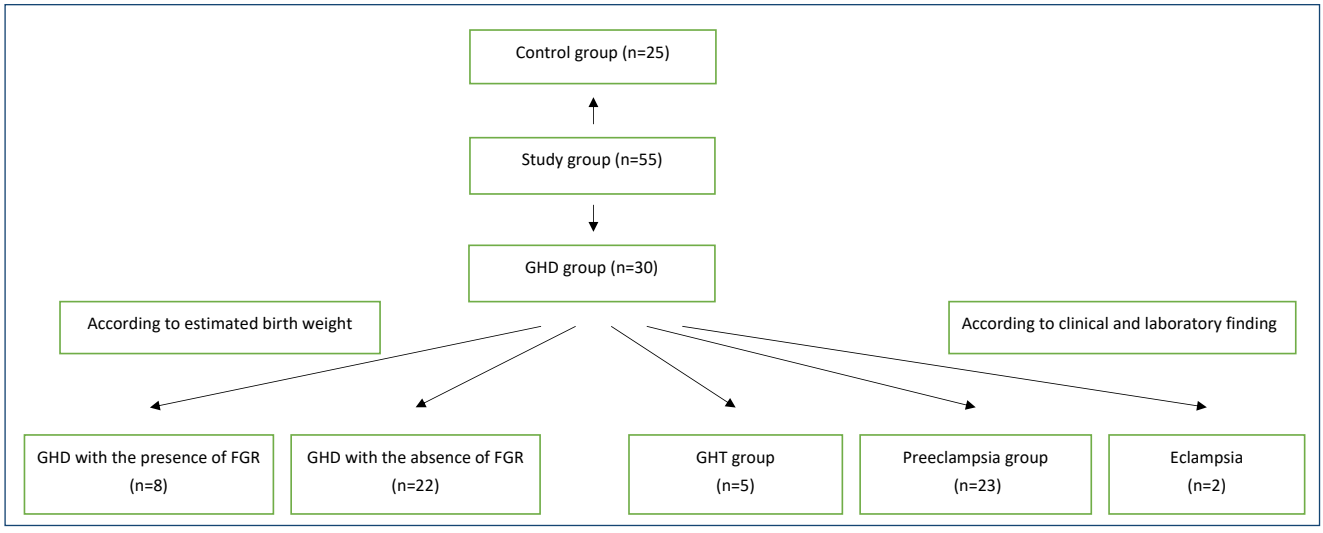

Figure 1. Study plan and flowchart. GHT: gestational hypertension; GHD: gestational hypertensive disease; FGR: fetal growth restriction.

(intra-measurement coefficient of variation) was <8%, while the inter-assay CV (inter-measurement coefficient of variation) was <10%.

Statistical analysis

Statistical analysis of the study data was performed using the IBM SPSS Statistics software (IBM Corporation, Armonk, NY, USA). Whether the data showed normal distribution or not was evaluated using the Shapiro-Wilk test. The data were observed to have a normal distribution, and Levene's test of homogeneity was used to evaluate whether the variances provided a homogeneous distribution. Then, comparisons between groups were made using parametric tests. The Student's t-test and one-way ANOVA were used to compare the analyzed findings of the groups. The Kruskal-Wallis and the Mann-Whitney U tests were used for data with inhomogeneous variances and small sample size. Statistical significance was considered to be $p<0.05$ at a 95% confidence interval.

RESULTS

A total of 55 pregnant women who met the inclusion and exclusion criteria of the study were included. Notably, 25 pregnant women were included in the control group and 30 pregnant women were included in the GHD group, which was further sub-classified according to laboratory parameters and physical examination findings: 23 pregnant women were in preeclampsia subgroup, 5 pregnant women were in the GHT subgroup, and 2 pregnant women were in the eclampsia subgroup. Moreover, the GHD group was further subclassified according to fetal birth measurement: FGR was present in 8 participants and absent in 22 participants.

Demographic parameters, obstetric examinations, and neonatal outcomes were similar in the control and GHD groups, except for the gestational week at delivery, which was statistically significantly earlier in the GHD group (p=0.002) (Table 1). Maternal blood pressure and laboratory parameters of the control and GHD groups were compared, and systolic and diastolic blood pressure was significantly higher in the GHD group (p<0.001). Laboratory parameters, including serum albumin levels, serum calcium levels, albumin-corrected calcium levels, and serum phosphate levels, were similar in the two groups (Table 1).

Comparison of the first three gestational hypertensive disease subgroups with the control group (Table 2)

The gestational week at delivery of the preeclampsia and eclampsia subgroups was earlier than that of the control group, while the gestational week at delivery in the GHT subgroup was similar

Table 1. Comparison of the gestational hypertensive disease and the control groups in terms of demographic parameters and laboratory values.

	Control (25)	GHD (30)	p-value
	Mean±standard deviation		
Age (years)	30.72±6.33	29.97±6.36	NS
Gravity	2.60±1.44	2.57±1.81	NS
Parity	0.80±0.76	0.93±1.08	NS
Abortus	0.84±0.98	0.77±1.40	NS
Birth weight (g)	3126.60±371.24	2903.00±775.62	0.379
Birth weight percentile (%)	45.08±28.48	46.33±34.33	0.735
Gestational week at delivery	38.00±1.53	36.50±2.03	0.002*
Preop Hgb (g/dl)	12.62±1.11	12.1±0.95	0.082
Postop Hgb (g/dL)	11.12±1.26	10.56±1.43	0.137
Serum Ca (mg/dL)	8.80±0.48	8.47±0.68	0.052
Corrected Ca (mg/dL)	9.33±0.38	9.18±0.55	0.253
Serum Pi (mg/dL)	3.54±0.53	3.78±0.91	0.577
Systolic (mmHg)	104.40±12.61	153.33±16.47	<0.001*
Diastolic (mmHg)	67.20±7.91	99.00±11.55	<0.001*
FGF23 (pg/mL)	285.09±218.18	248.39±305.45	0.265

GHD: gestational hypertensive disease; Ca: calcium; Pi: phosphate; FGF: fibroblast growth factor. *p<0.05. NS: non-significant.

to the control group. Serum calcium levels in the preeclampsia and eclampsia groups had lower values compared with the control group and the GHT group but were similar to those of the preeclampsia group. However, albumin-corrected calcium levels were similar in all groups. Systolic blood pressure was found to be higher in the GHT, preeclampsia, and eclampsia subgroups compared with the control group (p<0.001). In addition, the preeclampsia subgroup had higher systolic blood pressure than the GHT subgroup (p=0.015). Diastolic blood pressure was higher in the GHT and preeclampsia subgroups than in the control group. However, in the eclampsia group, diastolic blood pressure had higher values than the control group, although there was no statistically significant difference due to the small number of participants. Serum FGF23 levels were similar in the control group and the GHD group. In addition, there was no statistically significant difference in serum FGF23 levels between the control group and the GHT, eclampsia, and preeclampsia subgroups.

Comparison of the second two gestational hypertensive disease subgroups with the control group (Table 3)

In a comparison of the GHD without FGR group with the control group, the former had a lower gestational week at delivery and a higher BW percentile (p=0.023 and p=0.01, respectively). In addition, higher diastolic and systolic blood pressure values were determined in the GHD without FGR group compared with the control group (p<0.001). FGF23 levels were statistically similar between the GHD without the FGR group and the control group (p=0.073) (Table 3).

In a comparison of the GHD with the FGR group and the control group, the former had a lower gestational week, lower BW, and lower BW percentile (p<0.001). Although serum calcium values were found to be statistically lower in the GHD with the FGR group, the albumin-corrected calcium values were similar when compared with the control group. The GHD with the FGR group had higher systolic and diastolic blood pressure values (p<0.001), while FGF23 levels were statistically significantly lower than those of the control group (p=0.043) (Table 3).

DISCUSSION

This study showed that serum FGF23 levels detected in normotensive pregnant women and pregnant women with GHT disease were statistically similar, but serum FGF23 levels were found to be low in pregnancies with GHT disease with the presence of FGR. A study on rats reported that maternal

Table 2. Comparison of the gestational hypertensive subgroup and the control group in terms of demographic parameters and laboratory values.

	Control (25)	GHT (5)	Preeclampsia (23)	Eclampsia (2)	p-value
	Mean±standard deviation				
Age (years)	30.72±6.33	25.80±0.77	31.43±6.33	23.50±6.34	NS
Gravity	2.60±1.44	2.20±1.78	2.74±1.88	1.50±0.70	NS
Parity	0.80±0.76	0.60±0.89	1.04±1.10	1.50±0.70	NS
Abortus	0.84±0.98	0.60±0.89	0.87±1.54	0	NS
Birth weight (g)	3126.60±371.24	3048.00±610.98	2995.00±726.20	1482.50±123.74	0.116
Birth weight percentile (%)	45.08±28.48	48.80±35.56	50.21±33.72	3.00±2.82	0.188
Gestational week at delivery	38.13±1.50	37.66±0.84	36.64±1.77	32.00±0	<0.001[a]
Preop Hgb (g/dl)	12.62±1.11	12.56±0.61	12.04±1.04	12.00±0.28	0.261
Postop Hgb (g/dl)	11.12±1.26	11.06±1.41	10.35±1.46	11.70±0.14	0.191
Serum Ca (mg/dl)	8.80±0.48	8.97±0.53	8.46±0.63	7.42±0.36	0.003[b]
Corrected Ca (mg/dl)	9.33±0.38	9.40±0.34	9.14±0.59	9.10±0.58	0.470
Serum Pi (mg/dL)	3.54±0.53	3.86±0.61	3.66±0.82	5.08±1.71	0.283
Systolic (mmHg)	104.40±12.61	142.00±4.47	156.09±17.77	150.00±0.00	<0.001[c]
Diastolic (mmHg)	67.20±7.91	94.00±5.47	99.57±12.60	105.00±7.07	<0.001[d]
FGF23 (pg/mL)	285.09±218.18	330.45±458.00	236.49±287.38	180.10±55.69	0.721

GHT: gestational hypertension; Ca: calcium; Pi: phosphate; FGF: fibroblast growth factor. [a]p-value is significant between eclampsia and other groups (p<0.001) and between preeclampsia and control (p=0.035) by Tukey HSD. [b]p-value is significant between eclampsia and control (p=0.007) and between eclampsia and GHT (p=0.008) by Tukey HSD. [c]p-value is significant between control and other groups (p<0.001) and between preeclampsia and GHT (p=0.015) by Tukey HSD. [d]p-value is significant between control and GHT (p<0.001) and between control and preeclampsia (p<0.001) by Tukey HSD. NS: non-significant.

Table 3. Comparisons of (1) the gestational hypertension with the absence of the fetal growth restriction group and the control group and (2) the gestational hypertension with the presence of the fetal growth restriction group and the control group in terms of demographic parameters and laboratory values.

	Control (25)	GHD with the absence of FGR (22)	p-value	GHD with the presence of FGR (8)	p-value
	Mean±SD			Mean±SD	
Age (years)	30.72±6.33	30.09±6.14	0.732	29.62±7.34	NS
Gravity	2.60±1.44	2.23±1.47	0.278	3.50±2.39	NS
Parity	0.80±0.76	0.91±1.10	0.900	1.00±0.92	NS
Abortus	0.84±0.98	0.50±0.74	0.232	1.50±2.39	NS
Birth weight (g)	3126.60±371.24	3248.86±553.58	0.321	1951.88±403.83	**<0.001**
Birth weight percentile (%)	45.08±28.48	62.40±24.74	**0.010***	2.12±1.45	**<0.001**
Gestational week at delivery	38.00±1.53	37.06±1.58	**0.023***	34.95±2.41	**<0.001**
Preop Hgb (g/dl)	12.62±1.11	12.17±1.11	0.135	12.12±0.30	0.054
Postop Hgb (g/dl)	11.12±1.26	10.54±1.48	0.154	10.65±1.27	0.367
Serum Ca (mg/dL)	8.80±0.48	8.65±0.65	0.329	8.04±0.56	**0.001**
Corrected Ca (mg/dL)	9.33±0.38	9.20±0.57	0.305	9.17±0.47	0.343
Serum Pi (mg/dL)	3.54±0.53	3.60±0.76	0.924	4.30±1.13	0.115
Systolic (mmHg)	104.40±12.61	150.00±20.44	**<0.001**	156.25±13.02	**<0.001**
Diastolic (mmHg)	67.20±7.91	96.96±9.26	**<0.001**	101.25±19.59	**<0.001**
FGF23 (pg/mL)	285.09±218.18	288.26±337.56	0.701	118.95±77.35	**0.044***

GHD: gestational hypertensive disease; FGR: fetal growth restriction; Ca: calcium; Pi: phosphate; FGF: fibroblast growth factor. *p<0.05. NS: non-significant. Bold indicates statistically significant p-values.

malnutrition in the prenatal period resulted in low FGF23 levels. The strong relationship between this FGF23 decrease and FGR was explained in another study[13]. A study conducted to clarify the etiologies of FGR, the direct relationship of FGF23 levels with BW, length, and head circumference determined that the risk of FGR was lower in pregnant women with high FGF23 levels[14]. Similarly, this study showed that maternal serum FGF23 levels were associated with fetal BW, and low FGF23 levels may be a risk factor for FGR.

The relationship between GDM and serum FGF23 levels has been investigated in previous studies. Serum FGF23 levels were found to be high in pregnancies complicated with GDM[15]; however, FGF23 levels were examined in pregnant women at an early gestational week. In addition, neither estimated fetal weight nor BW were noted in the study. Another reason that FGF23 levels have been found to be higher in pregnancies complicated with GDM may be due to the high estimated fetal weight or BW. The result of this study is supported by the positive correlation between BW and BW percentile and FGF23 levels.

A recent study found that high α-Klotho values in human serum value can be considered a marker for preeclampsia[16]. Miranda et al. also found that maternal serum α-Klotho concentrations were higher than in non-pregnant women, but this increase was not significant in pregnant women with FGR fetuses regardless of preeclampsia[17]. In this study, we investigated FGF23, which is the obligate receptor of α-Klotho, but failed to detect significant differences in the serum FGF23 levels of the GHD group (including thepreeclampsia, eclampsia, and GHT subgroups) and the control group. However, there was a correlation between FGF23 levels and BW.

It is known that elevated FGF23 concentrations may occur due to iron deficiency and that iron replacement reverses this elevation. In addition, it has been shown that high FGF23 levels cause osteomalacia and rickets through phosphate and calcium metabolism and that iron replacement in pregnancy reverses the decrease in bone mineral density by decreasing FGF23 levels[18]. Although maternal serum ferritin levels were not identified in this study, maternal Hgb concentrations were found to be similar in all the groups. In addition, it should be noted that oral iron therapy is administered to all pregnant women in our country.

Previous studies have shown that, especially in the last trimester, there is a greater increase in blood pressure in pregnancies complicated with preeclampsia than in pregnancies complicated with GHT[19]. Davis et al. found that pregnant women with GHT

who progressed to preeclampsia had higher blood pressure values than GHT patients who did not progress to preeclampsia[20].

In our study, similar to the literature, we found that systolic blood pressure was higher in the preeclampsia group than in the GHT group, but diastolic blood pressure was similar in the two groups. This may be due to the small number of participants in the GHT group. Studies with a larger number of participants are needed to determine the accuracy of this finding.

Another interesting finding of our study was that the total serum calcium level was lower in the eclampsia subgroup than in the control group and the GHT subgroup. However, we found that albumin-corrected calcium levels were similar in all groups. The relationship between low maternal calcium levels and pregnancy-induced hypertensive diseases has been explained, but the protectiveness of calcium replacement in pregnancy for GHD has not yet been clearly demonstrated[21]. In a recent study, serum calcium levels were found to be lower in preeclamptic pregnant women compared with healthy pregnant women[22]. On the contrary, in our study, neither serum total calcium levels nor albumin-corrected calcium levels of the preeclampsia group were similar to those of the control group. A decrease in total serum calcium levels was demonstrated only in the eclamptic pregnant women.

Although the strongest limitation of our study was the inability to evaluate FGF23 levels together with serum and/or placental α-Klotho values, the relationship between FGF23 levels and FGR was demonstrated. Additional limitations include not knowing whether the participants took oral iron and/or multivitamin supplements and the small number of participants, especially in the eclampsia group. In addition, the index study group is small; therefore, further research is needed to validate this approach.

In conclusion, in this study, serum FGF23 levels were similar in pregnancies complicated by GHD and normotensive pregnancies. However, serum FGF23 levels were found to be lower in pregnancies complicated with GHD with the presence of FGR.

ETHICS

The study was performed in accordance with the ethical standards for human research established by the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the local Ethics Committee of Süleyman Demirel University School of Medicine.

INFORMED CONSENT

All patients provided written informed consent for the application of this technique.

AUTHORS' CONTRIBUTIONS

EK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization. **ÜKT:** Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **HİB:** Data curation, Funding acquisition, Methodology, Project administration, Validation. **MG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization.

REFERENCES

1. ACOG. Gestational hypertension and preeclampsia: ACOG Practice Bulletin, number 222. Obstet Gynecol. 2020;135(6):e237-60. https://doi.org/10.1097/AOG.0000000000003891
2. Liu CM, Cheng PJ, Chang SD. Maternal complications and perinatal outcomes associated with gestational hypertension and severe preeclampsia in Taiwanese women. J Formos Med Assoc. 2008;107(2):129-38. https://doi.org/10.1016/S0929-6646(08)60126-6
3. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013;25(2):124-32. https://doi.org/10.1097/GCO.0b013e32835e0ef5
4. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol. 2013;209(6):544.e1-12. https://doi.org/10.1016/j.ajog.2013.08.019
5. Cecati M, Giannubilo SR, Saccucci F, Sartini D, Ciavattini A, Emanuelli M, et al. Potential role of placental klotho in the pathogenesis of preeclampsia. Cell Biochem Biophys. 2016;74(1):49-57. https://doi.org/10.1007/s12013-015-0710-9
6. Amaro-Gahete FJ, de-la-O A, Jurado-Fasoli L, Ruiz JR, Castillo MJ, Gutiérrez Á. Role of exercise on S-Klotho protein regulation: a systematic review. Curr Aging Sci. 2018;11(2):100-7. https://doi.org/10.2174/1874609811666180702101338
7. Giannubilo SR, Cecati M, Saccucci F, Emanuelli M, Tranquilli AL. PP008. Placental klotho gene in preeclampsia. Pregnancy Hypertens. 2013;3(2):70. https://doi.org/10.1016/j.preghy.2013.04.036
8. Loichinger MH, Towner D, Thompson KS, Ahn HJ, Bryant-Greenwood GD. Systemic and placental α-klotho: effects of preeclampsia in the last trimester of gestation. Placenta. 2016;41:53-61. https://doi.org/10.1016/j.placenta.2016.03.004
9. Itoh N, Ornitz DM. Functional evolutionary history of the mouse Fgf gene family. Dev Dyn. 2008;237(1):18-27. https://doi.org/10.1002/dvdy.21388

10. Mohammadi M, Olsen SK, Ibrahimi OA. Structural basis for fibroblast growth factor receptor activation. Cytokine Growth Factor Rev. 2005;16(2):107-37. https://doi.org/10.1016/j.cytogfr.2005.01.008
11. Quarles LD. Fibroblast growth factor 23 and α-Klotho co-dependent and independent functions. Curr Opin Nephrol Hypertens. 2019;28(1):16-25. https://doi.org/10.1097/MNH.0000000000000467
12. Andrukhova O, Slavic S, Smorodchenko A, Zeitz U, Shalhoub V, Lanske B, et al. FGF23 regulates renal sodium handling and blood pressure. EMBO Mol Med. 2014;6(6):744-59. https://doi.org/10.1002/emmm.201303716
13. Gui S-P, Zou H, Bai Y, Liu M, Wang T, Zhou R. Low expression of FGF23 and its effect on rats with intrauterine growth retardation. Matern Med. 2020;2(4):211-6. https://doi.org/10.1097/FM9.0000000000000066
14. Qamar H, Perumal N, Papp E, Gernand AD, Mahmud A, Roth DE. Higher maternal parathyroid hormone concentration at delivery is not associated with smaller newborn size. Endocr Connect. 2021;10(3):345-57. https://doi.org/10.1530/EC-21-0056
15. Kizilgul M, Kan S, Beysel S, Apaydin M, Ozcelik O, Caliskan M, et al. Is fibroblast growth factor 23 a new cardiovascular risk marker in gestational diabetes? Arch Endocrinol Metab. 2017;61(6):562-6. https://doi.org/10.1590/2359-3997000000287
16. Uzun Cilingir I, Varol F, Gurkan H, Sutcu H, Atli E, Eker D, et al. Placental and serum levels of human Klotho in severe preeclampsia: a potential sensitive biomarker. Placenta. 2019;85:49-55. https://doi.org/10.1016/j.placenta.2019.08.084
17. Miranda J, Romero R, Korzeniewski SJ, Schwartz AG, Chaemsaithong P, Stampalija T, et al. The anti-aging factor α-klotho during human pregnancy and its expression in pregnancies complicated by small-for-gestational-age neonates and/or preeclampsia. J Matern Fetal Neonatal Med. 2014;27(5):449-57. https://doi.org/10.3109/14767058.2013.818652
18. Braithwaite VS, Mwangi MN, Jones KS, Demir AY, Prentice A, Prentice AM, et al. Antenatal iron supplementation, FGF23, and bone metabolism in Kenyan women and their offspring: secondary analysis of a randomized controlled trial. Am J Clin Nutr. 2021;113(5):1104-14. https://doi.org/10.1093/ajcn/nqaa417
19. Macdonald-Wallis C, Lawlor DA, Fraser A, May M, Nelson SM, Tilling K. Blood pressure change in normotensive, gestational hypertensive, preeclamptic, and essential hypertensive pregnancies. Hypertension. 2012;59(6):1241-8. https://doi.org/10.1161/HYPERTENSIONAHA.111.187039
20. Davis GK, Mackenzie C, Brown MA, Homer CS, Holt J, McHugh L, et al. Predicting transformation from gestational hypertension to preeclampsia in clinical practice: a possible role for 24 hour ambulatory blood pressure monitoring. Hypertens Pregnancy. 2007;26(1):77-87. https://doi.org/10.1080/10641950601147952
21. Malas NO, Shurideh ZM. Does serum calcium in pre-eclampsia and normal pregnancy differ? Saudi Med J. 2001;22(10):868-71. PMID: 11744944
22. Gamal Mohammed El-Maghraby M, Mohammed Zakarya AE-M, EL-Latif Hashish M, El-Boghdady A. Comparative study between serum calcium and magnesium levels in pre eclampsia versus normal pregnancy. Al-Azhar Med J. 2022;51(2):999-1014.

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231539

Validity and reliability of Turkish pregnant women's preferences for mode of delivery questionnaire

Nurdan Kaya Yilmaz[1*], Funda Evcili[2]

SUMMARY

OBJECTIVE: The aim of this study was to determine whether Pregnant Women's Preferences for Mode of Delivery Questionnaire, created by Zamani-Alavijeh et al., is a valid and reliable measurement tool for Turkish pregnant women.

METHODS: This study has a methodological research design and was conducted with 139 pregnant women who were randomly selected from those aged 18–35 years, who applied to obstetric clinic,.who had no previous prenatal losses and no systemic diseases, and who had conceived naturally. The data for this study were collected with the Personal Information Form and the Pregnant Women's Preferences for Mode of Delivery Questionnaire. To test the reliability and validity of Pregnant Women's Preferences for Mode of Delivery Questionnaire, Cronbach's α, split-half method, item analysis, Kendall's coefficient of agreement (W), explanatory factor analysis, and confirmatory factor were used.

RESULTS: The study found that Cronbach's α was 0.94, the Spearman–Brown reliability coefficient was 0.883, and the Guttman split-half was 0.880. Explanatory factor analysis revealed an 18-item structure with three factors having an eigenvalue exceeding 1, explaining 67.593% of the total variability, and factor loading between 0.40 and 0.64.

CONCLUSION: Based on the scientific recommendations, the Turkish version of the Pregnant Women's Preferences for Mode of Delivery Questionnaire has adequate psychometric properties.

KEYWORDS: Delivery. Preferences. Questionnaire. Reliability. Validity.

INTRODUCTION

Delivery preference can be made when a woman voluntarily chooses between vaginal or cesarean delivery based on her knowledge, beliefs, and attitudes[1,2]. Vaginal birth is natural, normal, and suitable for female physiology[3], while cesarean is an obstetric surgical method preferred in cases where vaginal delivery cannot be applied because of some maternal or fetal reasons, or when it is quite risky for the mother and the fetus. Cesarean rate has been increasing rapidly all over the world, and the current cesarean rate is 21.1%, which is expected to increase to 28.5% by 2030[4].

During the last trimester, the healthcare team evaluates medical indications, discusses them with the mother and family, and decides on the mode of delivery. However, most women decide on the mode of delivery under the influence of social, psychological, and environmental factors, aside from medical indications[1]. Women's choice of elective cesarean is affected by their families, friends, media, hospital, previous birth experiences, and healthcare staff[5-8]. Cesarean can be lifesaving when necessary, but non-indicative and unnecessary cesarean has negative outcomes in terms of the health of the mother and the fetus/newborn[9,10].

No questionnaire was found in the literature review to evaluate the birth method preferences of pregnant women in Turkey. Therefore, a measurement tool that can be applied in a short time by healthcare staff working in the perinatal field, can be easily interpreted, and will determine the factors affecting women's birth method preference is extremely necessary. Determining all the factors that affect the birth preferences of women, having the right information about birth patterns, and developing the appropriate intervention may be beneficial in decreasing cesarean section rates and increasing normal birth preferences. This study aimed to determine the reliability and validity of PPMDQ, which has not been used yet in pregnant women in Turkey.

[1]Ondokuz Mayıs University, Faculty of Health Sciences, Department of Midwifery – Samsun, Turkey.
[2]Sivas Cumhuriyet University, Vocational School of Health Care Services, Child Care and Youth Services – Sivas, Turkey.
*Corresponding author: nurdan.kaya@omu.edu.tr, nrdn.kaya@windowslive.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 04, 2024. Accepted on March 03, 2024.

METHODS

Study design and participants

This study was conducted using a methodological design in an obstetric clinic of a tertiary hospital between September 2020 and June 2021. It recommends that the number of samples must be between 5 and 10 times the number of items[11]. The study was conducted with a total of 139 pregnant women selected randomly from those who aged 18–35 years, who had no previous prenatal losses and no systemic diseases, and who had conceived naturally.

Language adaptation protocol

In questionnaire adaptation studies, when the questionnaires are translated, the steps of "translation into the target language" and "translation back into the original language" are often followed. There must be compatibility between the original questionnaire and its translation, and the items are equivalent to each other[12]. First, the original questionnaire was translated from English into Turkish by three experts to ensure language and content validity. Second, the translated text was then translated back into the original language. Then, the translated form and the original questionnaire were sent to 10 different experts to select the most appropriate translation. These experts were asked to evaluate the compatibility of the translation items with the original items.

Data collection instruments

The personal information form was used to collect the participants' characteristics. The original questionnaire developed by Zamani-Alavijeh et al.[13] consists of 21 items and 7 sub-dimensions. The questionnaire is in the 5-point Likert style (1= I Totally Disagree and 5=I Totally Agree). The Cronbach's α of the original questionnaire was determined to be 0.747[13].

Ethics statement

First, permission was obtained from the questionnaire developer. Second, this study was performed in accordance with the Helsinki Declaration and has been approved by the Ondokuz Mayıs University Clinical Research Ethics Committee (on February 13,.2019, with number B.30.2.ODM.0.20.08/48-184). All participants' written consents were obtained.

Statistical analysis

The data were transferred to the computer with the LISREL 8.54 and SPSS 22.0 package programs, and psychometric analyses were conducted. In this study, Cronbach's α, split-half method, and item analysis were used to test the reliability of PPMDQ. Kendall's coefficient of agreement (W) was calculated to determine whether the questionnaire's content was valid. The explanatory factor analysis was applied to test the construct validity of the questionnaire, and the confirmatory factor analysis was used to examine the relations between the questionnaire factors.

RESULTS

The study found that the mean age of pregnant women was 28±4.17, with 93.5% living in nuclear families and 3.6% having low incomes. Women's gestational age was 24.64±10.42, with 47.5% in the third trimester, 63.3% preferred to have a vaginal delivery, 31.1% had a history of planned cesarean, and 71.2% received information about delivery.

Reliability analysis

First, the study evaluated the item total score correlations of the 21-item questionnaire and three items that had a correlation coefficient below r=0.30 were removed from the questionnaire. After analysis, the number of items in the questionnaire decreased to 18. The remaining items had item total score correlation coefficients varying between 0.34 and 0.65, and the questionnaire items were adequate to represent the questionnaire (Table 1). After the item analysis, it was found that the Cronbach's α was 0.94, indicating high reliability. The Spearman–Brown and Guttmann split-half reliability coefficients obtained with the split-half method of the questionnaire were examined. It was found that the internal consistency coefficient of PPMDQ was Spearman–Brown (0.883) and Guttmann split-half (0.880).

Validity analysis

The Kaiser–Meyer–Olkin (KMO) test (0.881) and Bartlett's test (^{2}= 1894.713, SD=153, p=0.000) were found to be significant for PPMDQ, indicating that the data were suitable for factor analysis. The Kendall's W test was used to determine whether PPMDQ is valid in terms of content, and it was found that there were no statistical differences between expert opinions (Kendall's W: 0.176; p: 0.240>0.05).

The explanatory factor analysis (EFA) was made to test the construct validity and to determine the factors of the questionnaire. After the varimax factor rotation, a three-factor structure that had an eigenvalue above 1 and a factor load above 0.64 emerged, explaining 67.593% of the variance (factor 1: 52.370, factor 2: 8.262%, and factor 3: 6.961). The eigenvalues of the factors were found to be factor 1: 9.427, factor 2: 1.487, and factor 3: 1.253. After factor rotation, it was determined that 10 items were under the 1st factor, 5 items under the 2nd factor, and 3 items under the 3rd factor. Although the

Table 1. Distribution of Pregnant Women's Preferences for Mode of Delivery Questionnaire outline according to item total point correlation (n=139).

Items	Mean	Standard deviation	Corrected ttem total correlation	Cronbach's α if item deleted
i1	4.78	0.71	0.56	0.93
i2	4.85	0.50	0.74	0.93
i3	4.77	0.61	0.68	0.93
i4	4.71	0.71	0.68	0.93
i5	4.65	0.83	0.70	0.94
i6	4.66	0.83	0.70	0.93
i7	4.67	0.82	0.73	0.93
i8	4.67	0.71	0.69	0.93
i9	4.74	0.74	0.78	0.93
i10	4.71	0.76	0.70	0.93
i11	4.58	0.94	0.61	0.94
i12	4.65	0.74	0.67	0.94
i13	4.66	0.81	0.65	0.93
i14	4.69	0.75	0.75	0.93
i15	4.76	0.65	0.62	0.93
i16	4.64	0.86	0.62	0.94
i20	4.71	0.73	0.64	0.93
i21	4.65	0.72	0.64	0.94

PPMDQ: Pregnant Women's Preferences for Mode of Delivery Questionnaire.

original questionnaire consisted of seven factors, a three-factor structure emerged in the Turkish validity and reliability study. These factors were named "belief," "self-efficacy," and "preferences" (Table 2).

Based on the EFA, the questionnaire that had a three-factor structure was tested with the confirmatory factor analysis (CFA). In the study, ^{2}/SD=3.00, and the data fit of the model was found to be adequate. It was found that there was an agreement between the model and the observed data in terms of goodness-of-fit index values, and the validity and reliability study of the questionnaire for Turkish showed an acceptable level of fit (Table 3).

DISCUSSION

The item total score correlation is used to determine the relationship between the scores obtained from individual test items and the total test score[14]. There are various evaluations for the lower correlation coefficient (r) limit. According to Buyukozturk[15], the item total score correlation must be positive and greater than 0.30[15]. In the present study, three items that had a correlation coefficient below r=0.30 were removed from the questionnaire, and the 18 items with a correlation coefficient above r=0.30 were retained. The reliability criterion, also known as Cronbach's α, is used to evaluate the internal consistency of a Likert-type questionnaire. A Cronbach's α below 0.40 shows that the questionnaire is not "reliable," and if it is between 0.80 and 1.00, it shows that the questionnaire is "highly reliable"[12,14]. In the present study, the Cronbach's α of the 18-item questionnaire was calculated to be 0.94, and it was decided that the internal consistency of the questionnaire was highly reliable.

Another method employed to test the reliability of a questionnaire is the split-half method, which is the most widely used method for estimating test reliability[16]. The Spearman–Brown and Guttmann split-half reliability coefficients were examined in this study, and the internal consistency coefficient was found to be 0.883 and 0.880, respectively.

To test the validity of the questionnaire, the Kendall's W test and explanatory and confirmatory factor analyses were used. The Kendall's W goodness-of-fit test was used to determine the content validity of a questionnaire. It is aimed at determining whether there is agreement between expert opinions[15]. No significant differences were detected in the study in terms of expert opinions.

In this study, it was determined that although the original questionnaire had a seven-factor structure, the adapted questionnaire had a three-factor structure. When the questionnaire

Table 2. The results of explanatory factor analysis of Pregnant Women's Preferences for Mode of Delivery Questionnaire (n=139).

Factor	Item	Factor values	Eigenvalue	Variance
Factor 1. Belief	i6	0.583	9.427	52.370
	i7	0.442		
	i8	0.400		
	i9	0.563		
	i10	0.609		
	i11	0.817		
	i12	0.771		
	i13	0.791		
	i14	0.586		
	i15	0.799		
Factor 2. Self-efficacy	i1	0.790	1.487	8.262
	i2	0.585		
	i3	0.420		
	i4	0.697		
	i5	0.594		
Factor 3. Preferences	i16	0.841	1.253	6.961
	i20	0.758		
	i21	0.700		

PPMDQ: Pregnant Women's Preferences for Mode of Delivery Questionnaire.

Table 3. Fit index values of the scale and standard fit index value ranges*.

Compliance measures	Standard fit index values	Acceptable fit index values	Scale's fit index values
RMSEA	0≤RMSEA≤0.05	0.05<RMSEA≤0.08	0.074
SRMR	0≤SRMR≤0.05	0.05<SRMR≤0.10	0.94
NFI	0.95≤NFI≤1.00	0.90≤NFI<0.95	0.93
CFI	0.97≤CFI≤1.00	0.95≤CFI<0.97	0.95
AGFI	0.90≤AGFI≤1.00	0.85≤AGFI<0.90	0.87
GFI	0.95≤AGFI≤1.00	0.90≤AGFI<0.95	0.91
x2/df	0≤x2/df≤2	2<x2/df≤5	396.01/132=3.00

*Standard fit index value[17].

items were determined with the EFA, attention was paid to the fact that the eigenvalues of the items were 1, the load values were at least 0.30, the items were included in one single factor, and there was at least 0.10 difference between two factors[15]. The rotation of the factor load matrix helps find a more interpretable factor structure. The most commonly used technique in the rotation is the varimax, in which a rotation can be made with fewer variables so that the factor variances are maximized[14,15]. In this study, the "varimax method" was used as the factor rotation method. After varimax rotation, a three-factor structure that had an eigenvalue above 1 and a factor load above 0.64 emerged, explaining 67.593% of the variance.

The CFA was used to evaluate the accuracy of a structure determined by EFA. Goodness-of-fit tests are the steps at which the decision to accept or reject the model is made[14]. According to the literature data[17], the standard fit index values show acceptable and good fit, and the fit index values of the questionnaire are given in Table 3. With the CFA, the fit index values of the questionnaire were found to be within the "acceptable" range. Depending on the degree of freedom, the low chi square value (^{2}/SD) of 5 or fewer showed that the data fit of the proposed type is adequate[14]. In our study, ^{2}/SD=3.00, and the model's data fit was found to be adequate.

This study has some limitations. First, the test–retest reliability analysis of the scale could not be performed due to the COVID-19 pandemic conditions. Second, the results are sample-specific. The research was carried out in a city. Therefore, researchers should also validate the PPMDQ in rural parts of Turkey. The PPMDQ is understandable and appropriate to the Turkish cultural context and can be reliable and valid for Turkish pregnant women.

CONCLUSION

The Turkish version of the PPMDQ has adequate psychometric properties according to the best scientific recommendations. It was determined in our study that the data fit of PPMDQ according to fit index values was adequate, and the questionnaire could be used to determine women's types of delivery preferences. The questionnaire has 18 items and 3 sub-dimensions. The lowest score that can be obtained from the questionnaire is 18, and the highest score is 90. The questionnaire has no cutoff value. It is accepted that women prefer the normal delivery method as the score obtained from the questionnaire decreases, and the cesarean section method more strongly as the score increases. The questionnaire can be used by healthcare staff to evaluate women's beliefs about birth patterns, self-efficacy perceptions, and preferences for delivery methods, and to structure the contents of the training programs.

ACKNOWLEDGMENTS

The authors would like to thank all the pregnant women for their participation in this research.

LIMITATIONS

Owing to its single-centered nature, the results cannot be generalized to all pregnant women.

AUTHORS' CONTRIBUTIONS

NKY: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft. **FE:** Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

REFERENCES

1. Padua GR, Lima LC, Fernandes MF, Aquino Gandra AB, Polido CG. Factors that influence women in the choice of birth delivery. Braz J Dev. 2022;8(3):16612-23.
2. Welay FT, Gebresilassie B, Asefa GG, Mengesha MB. Delivery mode preference and associated factors among pregnant mothers in Harar Regional State, Eastern Ethiopia: a cross-sectional study. Biomed Res Int. 2021;2021:1751578. https://doi.org/10.1155/2021/1751578
3. Desai NM, Tsukerman A. Vaginal delivery. In: Stat pearls [Internet]. StatPearls Publishing; 2023.
4. Betran AP, Ye J, Moller AB, Souza JP, Zhang J. Trends and projections of caesarean section rates: global and regional estimates. BMJ Glob Health. 2021;6(6):e005671. https://doi.org/10.1136/bmjgh-2021-005671
5. Suwanrath C, Chunuan S, Matemanosak P, Pinjaroen S. Why do pregnant women prefer cesarean birth? A qualitative study in a tertiary care center in Southern Thailand. BMC Pregnancy Childbirth. 2021;21(1):23. https://doi.org/10.1186/s12884-020-03525-3
6. Sluijs AM, Wijma K, Cleiren MPHD, Lith JMM, Wijma B. Preferred and actual mode of delivery in relation to fear of childbirth. J Psychosom Obstet Gynaecol. 2020;41(4):266-74. https://doi.org/10.1080/0167482X.2019.1708319
7. Mehrotra A, Wolfberg A, Shah NT, Plough A, Weiseth A, Blaine AI, et al. Impact of an educational program and decision tool on choice of maternity hospital: the delivery decisions randomized clinical trial. BMC Pregnancy Childbirth. 2022;22(1):759. https://doi.org/10.1186/s12884-022-05087-y
8. Zewude B, Siraw G, Adem Y. The preferences of modes of child delivery and associated factors among pregnant women in Southern Ethiopia. Pragmat Obs Res. 2022;13:59-73. https://doi.org/10.2147/POR.S370513
9. Rahman M, Khan N, Rahman A, Alam M, Khan A. Long-term effects of caesarean delivery on health and behavioural outcomes of the mother and child in Bangladesh. J Health Popul Nutr. 2022;41(1):45. https://doi.org/10.1186/s41043-022-00326-6
10. Słabuszewska-Jóźwiak A, Szymański JK, Ciebiera M, Sarecka-Hujar B, Jakiel G. Pediatrics consequences of caesarean section-a systematic review and meta-analysis. Int J Environ Res Public Health. 2020;17(21):8031. https://doi.org/10.3390/ijerph17218031
11. Sousa VD, Rojjanasrirat W. Translation, adaptation and validation of instruments or scales for use in cross-cultural health care research: a clear and user-friendly guideline. J Eval Clin Pract. 2011;17(2):268-74. https://doi.org/10.1111/j.1365-2753.2010.01434.x
12. Capik C, Gozum S, Aksayan S. Intercultural scale adaptation stages, language, and culture adaptation: updated guideline. Florence Nightingale J Nurs. 2018;26(3):199-210. https://doi.org/10.26650/FNJN397481
13. Zamani-Alavijeh F, Shahry P, Kalhori M, Araban M. Pregnant women's preferences for mode of delivery questionnaire: psychometric properties. J Educ Health Promot. 2017;6:20. https://doi.org/10.4103/2277-9531.204738
14. Karagoz Y. SPSS AMOS METU applied biostatistics. Nobel Academic Press; 2021.
15. Buyukozturk S. Manual of data analysis for social sciences. 21st ed. Ankara: Pegem Press; 2015.
16. Ajayi BK. A comparative analysis of reliability methods. J Educ Pract. 2017;8(25):160-3.
17. Schermelleh-Engel K, Moosbrugger H, Müller H. Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. Methods Psychol Res. 2003;8(2):23-74.

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231559

Preoperative promestriene for hysteroscopy: a randomized clinical trial

Isabela Maciel Caetano[1*], Agnaldo Lopes da Silva Filho[2], Rivia Mara Lamaita[2], Bernardo Avila Maia[3], Eduardha Santos Temponi Barroso[4], Eduardo Batista Candido[2]

SUMMARY

OBJECTIVE: Intraoperative complications of hysteroscopy, such as the creation of a false passage, cervix dilatation failure, and uterine perforation, may require suspension of the procedure. Some patients refuse a new procedure, which delays the diagnosis of a possible serious uterine pathology. For this reason, it is essential to develop strategies to increase the success rate of hysteroscopy. Some authors suggest preoperative use of topical estrogen for postmenopausal patients. This strategy is common in clinical practice, but studies demonstrating its effectiveness are scarce. The aim of this study was to evaluate the effect of cervical preparation with promestriene on the incidence of complications in postmenopausal women undergoing surgical hysteroscopy.

METHODS: This is a double-blind clinical trial involving 37 postmenopausal patients undergoing surgical hysteroscopy. Participants used promestriene or placebo vaginally daily for 2 weeks and then twice a week for another 2 weeks until surgery.

RESULTS: There were 2 out of 14 (14.3%) participants with complications in the promestriene group and 4 out of 23 (17.4%) participants in the placebo group (p=0.593). The complications were difficult cervical dilation, cervical laceration, and vaginal laceration.

CONCLUSION: Cervical preparation with promestriene did not reduce intraoperative complications in postmenopausal patients undergoing surgical hysteroscopy.

KEYWORDS: Hysteroscopy. Hormone replacement therapy. Cervical erosion. Lacerations. Uterine perforation.

INTRODUCTION

Approximately 50% of hysteroscopy complications are related to difficult cervix dilation[1]. Postmenopausal status is a risk factor for cervical stenosis[2]. When the cervix is stenosed, there is an increased risk of false passage, cervix dilatation failure, cervical laceration, and uterine perforation[3]. A false passage, if not identified, will most likely lead to uterine perforation and its associated complications. Thus, when a false passage is created during cervical dilation, the procedure must be discontinued[4].

In four studies that evaluated 554, 976, 5000, and 31052 patients undergoing office hysteroscopy, it was not possible to complete the procedure in 9.5, 8.9, 5.2, and 6.2% of patients, respectively. The four studies reported pain and cervical stenosis as the major causes of failure[5-8]. One of them reported cervical stenosis in 32.7% of the patients, most of them managed successfully (70.1% of them in postmenopausal women)[8].

In a retrospective study of 516 patients undergoing office hysteroscopy, the authors described failure to access the uterine cavity in 62 patients (12%). Of these 62 patients, 36 refused to undergo a new procedure and 26 underwent a new procedure. All patients who underwent a new procedure had an anatomopathological diagnosis of an endometrial pathology, including endometrioid carcinoma and endometrial hyperplasia. Thus, it is extrapolated that an endometrial pathology may have been belatedly diagnosed in 36 patients who refused a new procedure. For this reason, the authors suggested that measures to increase the success rate of the first hysteroscopy be taken, such as for postmenopausal patients, the preoperative use of topical estrogen[9].

[1]Universidade Federal de Minas Gerais, Hospital das Clínicas, Woman's Health Department – Belo Horizonte (MG), Brazil.
[2]Universidade Federal de Minas Gerais, Department of Gynecology and Obstetrics – Belo Horizonte (MG), Brazil.
[3]Hospital Felicio Rocho – Belo Horizonte (MG), Brazil.
[4]Universidade Federal de Minas Gerais – Belo Horizonte (MG), Brazil.
*Corresponding author: isabelamcaetano@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 03, 2024. Accepted on March 02, 2024.
Research center
Hospital da Baleia, Rua Juramento, 1464 - Saudade, Belo Horizonte - MG, 30285-408

Some of the strategies already studied are cervical preparation with misoprostol alone or associated with topical estrogen, as presented below. It must be highlighted that misoprostol in Brazil is only available in maternity hospitals.

A systematic review evaluated the use of misoprostol in cervical preparation for hysteroscopy and showed that intraoperative complications were less common in the misoprostol group than in the placebo group. There was a significant reduction in the incidence of cervical laceration and creation of a false passage, but only for patients in their reproductive years. This procedure was not beneficial for postmenopausal women. Side effects were observed in 24 out of 136 (19%) participants of the misoprostol group and 12 out of 136 (9%) participants of the control group. Misoprostol was associated with mild abdominal pain, increased body temperature, and vaginal bleeding. There was no conclusive evidence regarding nausea, shivering, or diarrhea[1].

A randomized clinical trial compared the use of estradiol alone with the use of estradiol plus misoprostol in cervical preparation for hysteroscopy in postmenopausal patients. Patients were divided into two groups: (a) those who used topical estrogen for 14 days before hysteroscopy and misoprostol 12 h before surgery and (b) those who used topical estrogen for 14 days before hysteroscopy and placebo 12 h before surgery. Group (a) had significantly better cervical ripening compared to group (b). There was one uterine perforation in each group. There was a significant difference between the initial measurement of the diameter of the external orifice of the cervix in the office evaluation before the medications and the measurement at the time of hysteroscopy, in both groups: in group (a), from 2.6 mm to 5.7 mm, and in group (b), from 2.1 mm to 4.7 mm. There was no control group. They found that estradiol alone could increase the diameter of the external orifice of the cervix, but it is not known if it would imply a clinically significant outcome (i.e., reduction of intraoperative complications)[10].

There exists a gap in the medical literature on the use of isolated topical estrogen for cervical preparation for hysteroscopy. Conversely, it is common in clinical practice.

Topical estrogen plays an important role in the treatment of genitourinary syndrome of menopause[11]. The improvement of urogenital symptoms and cytology usually occurs after 5–14 days of local therapy[12]. Estriol, estradiol, and conjugated estrogen, even when applied vaginally, reduce FSH. Promestriene does not reach the systemic circulation, cannot be converted to estradiol, does not alter the serum level of gonadotropins and estradiol, and does not stimulate the endometrium. Its systemic inactivity justifies its use when active estrogens are contraindicated, as in patients with estrogen-sensitive cancers[12]. Studies have already evaluated the efficacy and safety of promestriene in such patients[12-15].

The aim of this study was to evaluate the effect of promestriene on the incidence of intraoperative complications in postmenopausal women undergoing surgical hysteroscopy.

METHODS

The study was approved by the ethics committee of the research center and by Plataforma Brasil of Brazil's National Health Department (CAAE 38240720.0.0000.5123, deliberation number 4.508.539 and 4.984.613). It is registered in The Brazilian Registry of Clinical Trials (ReBEC) and is reported according to the CONSORT guidelines.

The research center of this randomized clinical trial was a tertiary hospital in Belo Horizonte, Brazil. This is a pilot study so the sample size was not previously determined. Over a 12-month period, postmenopausal patients who met the criteria were invited to participate in the trial. The promestriene or placebo and vaginal applicators were placed in numbered opaque envelopes, according to the randomization. Randomization was made in Microsoft Excel® in blocks of 50 and in a 1:1 ratio. The envelopes were packed and sealed by a physician who did not meet the participants. Then, the envelopes were delivered to the participants by one of the authors, along with the consent form and written instructions. Participants were supposed to use the vaginal cream (promestriene or placebo) once a day for 2 weeks and, after that period, twice a week for another 2 weeks, until the procedure was done.

The placebos and their vaginal applicators were funded by the researchers. All the promestriene needed for the study were provided by Eurofarma®. There was no transfer of cash.

Participants' flowchart with inclusion and exclusion criteria are described in Figure 1. The shortage of supplies and the suspension of elective procedures due to the COVID-19 pandemic limited the number of participants.

All procedures were performed by the same team with a Kalz Stoltz 27050 SL outer sheath and a 4 mm/30º telescope. The surgeons did not know which group each participant belonged to. The participants were asked over telephone about the use of the vaginal cream in the postoperative appointment or later. The authors read the participants' operative report and noted any described complications.

Statistical analysis was conducted to describe the baseline characteristics of the study participants. For continuous

variables, the mean, standard deviation, median, quartiles, and minimum and maximum values were used. For categorical variables, the absolute and relative frequencies were described. In the evaluation of categorical variables, the authors used the chi-square and Fisher's exact tests. The Mann–Whitney test was used to compare continuous variables between groups since the variables did not show normal distribution by the Shapiro-Wilk test. All analyses were performed using the Stata software, version 16 and a 5% significance level was considered.

RESULTS

Participants were enrolled from August 2021 to July 2022. Data analysis of baseline characteristics is described in Table 1.

Exactly five participants were reallocated to the placebo group because they used promestriene for only 0 to 4 times. Two participants reported that they discontinued after using it three and four times due to discomfort associated with the applicator, while the other three reported using it 0, 1, and 3 times because they forgot to use it. No participant had any serious adverse event to the medications.

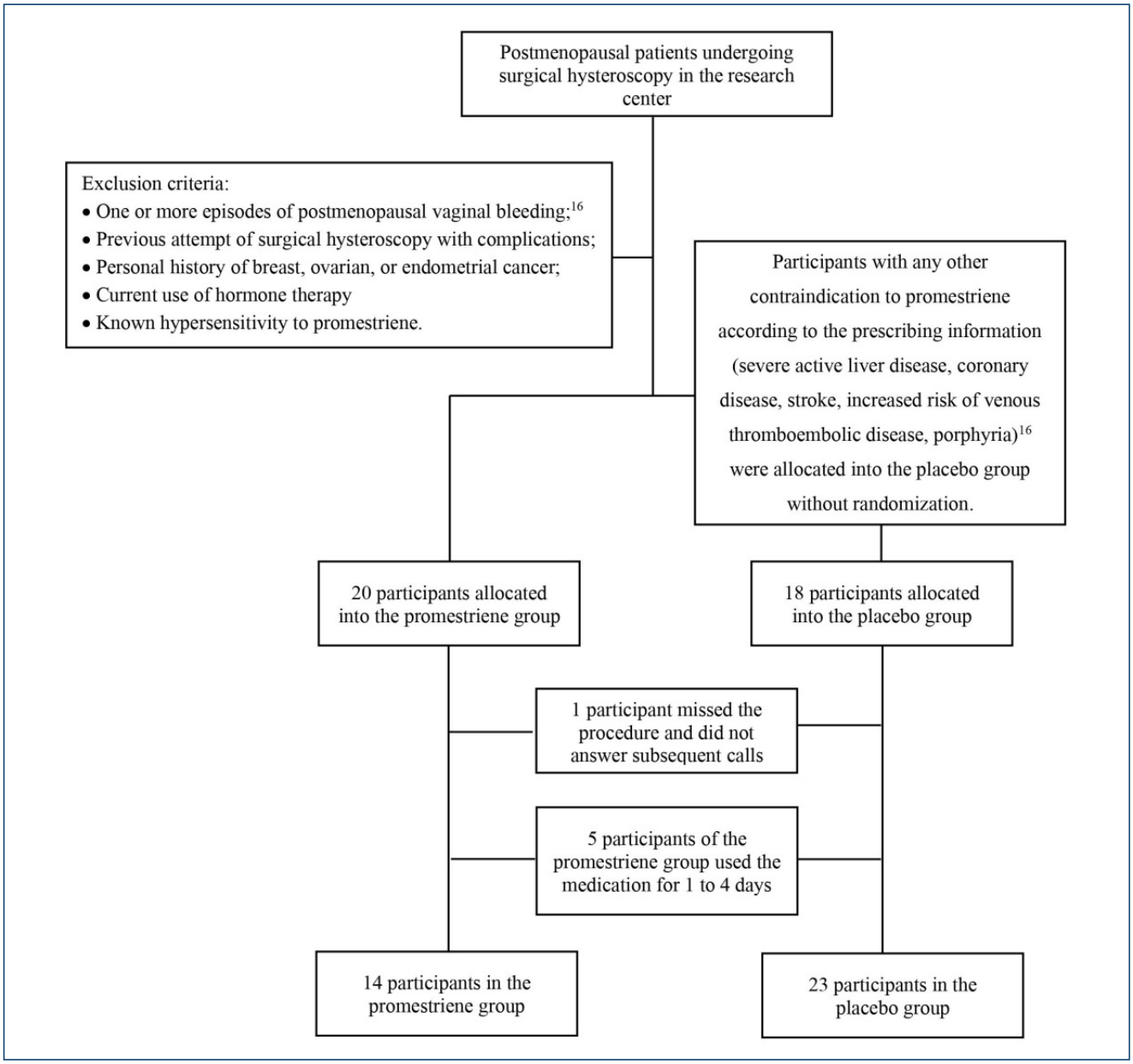

Figure 1. Participants' flowchart.

Of the 37 participants, 6 (16.21%) experienced complications, all of which were minor (Table 2). No complications resulted in the suspension of the procedure, prolonged hospital stay, or involved laparotomy. The participants who experienced complications were as follows:

- Promestriene group, 54 years old, cervical laceration requiring suture
- Promestriene group, 65 years old, cervical laceration not requiring suture
- Placebo group, 71 years old, difficult dilation
- Placebo group, 71 years old, difficult dilation
- Placebo group, 71 years old, difficult dilation and vaginal laceration requiring suture
- Placebo group, 74 years old, cervical laceration requiring suture

In the placebo group, participants with complications were significantly older (mean age 72.0 years; SD ±1.4) than participants who did not have experience complications (mean age 63.3 years; SD ±6.3) (p=0.005). There was no age difference observed between patients who had complications and patients who did not in the promestriene group.

DISCUSSION

Until August 2023, there were no studies in Portuguese or English evaluating topical estrogen versus placebo or control group for preparation for hysteroscopy. This is a gap in medical literature, even though it is common in clinical practice.

Table 1. Participants' baseline characteristics.

Characteristics		Promestriene group Mean (±SD)	Placebo group Mean (±SD)	p-value
Body weight		69.2 (±9.6)	70.2 (±13.7)	0.820
BMI		26.4 (±3.7)	27.3 (±5.1)	0.805
Number of pregnancies		2.9 (±2.2)	3.1 (±2.0)	0.423
Number of vaginal deliveries		1.8 (±2.2)	2.3 (±2.2)	0.404
Number of cesarian sections		0.6 (±0.8)	0.6 (±1.1)	0.664
Number of pregnancy losses		0.5 (±0.7)	0.2 (±0.7)	0.094
Menopause		50.5 (±3.2)	48.8 (±4.6)	0.291
Duration of medication use		30.6 (±1.7)	–	–
Age at surgery		59.4 (±5.8)	64.8 (±6.6)	0.019
Ethnicity	White	7	12	0.999
	Pardo	4	8	
	Black	2	3	
Educational qualification	Middle school	7	15	0.693
	High school	5	6	
	Bachelor's degree	1	1	

Table 2. Incidence of complications.

Complications		Promestriene group		Placebo group		p-value
		n	%	n	%	
Number of participants with laceration of the cervix or vagina	Yes	2/14	14.3	2/23	8.7	0.491
	No	12/14	85.7	21/23	91.3	
Number of participants with difficulty in cervical dilation	Yes	0/14	0	3/23	13	0.228
	No	14/14	100	20/23	87	
Number of participants with any complication	Yes	2/14	14.3	4/23	17.4	0.593
	No	12/14	85.7	19/23	82.6	

The hypothesis that topical estrogen alone reduces complications in postmenopausal patients is biologically plausible, as shown by a study that demonstrated that the use of topical estrogen increases the diameter of the external orifice of the cervix of postmenopausal women[10].

There is a concern about prescribing promestriene to patients with endometrial thickening due to the possibility of endometrial malignancy. However, the literature review found studies that demonstrate the safety of promestriene in patients with estrogen-dependent cancer[12-15]. The drug was used by the participants for 4 weeks, which is the usual (4–6 weeks) time of use in preparation for prolapse correction surgeries[17].

There was a significant difference between the groups in terms of age. This difference was attributed to the fact that patients with contraindications to hormone therapy were placed into the placebo group without randomization (i.e., all patients with coronary disease, stroke, and high risk of venous thromboembolic disease). As these diseases become increasingly prevalent with aging, patients with these diseases are expected to be older.

There was no significant difference between the groups in terms of number of participants with any complication, number of participants with cervix/vaginal laceration, and number of participants with difficulty in cervical dilation.

In the placebo group, participants who experienced complications were significantly older than those who did not experience complications. This finding is consistent with the literature: a higher rate of complications in hysteroscopy is expected in patients with hypoestrogenism[8]. On the contrary, in the promestriene group, there was no significant difference in terms of age between the participants who had complications and the participants who did not. Once genital atrophy had been treated, age ceased to be a risk factor for complications, which is a remarkable finding.

The sample size of this study was a limiting factor. Considering that hysteroscopy is a procedure with a low rate of complications, a large number of participants are necessary to show any significant difference.

CONCLUSION

Cervical preparation with promestriene did not demonstrate a reduction in the incidence of intraoperative complications in postmenopausal patients undergoing surgical hysteroscopy.

ETHICAL APPROVAL

The study was approved by the ethics committee of the research center and by Plataforma Brasil of Brazil's National Health Department on January 21, 2021 (CAAE 38240720.0.0000.5123, deliberation number 4.508.539 and 4.984.613. It is registered in The Brazilian Registry of Clinical Trials (ReBEC).

AUTHORS' CONTRIBUTIONS

IMC: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Writing – original draft. **ALSF:** Writing – review & editing. **RML:** Writing – review & editing. **BAM:** Methodology. **ESTB:** Writing – review & editing. **EBC:** Formal Analysis, Investigation, Project administration, Supervision, Writing – original draft.

REFERENCES

1. Al-Fozan H, Firwana B, Kadri H, Hassan S, Tulandi T. Preoperative ripening of the cervix before operative hysteroscopy. Cochrane Database Syst Rev. 2015;2015(4):CD005998. https://doi.org/10.1002/14651858.CD005998.pub2
2. Linda D, Bradley, MD. Overview of hysteroscopy. In: Post TW, editor. UpToDate. Waltham (MA): UpToDate Inc; 2019. [cited on 2019 Dec 02]. Available from: https://www.uptodate.com
3. Bradley LD. Complications in hysteroscopy: prevention, treatment and legal risk. Curr Opin Obstet Gynecol. 2002;14(4):409-15. https://doi.org/10.1097/00001703-200208000-00008
4. Song D, Xia E, Xiao Y, Li TC, Huang X, Liu Y. Management of false passage created during hysteroscopic adhesiolysis for Asherman's syndrome. J Obstet Gynaecol. 2016;36(1):87-92. https://doi.org/10.3109/01443615.2015.1030601
5. Kremer C, Barik S, Duffy S. Flexible outpatient hysteroscopy without anaesthesia: a safe, successful and well tolerated procedure. Br J Obstet Gynaecol. 1998;105(6):672-6. https://doi.org/10.1111/j.1471-0528.1998.tb10185.x
6. La Sala GB, Sacchetti F, Dessanti L, Torelli MG, Sartori F. Panoramic diagnostic microhysteroscopy: analysis of results obtained from 976 patients. Acta Eur Fertil. 1986;17(5):369-75. PMID: 3577603
7. Spiezio Sardo A, Taylor A, Tsirkas P, Mastrogamvrakis G, Sharma M, Magos A. Hysteroscopy: a technique for all? Analysis of 5,000 outpatient hysteroscopies. Fertil Ster. 2008;89(2):438-3.
8. Bettocchi S, Bramante S, Bifulco G, Spinelli M, Ceci O, Fascilla FD, et al. Challenging the cervix: strategies to overcome the anatomic impediments to hysteroscopy: analysis of 31,052 office hysteroscopies. Fertil Steril. 2016;105(5):e16-7. https://doi.org/10.1016/j.fertnstert.2016.01.030

9. Genovese F, D'Urso G, Guardo F, Insalaco G, Ciotta L, Carbonaro A, et al. Failed diagnostic hysteroscopy: analysis of 62 cases. Eur J Obstet Gynecol Reprod Biol. 2019;245:193-7. https://doi.org/10.1016/j.ejogrb.2019.10.031
10. Casadei L, Piccolo E, Manicuti C, Cardinale S, Collamarini M, Piccione E. Role of vaginal estradiol pretreatment combined with vaginal misoprostol for cervical ripening before operative hysteroscopy in postmenopausal women. Obstet Gynecol Sci. 2016;59(3):220-6. https://doi.org/10.5468/ogs.2016.59.3.220
11. Pompei LM, Machado RB, Wender MC, Fernandes CE. Consenso Brasileiro de Terapêutica Hormonal da Menopausa – Associação Brasileira de Climatério (SOBRAC). São Paulo (SP): Leitura Médica; 2018.
12. Pup L, Francia R, Cavaliere C, Facchini G, Giorda G, Paoli P, et al. Promestriene, a specific topic estrogen. Review of 40 years of vaginal atrophy treatment: is it safe even in cancer patients? Anticancer Drugs. 2013;24(10):989-98. https://doi.org/10.1097/CAD.0b013e328365288e
13. Pup L. Management of vaginal dryness and dyspareunia in estrogen sensitive cancer patients. Gynecol Endocrinol. 2012;28(9):740-5. https://doi.org/10.3109/09513590.2011.652717
14. Oyarzún MFG, Castelo-Branco C. Local hormone therapy for genitourinary syndrome of menopause in breast cancer patients: is it safe? Gynecol Endocrinol. 2017;33(6):418-20. https://doi.org/10.1080/09513590.2017.1290076
15. Pup L, Postruznik D, Corona G. Effect of one-month treatment with vaginal promestriene on serum estrone sulfate levels in cancer patients: a pilot study. Maturitas. 2012;72(1):93-4. https://doi.org/10.1016/j.maturitas.2012.01.017
16. ANTROFI. ANTROFI: creme vaginal. Responsável técnico Dra. Ivanete A. Dias Assi. São Paulo: Eurofarma Laboratórios S.A., 2022. 1 bula de remédio. 10 p. 2022. [cited on 2022 Nov 19]. Available from: https://eurofarma.com.br/produtos/bulas/patient/pt/bula-antrofi.pdf
17. Rahn DD, Good MM, Roshanravan SM, Shi H, Schaffer JI, Singh RJ, et al. Effects of preoperative local estrogen in postmenopausal women with prolapse: a randomized trial. J Clin Endocrinol Metab. 2014;99(10):3728-36. https://doi.org/10.1210/jc.2014-1216

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231571

Menopause and metabolic syndrome: anthropometric, lipid, and dietary profiles

Virginia Barros Sgorlon Gonçalves[1*], Sônia Maria Rolim Rosa Lima[1]

SUMMARY

OBJECTIVE: The aim of this study was to characterize the anthropometric, lipid, and dietary profiles of postmenopausal women with metabolic syndrome attending a public health service and compare them with a group of women without metabolic syndrome.

METHODS: A cross-sectional study was conducted with 60 postmenopausal women who were divided into two groups: control group and metabolic syndrome group, attending the Climacteric Outpatient Clinic at Santa Casa de São Paulo Hospital, Brazil, between February 2019 and December 2021. Participants were evaluated using a validated semi-quantitative food frequency questionnaire, body mass index, waist circumference, and serum laboratory tests.

RESULTS: Significant differences were observed between the groups regarding body mass index and all parameters of metabolic syndrome. The nutritional profile revealed an imbalance in the number of food portions consumed, particularly in the intake of carbohydrates in the form of flour and sweets, which was higher in the metabolic syndrome group.

CONCLUSION: The analysis of the three profiles of postmenopausal women revealed significant imbalances, particularly in the metabolic syndrome group, highlighting the importance of regular adjustments and evaluations during this phase of a woman's life.

KEYWORDS: Diet. Questionnaire. Metabolic syndrome. Menopause.

INTRODUCTION

Metabolic syndrome (MetS) is a risk factor for a number of chronic noncommunicable diseases with high levels of mortality and decreased quality of life. Prevalence in women is around 1.5 to 2 times higher than in men[1].

Metabolic syndrome appears more frequently during the menopausal transition and worsens after menopause. It represents a public health problem, with significant implications for quality of life, healthcare, as well as social and economic aspects[2].

In clinical practice, the criteria for MetS defined by the US National Cholesterol Education Program Adult Treatment Panel III (ATP III) are widely used for their simplicity and practicality. MetS is diagnosed based on the presence of three out of five factors[3].

The consumption of a diet rich in saturated fat, starchy carbohydrates, and high daily caloric intake is associated with numerous chronic noncommunicable diseases[4].

To investigate possible relationships between dietary habits and MetS, we employed anthropometric assessment; conducted routine serum tests for total cholesterol, lipid fractions, triglycerides, and glucose; and applied a validated semi-quantitative food frequency questionnaire (FFQ)[5,6].

METHODS

The protocols followed the ethical standards of the Declaration of Helsinki and were approved by the Research Ethics Committee of the Irmandade da Santa Casa de Misericórdia de São Paulo (CEP: 5.322.400). All volunteers recruited signed the informed consent form. Data collection was performed at the moment the patient agreed to participate in the study.

The sample consisted of 60 postmenopausal women aged between 44 and 64 years, who were divided into two groups: MetS group (MetSG, n=30) and control group (CG, n=30). A cross-sectional study was conducted in the public health service with patients attending the Climacteric Outpatient Clinic of Santa Casa de São Paulo Hospital between February 2019 and December 2021.

Inclusion criteria were as follows: postmenopausal women aged up to 64 years, BMI≥18.5 to <35 kg/m^2, amenorrhea ≥12 months, and FSH≥30 mU/mL. Exclusion criteria were as follows: BMI>35 kg/m^2, illicit drug use or alcohol abuse, a history of bariatric surgery, cancer, or cardiovascular disease. The diagnosis of MetS was determined according to the ATP III (Adult Treatment Panel) guidelines, widely used for their simplicity and practicality: (1) waist circumference>88 cm; (2) HDL cholesterol concentrations <50 mg/dL; (3) triglycerides

[1]Santa Casa de São Paulo School of Medical Sciences, Department of Obstetrics and Gynecology – São Paulo (SP), Brazil.
*Corresponding author: vibarros00@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 17, 2024. Accepted on March 02, 2024.

≥150 mg/dL; (4) blood pressure levels ≥130/85 mmHg; and (5) fasting blood glucose ≥110 mg/dL. MetS is diagnosed based on the presence of three out of five factors[3].

Through a standardized anamnesis, the following were observed: anthropometric profile, in which weight was measured in kilograms (Balmak digital scale, model BK 200, accuracy of 0.1 kg) with the patient in an upright position and minimally dressed, height was measured in centimeters using a vertical stadiometer, and waist circumference (WC) was measured in centimeters at the midpoint between the last rib and the iliac crest using a measuring tape. Body mass index (BMI) was calculated as weight in kilograms divided by the height in square meters (kg/m^2)[7]. For the nutritional profile, a validated semi-quantitative FFQ was completed in a person, which contains nine frequency options: never or<1 month, 1–3 per month, 1 per month, 2–4 per month, 5–6 per month, 1 per day, 2–3 per day, 4–5 per day, and 6+ per day, with 103 questions and 98 foods[7]. Based on the Food Guide for the Brazilian Population, an average intake of food was found to be 2,000 kcal/day[8].

In the lipid profile, blood samples were analyzed by enzymatic method using the BT 3000 plus device (Wiener lab®, Rosario, Argentina). The LDL value was calculated and obtained using the Friedewald formula (LDLc= CT-HDLc – TG/5)[9]. Blood pressure was measured using a semi-automatic oscillometer (Omron Hbp-112) while the participant was in a seated position[10].

Sample size calculation and statistical analysis

The criterion used was a test power of 80%, and a significance level of 5% was considered adequate for evaluating the frequency of the main study variables. Analyses were performed using SPSS version 25.0 (IBM Corp. 2017, Armonk, NY, USA). To compare qualitative variables, we used the chi-square test and, for quantitative variables, we used the Mann-Whitney nonparametric test and the Student's t-test.

RESULTS

With respect to the analyzed characteristics, there were significant differences between the groups in terms of weight, BMI, VLDL cholesterol, and clinical parameters for MetS, as shown in Table 1. The most prevalent parameter in this study was WC>88 cm, which was present in 100% of the MetSG participants. The results of the FFQ with 19 most common foods on the Brazilian table revealed significant differences in the consumption frequencies

Table 1. Socio-demographic, anthropometric, and lipid profiles and clinical parameters of postmenopausal women in the control group and the metabolic syndrome group (Ambulatório de climatério- FCMSCSP–2022).

Sample characteristics	Control group	MetS group[1]	p-value
Age (years)*	55.0 (±4.9)	56.0 (±5.3)	0.339
Weight (kg)*	64.0 (±10.2)	76.0 (±8.0)	0.000
Height (m)**	1.55 (1.48–1.77)	1.59 (1.44–1.72)	0.239
BMI (kg/m^2)**	25.4 (18.8–33.2)	29.7 (24.0–35.0)	0.000
Time after menopause (years)**	4.5 (2.0–16.0)	4.5 (2.0–23.0)	0.922
HDL cholesterol (mg/dL)**	59.0 (102.0–42.0)	50.5 (84.0–34.0)	0.002
LDL cholesterol (mg/dL)**	120.5 (236.0–81.0)	121.0 (161.0–50.2)	0.217
VLDL cholesterol (mg/dL)**	21.5 (40.6–10.0)	32.1 (69.2–17.2)	0.001
Non-HDL cholesterol (mg/dL)**	150.4 (269.6–101.2)	151.2 (201.4–73.6)	0.929
Total cholesterol (mg/dL)**	209.6 (325.6–168.2)	198.3 (249.4–158.0)	0.079
Triglycerides (mg/dL)**	107.5 (203.0–50.0)	158.5 (346.0–15.8)	0.001
Systolic blood pressure (≥130 mmHg)§	12.0 (±1.6)	13.6 (±1.2)	0.024
Diastolic blood pressure (≥85 mmHg)§	7.7 (±1.1)	8.4 (±0.8)	0.024
Waist circumference (>88 cm)§	88.8 (±8.1)	102.0 (±8.7)	0.000
Fasting blood glucose (≥110 mg/dL)§	93.5 (±9.7)	105.6 (±14.0)	0.001
Triglycerides (≥150 mg/dL)§	115.3 (±38.6)	161.7 (±52.9)	0.002
HDL cholesterol (<50 mg/dL)§	63.5 (±14.1)	52.5 (±12.5)	0.002

p<0.05; *Student's t-test (mean and SD); **Mann–Whitney test (median, minimum, and maximum); §Chi-square test (mean and SD); BMI: body mass index; kg: kilograms; m: meter; kg/m^2: kilogram per square meter; mg/dL: milligram per deciliter; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; mmHg: millimeter of mercury; cm: centimeters; MetS[1]: metabolic syndrome; FCMSCSP: Faculdade de Ciências Médicas da Santa Casa de São Paulo. Bold indicates statistically significant p-value.

of up to three times a day analyzed between the two groups with regard to vegetables, fruit juice, wheat flour, sugary cereals, cakes, cookies, mono- and polyunsaturated fats, sweets in general, fast food, and added sugar, as described in Table 2.

DISCUSSION

To help promote a better understanding of MetS and its possible treatments in postmenopausal women, we analyzed a series of variables that constitute risk factors for chronic noncommunicable diseases, as previous studies have adopted a selective approach, considering the individual's nutritional, anthropometric, and lipid profiles separately[11,12].

The overweight and obesity in women with MetS were also found in other studies[13-15]. The prevalence of WC >88 cm present in 100% of MetSG can be observed in studies that analyzed the anthropometric profile of participants[13,16]. Regarding changes in the lipid profile, we found similar reports in the literature concerning postmenopausal women with MetS[17,18].

When we analyzed the nutritional profile of postmenopausal women using the FFQ and examined the consumption of 19 foods up to three times a day, we observed differences between the two groups in relation to the intake of certain nutrients. The MetSG had higher carbohydrate consumption, mainly refined starchy, bakery products, sugary cereals, cakes, sweet biscuits, sweets in general, above the recommended limit (up to 55% of total daily energy intake), and added sugar, above the recommended limit (up to 10% of the total energy ingested), than the CG. The fat intake for this group was also higher than recommended (up to 35% of total daily energy intake), mainly due to consumption of fast food rich in saturated fat and sodium according to the Brazilian Food Guide[8].

The World Health Organization recommends that sugar consumption be <10% of daily energy intake[19], while <5% is recommended in the United States and the United Kingdom[20]. Simple sugar intake is associated with increased blood pressure, WC, serum triglyceride, glucose concentrations, and a significantly increased risk of developing MetS[18].

Table 2. Frequency of ingestion of 19 food items from the FFQ, up to three times a day, of postmenopausal women in the control group (n=30) and the metabolic syndrome group (n=30) (Ambulatório de Climatério – FCMSCSP–2022).

Foods	Partial number	Control group	MS[1] group	Total n=60	Chi square
	nº	%	%	%	p
Assorted meats	11	20.0	16.6	18.3	0.238
Fish	1	0.0	3.3	1.6	0.339
Eggs	18	23.3	36.3	30.0	0.364
Whole animal derivatives	33	40.0	70.0	55.0	0.084
Semi-skimmed and skimmed	11	20.0	16.6	18.3	0.238
Greens and vegetables	22	46.6	26.6	36.6	0.007
Roots	5	6.6	10.0	8.3	0.309
Legumes	38	66.6	60.0	63.3	0.191
Cereals	42	70.0	70.0	70.0	0.366
Fruits	23	50.0	26.6	38.3	0.059
Fruit juice	3	6.6	3.3	5.0	0.009
White and whole wheat flour	31	33.3	70.0	51.6	<0.001
Sugary cereal, cake, cookies, and candy	6	3.3	16.6	10.0	<0.001
Mono- and polyunsaturated fat	11	36.6	0.0	18.3	0.001
Sweets in general	13	16.6	26.6	21.6	<0.001
Fast food	4	0.0	13.3	6.66	0.009
Alcoholic beverage	0	0.0	0.0	0.0	0.079
Regular soda and artificial juice	9	10.0	20.0	60.0	0.079
Sugar for added	25	36.3	46.6	41.6	0.004

p<0.05; Chi-square test (mean and SD); N partial: number of participants who responded positively to the frequency of consumption of the 19 food items; MS[1]: metabolic syndrome group; FFQ: Food Frequency Questionnaire; FCMSCSP: Faculdade de Ciências Médicas da Santa Casa de São Paulo. Bold indicates statistically significant p-value.

The CG had a significantly higher consumption of greens and vegetables. According to the results of a meta-analysis, there is a possible relationship between the decrease in fiber intake from vegetables and increased risk factors for MetS[21,22]. The CG also showed higher consumption of items such as good sources of fat like olive oil, compared to the MetSG. These results are similar to other studies[23-25].

Diet analysis and its impact on anthropometric assessment and laboratory tests are of fundamental importance for planning health promotion interventions and managing comorbidities in postmenopausal women. Adequate nutrition and a healthy lifestyle should be taken into consideration as important factors in this phase of a woman's life[16].

However, it is important to highlight that the completion of the FFQ regarding the last year can introduce some dispersion in the results, considering that it relies on the participants' good memory and honesty during completion, which may compromise the analysis performed.

Therefore, we hope that our study opens new horizons for further research and scientific investigations in this field.

CONCLUSION

The analysis of the anthropometric, lipid, and dietary profiles of postmenopausal women with and without MetS revealed an imbalance in macronutrient intake and portion sizes consumed. The MetSG had a higher prevalence of physical and laboratory alterations compared to the CG, which exhibited profiles closer to the ideal.

Therefore, there is a need to emphasize the importance of nutritional adjustments and regular evaluations during this phase of life in order to minimize the risks of health complications associated with this condition.

AUTHORS' CONTRIBUTIONS

VBSG: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft. **SMRRL:** Conceptualization, Methodology, Supervision, Writing – review & editing.

REFERENCES

1. World Health Organization (WHO). World health statistics. 2023. Available from: https://www.who.int/data/gho/publications/world-health-statistics
2. Xu H, Li X, Adams H, Kubena K, Guo S. Etiology of metabolic syndrome and dietary intervention. Int J Mol Sci. 2018;20(1):128. https://doi.org/10.3390/ijms20010128
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA. 2001;285(19):2486-97. https://doi.org/10.1001/jama.285.19.2486
4. Canhada SL, Luft VC, Giatti L, Duncan BB, Chor D, Fonseca MJMD, et al. Ultra-processed foods, incident overweight and obesity, and longitudinal changes in weight and waist circumference: the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Public Health Nutr. 2020;23(6):1076-86. https://doi.org/10.1017/S1368980019002854
5. Harraqui K, Oudghiri DE, Hannoun Z, Naceiri Mrabti H, Aboulghras S, Assaggaf H, et al. frequency of metabolic syndrome and study of anthropometric, clinical and biological characteristics in peri- and postmenopausal women in the City of Ksar El Kebir (Northern Morocco). Int J Environ Res Public Health. 2022;19(10):6109. https://doi.org/10.3390/ijerph19106109
6. Furlan-Viebig R, Pastor-Valero M. [Development of a food frequency questionnaire to study diet and non-communicable diseases in adult population]. Rev Saude Publica. 2004;38(4):581-4. https://doi.org/10.1590/s0034-89102004000400016
7. Weir CB, Jan A. BMI classification percentile and cut off points. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. PMID: 31082114.
8. Ministério da Saúde. Guia alimentar para a população Brasileira: promovendo a alimentação saudável. 2nd ed. Brasília: Ministério da Saúde; 2014. p. 156.
9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502. PMID: 4337382
10. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Brazilian guidelines of hypertension - 2020. Arq Bras Cardiol. 2021;116(3):516-658. https://doi.org/10.36660/abc.20201238
11. Jaballah A, Soltani I, Bahia W, Dandana A, Hasni Y, Miled A, et al. The relationship between menopause and metabolic syndrome: experimental and bioinformatics analysis. Biochem Genet. 2021;59(6):1558-81. https://doi.org/10.1007/s10528-021-10066-7
12. Hamoda H, Moger S, Board of Trustees and Medical Advisory Council of the British Menopause Society. Developing the Women's health strategy: the British Menopause Society's recommendations to the department of health and social care's call for evidence. Post Reprod Health. 2022;28(1):13-8. https://doi.org/10.1177/20533691211064037
13. Ebong IA, Wilson MD, Appiah D, Michos ED, Racette SB, Villablanca A, et al. Relationship between age at menopause, obesity, and incident heart failure: the atherosclerosis risk in communities study. J Am Heart Assoc. 2022;11(8):e024461. https://doi.org/10.1161/JAHA.121.024461
14. Lima, SMRR, Botogoski, SR, Reis BF. Menopausa - O que você precisa saber: abordagem prática e atual do período do climatério. São Paulo (SP): Atheneu; 2014. p. 565-9.
15. Paiva LHSC, Moraes AVG. FEBRASGO. Influência da obesidade no climatério: risco cardiovascular, câncer e obesidade na pós menopausa. In: FEBRASGO. Obesidade na mulher. São Paulo (SP): Federação Brasileira das Associações de Ginecologia e Obstetrícia (FEBRASGO); 2019. p. 86-105.
16. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52. https://doi.org/10.1016/S0140-6736(04)17018-9

17. Harraqui K, Oudghiri DE, Hannoun Z, Naceiri Mrabti H, Aboulghras S, M Assaggaf H, et al. frequency of metabolic syndrome and study of anthropometric, clinical and biological characteristics in peri- and postmenopausal women in the City of Ksar El Kebir (Northern Morocco). Int J Environ Res Public Health. 2022;19(10):6109. https://doi.org/10.3390/ijerph19106109

18. Gouvea TM, Serpa MA, Lopes ACF, Veloso VM, Carrillo MRGG, Lima AA. Avaliação de dislipidemia e risco cardiovascular em mulheres no climatério. Discipl Sci. 2020;21(2):311-25. https://doi.org/10.37777/dscs.v21n2-026

19. World Health Organization. Healthy diet. World Health Organization. Regional office for the eastern mediterranean. 2019. Available from: https://apps.who.int/iris/bitstream/handle/10665/325828/EMROPUB_2019_en_23536.pdf

20. Public Health England. Sugar reduction: from evidence into action. London: Public Health England; 2015.

21. Malik VS, Hu FB. Sugar-sweetened beverages and cardiometabolic health: an update of the evidence. Nutrients. 2019;11(8):1840. https://doi.org/10.3390/nu11081840

22. Steiner ML, Azevedo LH, Bonacordi CL, Barros AZ, Strufaldi R, Fernandes CE. [Food consumption in postmenopausal women and its relation with anthropometric measurements and time since menopause]. Rev Bras Ginecol Obstet. 2015;37(1):16-23. https://doi.org/10.1590/SO100-720320140005138

23. Cybulska AM, Schneider-Matyka D, Bosiacki M, Chlubek D, Panczyk M, Grochans E. The levels of bioelements in postmenopausal women with metabolic syndrome. Nutrients. 2022;14(19):4102. https://doi.org/10.3390/nu14194102

24. Ko SH, Kim HS. Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women. Nutrients. 2020;12(1):202. https://doi.org/10.3390/nu12010202

25. Geony HG, Park H. Distúrbios metabólicos na menopausa. Metabolitos. 2022;12(10):954. https://doi.org/10.3390/metabo12100954

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231574

The role of the *Chitinase 3-Like 1 (CHI3L1) genes* in the preeclampsia pathophysiology

Nigar Mammadova[1], Sibel Özler[2], Belma Gözde Özdemir[2*], Fazıl Avcı[2], Nadir Koçak[3], Ersin Çintesun[2], Gökçen Örgül[2], Çetin Çelik[2]

SUMMARY

OBJECTIVE: The aim of this study was to investigate the relationship between *Chitinase 3-Like 1 gene* polymorphisms and the occurrence of preeclampsia in a selected cohort of pregnant women.

METHODS: A total of 75 pregnant women participated in the study, 35 of whom were diagnosed with preeclampsia, while 40 served as healthy controls. The preeclamptic group was subdivided based on severity. Real-time polymerase chain reaction was employed to analyze the serum samples for variations in *Chitinase 3-Like 1 gene* polymorphisms.

RESULTS: The rs880633 polymorphism was found to be significantly more frequent in the control group (80%) compared with the overall preeclamptic group (60%) ($p<0.05$). In the severity-based subgroups, rs880633 appeared in 57.1% of non-severe and 61.9% of severe preeclamptics. Contrarily, the heterozygous form of rs7515776 polymorphism showed a significantly higher prevalence in the preeclamptic cohort ($p<0.05$), without distinctions in severity subgroups.

CONCLUSION: The study suggests that the rs880633 polymorphism may serve a protective role against the development of preeclampsia, whereas the rs7515776 polymorphism may be associated with an elevated risk. Further research is warranted to elucidate the clinical implications of these findings.

KEYWORDS: Chitinase-3-Like Protein 1. Etiology. Preeclampsia.

INTRODUCTION

One of the primary factors that contributes to maternal and perinatal mortality and morbidity during pregnancy is the presence of hypertensive disorders. These disorders manifest in approximately 5–10% of all pregnancies[1]. Hypertensive diseases during pregnancy can be classified into four categories: gestational hypertension, preeclampsia-eclampsia syndrome, chronic hypertension, and superimposed preeclampsia. According to the 2018 guidelines set forth by the International Society for the Study of Hypertension in Pregnancy (ISSHP), preeclampsia is defined as elevated systolic blood pressure (≥140 mmHg) and/or elevated diastolic blood pressure (≥90 mmHg), measured at least twice, at 4-h intervals, in otherwise healthy pregnant women. Moreover, preeclampsia is accompanied by one or more of the following new-onset conditions after the 20th week of gestation: proteinuria, evidence of other maternal organ dysfunction, or uteroplacental dysfunction[2]. Preeclampsia is a multisystemic disorder characterized by hypertension, proteinuria, or end-organ damage, which may manifest as thrombocytopenia, renal dysfunction, hepatic dysfunction, pulmonary edema, or neurological or visual impairments. A critical role in the etiopathogenesis of both uteroplacental and systemic endothelial dysfunction is played by trophoblastic invasion deficiencies, which are essential for proper placental development. The etiology of preeclampsia is complex and multifactorial, involving oxidative stress, an elevated maternal immune response, ischemia, inflammation, and various immunological and environmental factors. Genetic predispositions also contribute to the etiology of preeclampsia[3-7].

The *Chitinase 3-Like 1 (CHI3L1) gene* encodes for a glycoprotein, also known as YKL-40. This glycoprotein is produced by a myriad of human cells, including but not limited to macrophages, neutrophils, stem cells, bone cells, synoviocytes, chondrocytes, fibroblast-like cells, endothelial cells, vascular smooth muscle cells, hepatic stellate cells, mammary epithelial cells, and cancer cells. The CHI3L1 glycoprotein, secreted by activated neutrophils and macrophages, has been implicated in several crucial biological pathways, including angiogenesis,

[1]Afiyet Hospital, Private Clinic of Obstetrics and Gynecology – İstanbul, Turkey.
[2]Selcuk University, Faculty of Medicine, Department of Obstetrics and Gynecology – Konya, Turkey.
[3]Selcuk University, Faculty of Medicine, Department of Medical Genetics – Konya, Turkey.
*Corresponding author: gzdgrgn35@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this work was supported by the Scientific Research Project Department of our university with the decision number 20122016 (Selcuk University).
Received on January 13, 2024. Accepted on March 10, 2024.

extracellular matrix reorganization, oncogenesis, and inflammation[8-10]. Gene polymorphisms represent single base-pair variations within genomic DNA that differentiate normal individuals within a population. In certain instances, these polymorphisms can significantly impact the function of the protein encoded by the gene or alter enzymatic activity. Such genetic polymorphisms are responsible for individual variances in susceptibility to illness and can significantly influence how individuals respond to medical treatment. The *CHI3L1 gene* exhibits multiple polymorphisms, and these genetic variations may influence both the incidence and prognosis of inflammatory and neoplastic diseases[11]. The objective of this study is to investigate any potential correlation between preeclampsia and polymorphisms in the *CHI3L1 gene* and specifically focus on the influence of polymorphisms in the *CHI3L1 gene* on the incidence and prognosis of preeclampsia.

METHODS

Ethics approval

This prospective case-control study obtained the necessary ethical approval from the local ethics committee (Approval Number 2020/220, dated 13.05.2020).

Study design

The study was conducted between September 2020 and March 2021. It comprised 35 pregnant women diagnosed with preeclampsia who were admitted to our tertiary care center; these women constituted the study group (Group 1). The control group (Group 2) consisted of 40 healthy pregnant women. Preeclampsia was diagnosed based on elevated systolic blood pressure (≥140 mmHg) and/or diastolic blood pressure (≥90 mmHg), measured at least twice at 4-h intervals in otherwise healthy pregnant women, accompanied by one or more new-onset findings after the 20th week of gestation, such as proteinuria, evidence of other maternal organ dysfunction, or uteroplacental dysfunction[2]. Additionally, participants in Group 1 were further subdivided into severe and non-severe preeclamptic cases. The severity of the disease was assessed according to the recommendations of the American College of Obstetricians and Gynecologists[3]. Eligible participants for this study were females aged between 18 and 45 years with a singleton pregnancy. All patients were informed about the research objectives and procedures and signed an "Informed Voluntary Consent Form."

Exclusion criteria for the study included pregnant women with co-existing medical conditions such as diabetes mellitus, chronic hypertension, thromboembolism, thrombophilia, a history of liver or renal disease, structural or chromosomal fetal anomalies, multiple pregnancies, and gestational age less than 20 weeks. The control group comprised healthy pregnant women who did not present any additional features [e.g., placenta previa, intrauterine growth restriction (IUGR), and placental abruption] in their current or previous pregnancies.

Data, including age, gravidity, parity, blood pressure measurements, and complete blood count results [hemoglobin (Hb), white blood cell count (WBC), and platelets], were collected. Liver enzymes, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were also recorded for all study participants.

Genetic analysis

To analyze the *CHI3L1 gene* polymorphism in pregnant women, a 5 mL blood sample was collected from the antecubital brachial vein into an EDTA tube using a vacutainer. The blood samples were stored at +4°C until the completion of patient enrollment. The real-time polymerase chain reaction (PCR) technique was employed to study all the samples.

DNA isolation was performed using the DETAGEN Whole Blood DNA Isolation Kit (Detagen/Turkey). Following isolation, the densities (ng/µL) and absorption measurements (A260/280 ratio between 1.80 and 2.00) of the DNA samples were evaluated using a NanodropLite spectrophotometer (Thermo Scientific). The DETAGEN *CHI3L1 Gene* New Generation Sequencing Kit (Detagen/Turkey) was utilized for next-generation sequencing analysis. DNA purification was performed with AMPure XP Beads (Beckman Coulter, Indiana, USA), and sequencing was conducted on an Illumina MiSeq platform (Illumina, San Diego, California, USA).

The entire sequence of the *CHI3L1 gene* was screened in the serum of all participants. The distributions of both homozygous and heterozygous forms of the *CHI3L1 gene* polymorphisms were examined and compared between the two groups. Specifically, a total of 10 *CHI3L1 gene* polymorphisms were analyzed as follows:

c.433 A>G p.R145G (rs880633)
c.25+24 T>A (rs7515776)
c.-131 C>G (rs4950928)
c.56-19 T>C (rs1538372)
c.1092 T>C p.C364=(rs4950927)
c.55+32 C>T (rs111768615)
c.315-56 C>G (rs12410110)
c.257+5 G>A (rs201303588)
c.587+65 C>G (rs12409713)
c.894+9 G>T (no rs identification)

Statistical analysis

All statistical analyses were performed using the SPSS 20 statistical software package (SPSS Inc., Chicago, IL). The distribution of homozygous and heterozygous forms of the polymorphism was statistically compared in terms of age, gravida and parity, blood pressure, hemogram, WBC, serum biochemistry, and *CHI3L1 gene* polymorphism in the pregnant women in Groups 1 and 2. Cases with severe and non-severe preeclampsia in Group 1 were compared statistically with the control group and among themselves. Categorical data were compared with chi-square analysis, the Mann-Whitney U test was used to compare two groups of numerical data, and the Kruskal-Wallis H test was used to compare three or more groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

No statistically significant differences were observed between the two groups regarding age, gestational weeks, gravida, and parity. However, significant differences were identified in the levels of systolic blood pressure (SBP) and diastolic blood pressure (DBP) between the control and preeclamptic groups (refer to Table 1).

Among the nine analyzed *CHI3L1 gene* polymorphisms, only two showed statistically significant differences between the study groups: c.433 A>G p.R145G (rs880633) and c.25+24 T>A (rs7515776). For the remaining seven polymorphisms (rs4950928, rs1538372, rs4950927, rs111768615, rs12410110, rs201303588, rs12409713, and c.894+9 G>T), no significant differences were observed between the groups ($p>0.05$ for all).

Table 2 illustrates the distribution of homozygous and heterozygous forms of the c.433 A>G p.R145G (rs880633) polymorphism across groups. The prevalence of this polymorphism was 80% in the control group, 57.1% in the non-severe preeclampsia group, and 61.9% in the severe preeclampsia group. Notably, 8 out of 40 cases in the control group did not exhibit this polymorphism, representing 20% of the sample. The rate of homozygous polymorphisms was highest in severe preeclamptic cases (33.3%), compared with 17.5 and 21.4% in the control and non-severe groups, respectively. Conversely, the rate of heterozygous polymorphisms was higher in the control group (62.5% vs. 35.7 and 28.6%).

Table 1. Demographic variables and blood pressure of the cases in both groups.

	Control (n=40)	Preeclampsia			p1	p2
		All PE (n=35)	Non-severe (n=14)	Severe (n=21)		
Age, years	28.7±5.17	29.65±7.48	29.42±8.05	29.80±7.27	0.528	0.811
Gravida	2 (1–6)	3 (1–6)	1.5 (1–5)	3 (1–6)	0.498	0.295
Parity	1 (0.0–3)	1 (0.0–4)	0 (0–4)	2 (0–3)	0.690	0.261
Gestational weeks	32	32	32.2	32	0.369	0.608
SBP	110 (100–120)	160 (140–190)	140 (140–150)	160 (150–190)	<0.001*	<0.001*
DBP	70 (60–70)	100 (70–150)	90 (70–110)	100 (90–150)	<0.001*	<0.001*

SBP: systolic blood pressure; DBP: systolic blood pressure; p1: comparison of control group and preeclampsia patients; p2: comparison of control group, severe, and non-severe preeclampsia. *p<0.05 was accepted as statistically significant.

Table 2. Distribution of homozygous and heterozygous values of c.433 A>G p.R145G (rs880633) polymorphism between severe and non-severe preeclampsia group in preeclampsia and control group.

	Control (n=40)	Preeclampsia		p1	p2		OR 95%CI	p3	p4
		Non-severe (n=14)	Severe (n=21)						
No polymorphism	8 (20.0%)	6 (42.9%)	8 (38.1%)	0.026*	0.058		0.37 (0.13–1.04)	0.092	0.158
Polymorphism is present	32 (80.0%)	8 (57.1%)	13 (61.9%)						
Homozygous	7 (17.5%)	3 (21.4%)	7 (33.3%)						
Heterozygous	25 (62.5%)	5 (35.7%)	6 (28.6%)						

Data are given as n (%). p1=control and preeclampsia group with no polymorphism, comparison of homozygous and heterozygous; p2=control and preeclampsia group with no polymorphism comparing polymorphism exists; p3=control, severe, and non-severe preeclampsia group without polymorphism, comparison of homozygous and heterozygous; p4=control, comparison of severe and non-severe preeclampsia group with no polymorphism with polymorphism exists. *p<0.05 was accepted as statistically significant.

Regarding the c.25+24 T>A (rs7515776) polymorphism, its prevalence was lower in healthy pregnancies (65% vs. 35%), as depicted in Table 3. Within the preeclampsia cohort, the rates of this polymorphism were 57.1% for non-severe cases and 38.1% for severe cases. No instances of homozygous polymorphism were observed in the preeclamptic patients.

DISCUSSION

The primary finding of our study suggests that the polymorphism rs880633 in the *Chitinase-3-like protein 1 (CHI3L1) gene* may serve as a protective factor against preeclampsia. This is a pioneering study, as this is the first to explore the relationship between *CHI3L1 gene* polymorphisms and preeclampsia. Preeclampsia is a significant cause of maternal morbidity and mortality, and currently, no standard screening tests are available for its diagnosis, as indicated by previous studies[12,13].

CHI3L1, which is a biomarker involved in inflammation and tissue remodeling, plays a supportive role in angiogenesis, antiapoptosis, and cell proliferation[8-11]. Dina Nada et al. investigated the relationship of circulating YKL-40 levels and CHI3L1 variants with the risk of progression of scoliosis to spinal deformity in adolescent idiopathic scoliosis. It has been shown that the rs880633 polymorphism of the *CHI3L1 gene* is positively correlated with high YKL-40 levels and is protective against spinal deformity[14]. Huang et al. examined the polymorphisms of the *CHI3L1 gene* in patients with hepatocellular cancer and found a high rate of CHI3L1rs880633 polymorphism in patients with hepatocellular cancer[15]. CHI3L1 has been identified as a promoter of angiogenesis in neoplasms and has been shown to play a role in the activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway in endothelial cells[16,17]. CHI3L1 has been shown to modulate vascular endothelial cell morphology and stimulate migration. CHI3L1 plays a role in tumor angiogenesis[18]. In our study, CHI3L1rs880633 polymorphism was detected at a higher rate in the control group. This aligns with our observation that the rs880633 polymorphism was more prevalent in our control group, suggesting that it may confer protective effects, potentially mediated through angiogenesis.

El-Fattah et al. investigated the rs4950928 polymorphism of the *CHI3L1 gene* in patients with colorectal cancer (CRC). They could not find a significant relationship between CHI3L1 rs4950928 polymorphism and CRC[19]. Dai et al. investigated whether CHI3L1 polymorphisms and plasma level of protein are associated with Alzheimer's disease. This study showed that the CG+GG genotype of rs4950928 C>G is a protective factor for Alzheimer's disease and reduces the severity of Alzheimer's disease[20]. The CHI3L1 rs4950928 genotype was investigated in patients with glioblastoma (GBM), and no significant correlation was found between glioblastoma and the CHI3L1 rs4950928 genotype[20,21]. Our study found no significant relationship between *CHI3L1 gene* rs4950928 polymorphism and preeclampsia.

YKL-40 levels with CHI3L1 rs 1538372 polymorphism and its relationship to lung function were investigated. Tsai et al. found decreased lung functions in CHI3L1 rs1538372 CC carriers[22]. In our study, no significant relationship was found between the rs1538372 polymorphism of the *CHI3L1 gene* and preeclampsia.

The effects of rs7515776 polymorphism of *the CHI3L1 gene* and YKL-40 levels on the course of the disease were investigated in sarcoidosis patients[23]. There was no correlation between rs7515776 polymorphism and serum YKL-40 levels in sarcoidosis patients. This study observed that the rs7515776 polymorphism showed a higher heterozygosity rate in the preeclampsia group. However, the absence of homozygosity prevents us from attributing definitive clinical significance to this finding.

There are no studies in the literature associated with rs4950927, rs111768615, rs12410110, rs201303588, rs12409713, and c.894+9 G>T (no rs) polymorphisms of the *CHI3L1 gene*. In this

Table 3. Distribution of homozygous and heterozygous values of c.25+24T>A(rs7515776) polymorphism between severe and non-severe preeclampsia group in preeclampsia and control group.

	Control (n=40)	Preeclampsia			p1	p2	OR 95%CI	p3	p4
		All PE (n=35)	Non-severe (n=14)	Severe (n=21)					
No polymorphism	26 (65.0)	19 (54.3)	6 (42.9)	13 (61.9)	0.046*	0.345	1.56 (0.61–3.96)	0.109	0.339
Polymorphism is present	14 (35.0)	16 (45.7)	8 (57.1)	8 (38.1)					
Homozygous	4 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)					
Heterozygous	10 (25.0)	16 (45.7)	8 (57.1)	8 (38.1)					

Data are given as n (%). p1=control and preeclampsia group with no polymorphism, comparison of homozygous and heterozygous; p2=comparison of control and preeclampsia group with no polymorphism with polymorphism exists; p3=control, severe, and non-severe preeclampsia group with no polymorphism, comparison of homozygous and heterozygous; p4=control, comparison of severe and non-severe preeclampsia group with no polymorphism with polymorphism exists. *p<0.05 was accepted as statistically significant.

study, a significant correlation was not found between rs4950927, rs111768615, rs12410110, rs201303588, rs12409713, and c.894+9 G>T (no rs) polymorphisms of the *CHI3L1 gene* and preeclampsia.

One limitation of our research is the small sample size and the absence of detailed birth records and perinatal outcomes. These factors could have provided a more nuanced understanding of the clinical implications of these polymorphisms.

In summary, our study provides a promising direction for future research, suggesting that the rs880633 polymorphism in the *CHI3L1 gene* could act as a protective factor against preeclampsia. More comprehensive studies with larger sample sizes and a more extensive range of clinical data are required to confirm these initial findings.

AUTHORS' CONTRIBUTIONS

NM: Conceptualization, Software, Visualization, Writing – review & editing. **SÖ:** Data curation, Visualization. **BGÖ:** Formal Analysis, Supervision, Writing – original draft. **FA:** Funding acquisition, Validation. **NK:** Investigation. **EÇ:** Methodology. **GÖ:** Project administration. **ÇÇ:** Resources.

REFERENCES

1. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122-31. https://doi.org/10.1097/01.AOG.0000437382.03963.88
2. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. Pregnancy Hypertens. 2018;13:291-310. https://doi.org/10.1016/j.preghy.2018.05.004
3. Dekker GA, Geijn HP. Hypertensive disease in pregnancy. Curr Opin Obstet Gynecol. 1992;4(1):10-27. PMID: 1543821
4. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308(5728):1592-4. https://doi.org/10.1126/science.1111726
5. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10(8):466-80. https://doi.org/10.1038/nrneph.2014.102
6. Magee LA, Nicolaides KH, Dadelszen P. Preeclampsia. N Engl J Med. 2022;386(19):1817-32. https://doi.org/10.1056/NEJMra2109523
7. Thilaganathan B. Pre-eclampsia and the cardiovascular-placental axis. Ultrasound Obstet Gynecol. 2018;51(6):714-7. https://doi.org/10.1002/uog.19081
8. Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, et al. Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. J Biol Chem. 2003;278(45):44058-67. https://doi.org/10.1074/jbc.M306792200
9. Volck B, Price PA, Johansen JS, Sørensen O, Benfield TL, Nielsen HJ, et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Physicians. 1998;110(4):351-60. PMID: 9686683
10. Rehli M, Krause SW, Andreesen R. Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation. Genomics. 1997;43(2):221-5. https://doi.org/10.1006/geno.1997.4778
11. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr. 2004;79(6):935-45. https://doi.org/10.1093/ajcn/79.6.935
12. Peixoto-Filho FM, Costa FDS, Kobayashi S, Beitune PE, Garrido AG, Carmo AV, et al. Prediction and prevention of preeclampsia. Rev Bras Ginecol Obstet. 2023;45(1):49-54. https://doi.org/10.1055/s-0043-1763495
13. Jung E, Romero R, Yeo L, Gomez-Lopez N, Chaemsaithong P, Jaovisidha A, et al. The etiology of preeclampsia. Am J Obstet Gynecol. 2022;226(2S):S844-66. https://doi.org/10.1016/j.ajog.2021.11.1356
14. Nada D, Julien C, Rompré PH, Akoume MY, Gorman KF, Samuels ME, et al. Association of circulating YKL-40 levels and CHI3L1 variants with the risk of spinal deformity progression in adolescent idiopathic scoliosis. Sci Rep. 2019;9(1):5712. https://doi.org/10.1038/s41598-019-41191-4
15. Areshkov PO, Avdieiev SS, Balynska OV, Leroith D, Kavsan VM. Two closely related human members of chitinase-like family, CHI3L1 and CHI3L2, activate ERK1/2 in 293 and U373 cells but have the different influence on cell proliferation. Int J Biol Sci. 2012;8(1):39-48. https://doi.org/10.7150/ijbs.8.39
16. Antonelli M, Massimino M, Morra I, Garrè ML, Gardiman MP, Buttarelli FR, et al. Expression of pERK and pAKT in pediatric high grade astrocytomas: correlation with YKL40 and prognostic significance. Neuropathology. 2012;32(2):133-8. https://doi.org/10.1111/j.1440-1789.2011.01252.x
17. Pelloski CE, Lin E, Zhang L, Yung WK, Colman H, Liu JL, et al. Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. Clin Cancer Res. 2006;12(13):3935-41. https://doi.org/10.1158/1078-0432.CCR-05-2202
18. Huang WS, Lin HY, Yeh CB, Chen LY, Chou YE, Yang SF, et al. Correlation of Chitinase 3-Like 1 single nucleotide polymorphisms with hepatocellular carcinoma in Taiwan. Int J Med Sci. 2017;14(2):136-42. https://doi.org/10.7150/ijms.17754
19. El-Fattah AA, Sadik NA, Shaker OG, Kamal AM. Are SMAD7 rs4939827 and CHI3L1 rs4950928 polymorphisms associated with colorectal cancer in Egyptian patients?. Tumour Biol. 2016;37(7):9387-97. https://doi.org/10.1007/s13277-016-4813-8
20. Dai QH, Gong DK. Association of the polymorphisms and plasma level of CHI3L1 with Alzheimer's disease in the Chinese han population: a case-control study. Neuropsychobiology. 2019;77(1):29-37. https://doi.org/10.1159/000492536
21. Holst CB, Christensen IJ, Skjøth-Rasmussen J, Hamerlik P, Poulsen HS, Johansen JS. Systemic immune modulation in gliomas: prognostic value of plasma IL-6, YKL-40, and genetic variation in YKL-40. Front Oncol. 2020;10:478. https://doi.org/10.3389/fonc.2020.00478
22. Tsai Y, Ko Y, Huang M, Lin M, Wu C, Wang C, et al. CHI3L1 polymorphisms associate with asthma in a Taiwanese population. BMC Med Genet. 2014;15:86. https://doi.org/10.1186/1471-2350-15-86
23. Kruit A, Grutters JC, Ruven HJ, Moorsel CC, Bosch JM. A CHI3L1 gene polymorphism is associated with serum levels of YKL-40, a novel sarcoidosis marker. Respir Med. 2007;101(7):1563-71. https://doi.org/10.1016/j.rmed.2006.12.006

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231614

The investigation of relationship between serum melatonin levels with Beck Depression Inventory and Beck Scale for Suicidal Ideation in suicide patients

Aynur Yurtseven[1], Cemil Kavalci[2*], Yasemin Yilmaz Aydin[1], Kemal Aydin[1], Ömer Faruk Demir[1], Şeyda Özdemir[3], Gülsüm Kavalci[4]

OBJECTIVE: Melatonin plays a role in many biological and physiological events. There are studies in the literature relating melatonin levels to many psychiatric disorders such as schizophrenia, bipolar disorder, and major depressive disorder. We aimed to investigate the relationship between serum melatonin levels with the Beck Depression Inventory and the Beck Scale for Suicidal Ideation in suicide patients.

METHODS: The study was conducted prospectively with volunteer patients aged 20–50 years who were admitted to the emergency department after a suicide attempt. The social and occupational status, educational levels, marital status, and stressor factors of patients were questioned. Beck Depression Inventory and Beck Scale for Suicidal Ideation were applied to each patient included in the study. Blood melatonin levels were evaluated using the enzyme-linked immunosorbent assay method. The data were analyzed with the SPSS 23.00 statistical program. Descriptive values were expressed by the number of cases (n), percentage (%), median (interquartile range), and mean±standard deviation. The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables, and the Pearson or Spearman correlation test was used to assess the relationship between disease severity and melatonin level. A value of $p<0.05$ was considered statistically significant.

RESULTS: No statistically significant correlation was found between melatonin level and the Beck Depression Inventory score ($r=-0.098$, $p=0.44$). However, a statistically weak, inverse, and significant correlation was discovered between melatonin levels and the Beck Scale for Suicidal Ideation score ($r=-0.465$, $p=0.00$).

CONCLUSION: According to our results, it was determined that there was a significant negative relationship between melatonin level and the Beck Scale for Suicidal Ideation scoring.

KEYWORDS: Emergency. Depression. Suicide. Melatonin.

INTRODUCTION

Instances of suicide exhibit a wide range of clinical manifestations, which -can vary significantly, and in severe situations, may lead to fatality[1]. Based on data provided by the World Health Organization (WHO), the annual mortality resulting from suicide attempts exceeds 700,000 individuals, with a yearly escalation rate ranging from 10 to 20 times[2]. Suicide has been shown to account for 8.5% of fatalities in those aged 15–29 years, making it the world's second leading cause of death in this age group[3]. With the continuous growth of the global population, the emergence of suicide cases as a significant health issue has become an unavoidable reality.

The Beck Depression Inventory (BDI) is a psychometric instrument including 21 questions that have been systematically organized to assess various manifestations of depressive symptoms[4]. Numerous research studies have established a correlation between depression and suicide, with a crucial emphasis on the potential influence of emotions of despair, drawing upon the findings of prior research that assert the limited clarity of the BDI in assessing suicide risk.

The BSSI was created by Beck et al. in 1979 specifically for the assessment of individuals who had made suicide attempts[5]. The BSSI is a self-report instrument that utilizes the semi-structured interview format, drawing on the Scale for Suicidal Ideation[5]. The BSSI is a tool consisting of 21 items, especially designed for adult patients with psychiatric symptoms, and aims to evaluate individuals' levels of active suicidal ideation, passive suicide desire, and preparation phases[5].

Melatonin, which is scientifically referred to as N-acetyl 5-methoxy tryptamine, is a hormone that is produced and released

[1]Dışkapı Yıldırım Beyazıt Training and Research Hospital, Department of Emergency – Ankara, Turkey.
[2]Health Science of Turkey, Antalya Training and Research Hospital, Department of Emergency – Antalya, Turkey.
[3]Dışkapı Yıldırım Beyazıt Training and Research Hospital, Department of Biochemistry – Ankara, Turkey.
[4]Antalya Training and Research Hospital, Department of Anesthesiology – Antalya, Turkey.
*Corresponding author: cemkavalci@yahoo.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 22, 2024. Accepted on March 03, 2024.

by the pineal gland[6]. Melatonin is a hormone that plays a significant role in several biochemical and physiological processes inside the human body[6]. Melatonin, which is a hormone responsible for regulating the body's circadian rhythm, has been found to possess several therapeutic qualities including sleep regulation, antidepressant effects, anxiety reduction, neuroprotection, anti-inflammatory activity, and analgesic capabilities[7]. Various factors, including light exposure, medication use, endocrine diseases, and hormone levels, might potentially influence the amounts of melatonin in the blood plasma, thus impacting the circadian rhythm. The diurnal variation of melatonin levels in the bloodstream is also seen. The circadian rhythm, which is mostly secreted during nocturnal hours, experiences disruption among those engaged in shift work[8]. Psychotropic medications have the potential to influence the sleep–wake cycle and biorhythms, such as body temperature and hormone levels, by modulating the oscillation between states of sleep and alertness. Several studies have demonstrated the impact of lithium, imipramine, valproate, fluoxetine, selective serotonin reuptake inhibitors (SSRIs), and medications belonging to the opiate category on circadian rhythm[9]. Oral contraceptives have the potential to modify melatonin plasma concentrations through their inhibitory action on CYP1A2[10]. The observation that hypothyroidism results in a notable reduction in plasma melatonin levels implies a potential association between thyroid diseases and the pineal gland[11].

According to the reports, a decline in melatonin levels is observed in individuals with sleep difficulties, depression[12,13], and suicide[13-15]. Arioz et al. [16] have demonstrated that the administration of melatonin is associated with a reduction in depression-like behaviors. Melatonin is employed as a therapeutic intervention for the management of depression and sleep disturbances, as supported by scientific literature[17-19]. According to the findings of Leone et al. [20], it was observed that the administration of melatonin resulted in a reduction in the likelihood of self-harm among individuals in the young age group. The findings presented in this study indicate a potential association between the hormone melatonin and certain psychiatric disorders. The investigation of the association between melatonin levels and suicide remains limited in current research.

The objective of this study was to examine the correlation between serum melatonin levels and the BSSI and BDI scores in individuals who had attempted suicide.

METHODS

The design

The study was conducted in a prospective, observational manner after the consent of the ethical committee (decision no. 12/10, dated 31.05.2021) in the Emergency Department of Ankara Diskapi Yildirim Beyazit Training and Research Hospital. The study was conducted in accordance with the Helsinki Declaration. We obtained informed permission forms from the patients who volunteered to participate. The power analysis was conducted before the study using the G*power 3.1.9.4 software tool. The sample size required to achieve 80% statistical power was determined to be 63 patients, assuming a medium effect size and a significance level of $p<0.05$.

Participants

The study enrolled volunteer patients who were at least 18 years of age and had been admitted to the emergency department between the hours of 08:00 and 16:00. These patients had a history of using numerous medicines for the goal of self-harm. Blood samples were collected during the initial hour of admission to our department, with meticulous attention given to guaranteeing patient well-being.

The exclusion criteria were as follows:

- People who use drugs or refuse to take part in the study;
- Those having thyroid dysfunction;
- Those working in shifts, and taking melatonin or similar drugs, immunosuppressant drugs, and chemotherapy for cancer diagnosis;
- Those having a sleep problem, taking medical help and antipsychotics, and having had a mental diagnosis in the past;
- Those using oral contraceptive pills;
- Those having Cushing's syndrome and Addison's disease;
- Those who had radiation therapy for the pineal gland earlier.

The participants in the research were surveyed on their social and occupational standing, educational attainment, marital status, reproductive history, and sources of stress. The BDI and the BSSI were administered to each participant enrolled in the research within the emergency department, under the guidance of a psychiatrist. The rationale for concurrently using both measures lies in the literature's observation that the BDI scale does not consistently provide accurate predictive capabilities for suicide attempts.

Determination of serum melatonin levels

To assess the melatonin level, a total of 5 mL of venous blood samples were obtained from patients upon their arrival. These samples were then carefully put in a standard vacuum tube. Subsequently, the serum samples were subjected to centrifugation at a speed of 1,000 revolutions per minute for a duration of 15 min, all within a time frame of

30 min. Finally, the centrifuged serum samples were kept at a temperature of -70°C to facilitate further analysis. The assessment of melatonin levels was conducted utilizing the enzyme-linked immunosorbent assay (ELISA) technique. Before conducting the test, essential calibration investigations were performed. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold standard for melatonin measurement due to its high sensitivity and accuracy. In the context of comparing the LC-MS/MS assay and ELISA for the measurement of melatonin levels, the ELISA test was selected due to the observed satisfactory concordance within the lower range (<30 pmol/L). However, it is worth noting that for melatonin levels beyond 30 pmol/L, the ELISA assay yielded considerably lower measurements compared with the LC-MS/MS assay[19].

Statistical analysis

The data were analyzed using the IBM SPSS Statistics 23.0 software package. The descriptive data in this study were represented by the number of instances (n) and percentage (%), as well as the median and interquartile range (IQR). The distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Intergroup comparisons were assessed using the Mann-Whitney U and Kruskal-Wallis tests. The Spearman correlation test was utilized to examine the association between the BDI, BSSI, and the level of blood melatonin. A $p<0.05$ value was considered statistically significant.

RESULTS

The research comprised 63 patients who satisfied the criteria. The median age of the patients was 29 years[15], and 65.1% of the patients were female. The study revealed that there was no statistically significant disparity in melatonin levels across genders ($p>0.05$) (Table 1). Only 12.7% of the patient population have a higher level of education. The demographic details of the patients are presented in Table 1.

In the study, it was found that 76.4% (n=50) of participants reported using numerous types of drugs. Additionally, 19.1% (n=12) reported using only one type of drug. A small percentage, i.e., 1.6% (n=1), engaged in jumping from a height following drug use. Furthermore, 3.2% (n=2) reported engaging

Table 1. Melatonin levels based on variables.

Variables		n (%)	Melatonin	p-value
Gender	Male	22 (34.9)	148.38 (946.5)	0.63
	Female	41 (65.1)	203.76 (228.7)	
Chronic disease	Yes	9 (14.3)	122.43 (64.9)	0.03*
	No	54 (85.7)	218.44 (495.20)	
Educational status	No	1 (1.6)	102.55	0.63
	Elementary school	16 (25.4)	210.38 (409.4)	
	Middle school	12 (19)	149.17 (1,508.9)	
	High school	26 (41.3)	217.20 (323.5)	
	University	8 (12.7)	170.84 (236.7)	
Marital status	Single	26 (41.3)	218.44 (393.5)	0.73
	Divorced	6 (9.5)	192.98 (295.9)	
	Married	31 (49.2)	178.92 (418.3)	
Children	Yes	38 (60.3)	179.78 (413.6)	0.34
	No	25 (39.7)	223.18 (515.6)	
Mood	Anxious	9 (14.3)	196.55 (629.3)	0.63
	Depressed	54 (85.7)	210.80 (333.6)	
Stressor factor	Familial	46 (73)	184.73 (385.8)	0.28
	Environmental	17 (27)	243.38 (904.7)	
Thought content	Regular	23 (36.5)	210.38 (279.9)	0.83
	Regret	28 (44.4)	196.85 (698.7)	
	Stressor factor	12 (19)	207.73 (869.7)	
Hospitalization status	Discharged	43 (68.3)	243.38 (708.6)	0.088
	Ward	7 (11.1)	157.18 (1,144.8)	
	ICU	13 (20.6)	149.17 (76.1)	

*Statistical significance.

in suicidal self-mutilation, while another 1.6% (n=1) reported consuming rat poison.

The findings of this study indicate a statistically significant decrease in blood melatonin levels in those with chronic disorders compared with those without chronic diseases ($p<0.05$). The blood melatonin levels, as influenced by various circumstances, are presented in Table 1.

A significant correlation was found between familial stress variables and suicide in 73% of the individuals under observation. The study revealed that a significant majority of the patients, i.e., 85.7%, had symptoms of depression as seen in their emotional state. Upon analyzing the blood melatonin level in relation to marital status, it was determined that there was no statistically significant difference ($p>0.05$).

The median BDI score was 25[13], whereas the median BSSI score was 10[9]. The median value of melatonin level was found to be 197.15 (379.3). A statistically significant correlation was observed between the BDI and the BSSI score, indicating a weak positive relationship ($r=0.423$, $p<0.05$).

The Spearman correlation test was employed to ascertain the presence of an association between melatonin levels and the BDI as well as the BSSI. The results of the study indicate that there is no statistically significant relationship between melatonin levels and BDI scores ($r=-0.098$, $p>0.05$) (see Figure 1). Nevertheless, an analysis of the data revealed a statistically significant, moderate, and inverse association between melatonin levels and the BSSI ($r=-0.511$, $p<0.05$) (see Figure 2). It was observed that there was a positive correlation between the BSSI and the reduction in melatonin levels.

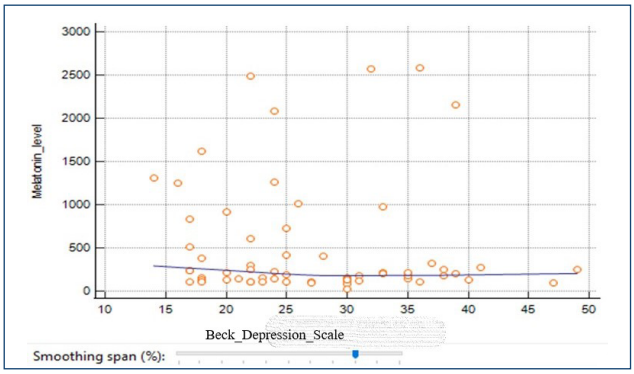

Figure 1. Relationship between Beck Depression Inventory and blood melatonin levels.

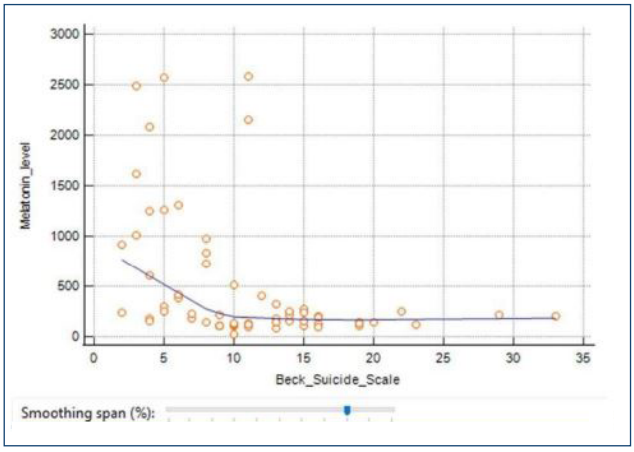

Figure 2. Relationship between Beck Suicide Idea Scale and blood melatonin levels.

DISCUSSION

Melatonin (N-acetyl-5-methoxytryptamine), which has both hydrophilic and lipophilic affinities, is produced by the pineal gland and easily diffuses into membranes, cytoplasm, nucleus, and mitochondria and can be used safely even at high doses[21,22]. Melatonin is involved in many physiological events, from the regulation of the sleep–wake cycle and circadian rhythms in the body, neural development, jet lag treatment, and improvement of the cardiovascular system to the regulation of the immune system and endocrine functions[23,24]. Melatonin is effective in the synthesis and release of hypothalamic gonadotropin-releasing hormone (GnRH) and is involved in the regulation of gonadal physiological responses[25]. Evidence for sex differences in melatonin levels has been reported in many previous studies and has been exhibited in women, suggesting that hormonal contraceptive pills may be a reason for the significant variability in women. Indeed, there is evidence that the use of oral contraceptive pills increases melatonin levels, although many studies do not consider female hormonal status[26]. In this study, in which OCS use was stopped in line with the information in the literature, it was observed that there was no statistically significant difference in melatonin levels between genders ($p>0.05$).

The findings collected from the study indicate that there is no statistically significant association between the outcomes of the BDI and blood melatonin levels. However, when the relationship between melatonin concentration in the blood and the BSSI score was examined, it was determined that there was a negative correlation, and among individuals with suicidal behavior, those with high blood melatonin levels received lower scores on the BSSI ($r=-0.511$, $p<0.05$).

Previous research has suggested that melatonin therapy is particularly effective in addressing sleep disturbances associated with psychiatric conditions, including anxiety and depression, and may even mitigate the risk of suicide[27,28]. Rao et al. [12]

have shown a reduction in melatonin levels among those with depression and those who have attempted suicide. According to Leone et al. [29], the likelihood of suicide attempts in early adolescence was shown to be highest prior to melatonin medication but decreased following the treatment. In their study, Høier et al. examined the association between the use of melatonin and suicidal behavior. The results indicated that those who were prescribed melatonin exhibited a suicide rate that was four times higher and a risk of initial suicide attempt that was five times greater in comparison with those who did not get melatonin prescription[30]. The study encompassed a substantial population sample in Denmark, yielding valuable insights into various psychiatric and sleep disorders linked to suicidal behaviors. However, the fact that almost all of the individuals who exhibited suicidal behavior and received melatonin treatment also had a history of an accompanying mental disorder was seen as a limitation of the study. This study was designed by reviewing previous studies and current literature information, and many factors that could affect blood melatonin levels were excluded from the study.

In this investigation, it was shown that a negative correlation existed between the concentration of melatonin and the BSSI score. There is a positive correlation between the reduction in melatonin levels and the increase in suicidal thoughts. Reduced levels of melatonin have been associated with sleep disruptions and the potential to elicit suicidal ideation.

CONCLUSION

According to our findings, there is an inverse relationship between the melatonin level and BSSI. As the melatonin level decreases, the BSSI score in creases. In line with the information obtained from this study, it is recommended to start exogenous melatonin treatment before an active suicidal attempt occurs in individuals who exhibit suicidal thoughts and decrease endogenous melatonin levels. We think that at least some of the suicide attempts can be prevented with exogenous melatonin treatment.

Limitations of the study

In this investigation, individuals with comorbid neuropsychiatric conditions undergoing treatment, engaging in shift work, experiencing sleep disturbances, or using drugs known to potentially induce sleep disturbances were excluded from participation. This study aimed to address the inadequacies and limitations identified in prior research, thus making a valuable contribution to the existing body of knowledge due to its accessibility.

INFORMED CONSENT

Written informed consent was obtained from all subjects before the study.

ETHICAL APPROVAL

Ethical approval for this study was obtained from Dişkapi Yildirim Beyazıt Training and Research Hospital (31.05.2021, 112/10). This study was performed according to the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

AY: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – original draft. **CK:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Supervision, Writing – review & editing. **KA:** Formal Analysis, Supervision, Writing – review & editing. **YYA:** Investigation, Validation, Writing – original draft. **ÖFD:** Data curation, Formal Analysis, Supervision, Writing – review & editing. **ŞÖ:** Formal Analysis, Methodology, Validation. **GK:** Conceptualization, Formal Analysis, Writing – original draft.

REFERENCES

1. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003;301(5631):386-9. https://doi.org/10.1126/science.1083968
2. Bryleva EY, Brundin L. Kynurenine pathway metabolites and suicidality. Neuropharmacology. 2017;112(Pt B):324-30. https://doi.org/10.1016/j.neuropharm.2016.01.034
3. Bachmann S. Epidemiology of suicide and the psychiatric perspective. Int J Environ Res Public Health. 2018;15(7):1425. https://doi.org/10.3390/ijerph15071425
4. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4(6):561-71. https://doi.org/10.1001/archpsyc.1961.01710120031004
5. Beck AT, Kovacs M, Weissman A. Assessment of suicidal intention: the scale for suicide ideation. J Consult Clin Psychol. 1979;47(2):343-52. https://doi.org/10.1037//0022-006x.47.2.343
6. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. Sleep Med Rev. 2005;9(1):11-24. https://doi.org/10.1016/j.smrv.2004.08.001
7. Mahmood D. Pleiotropic effects of melatonin. Drug Res (Stuttg). 2019;69(2):65-74. https://doi.org/10.1055/a-0656-6643

8. Grosswald B. The effect of shift work on the family satisfaction. J Contemp Soc Ser. 2004;85(3):413-23. https://doi.org/10.1177/104438940408500320
9. Selvi Y, Be□iro□lu L, Aydın A. Chronobiology and mood disorders. Curr Approach Psychiatr. 2011;3(3):368-86.
10. Gunn PJ, Middleton B, Davies SK, Revell VL, Skene DJ. Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. Chronobiol Int. 2016;33(1):39-50. https://doi.org/10.3109/07420528.2015.1112396
11. Baltaci AK, Mogulkoc R. Leptin, neuropeptide Y (NPY), melatonin and zinc levels in experimental hypothyroidism and hyperthyroidism: relation with melatonin and the pineal gland. Horm Mol Biol Clin Investig. 2018;34(3):20180009. https://doi.org/10.1515/hmbci-2018-0009
12. Rao AV, Parvathi DS, Kota RT, Prabakar ER, Baskaran S, Swaminathan R, et al. Depression, cognitive disorder and melatonin profile. Ann Natl Acad Med Sci (India). 1988;25:41-9.
13. Rao VA. Toward suicide prevention. Indian J Psychiatry. 1999;41(4):280-8. PMID: 21430799
14. Mann JJ. Neurobiology of suicidal behaviour. Nat Rev Neurosci. 2003;4(10):819-28. https://doi.org/10.1038/nrn1220
15. Kurtulus Dereli A, Demırci GN, Dodurga Y, Özbal S, Cankurt U, Boz B, et al. Evaluation of human pineal gland acetylserotonin O-methyltransferase immunoreactivity in suicide: a preliminary study. Med Sci Law. 2018;58(4):233-8. https://doi.org/10.1177/0025802418797178
16. Arioz BI, Tastan B, Tarakcioglu E, Tufekci KU, Olcum M, Ersoy N, et al. Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway. Front Immunol. 2019;10:1511. https://doi.org/10.3389/fimmu.2019.01511
17. Boafo A, Greenham S, Sullivan M, Bazaid K, Suntharalingam S, Silbernagel L, et al. Medications for sleep disturbance in children and adolescents with depression: a survey of Canadian child and adolescent psychiatrists. Child Adolesc Psychiatry Ment Health. 2020;14:10. https://doi.org/10.1186/s13034-020-00316-8
18. Bock DE, Roach-Fox E, Seabrook JA, Rieder MJ, Matsui D. Sleep-promoting medications in children: physician prescribing habits in Southwestern Ontario, Canada. Sleep Med. 2016;17:52-6. https://doi.org/10.1016/j.sleep.2015.10.003
19. Jan JE, O'Donnell ME. Use of melatonin in the treatment of paediatric sleep disorders. J Pineal Res. 1996;21(4):193-9. https://doi.org/10.1111/j.1600-079x.1996.tb00286.x
20. Leone M, Kuja-Halkola R, Lagerberg T, Bjureberg J, Butwicka A, Chang Z, et al. Melatonin use and the risk of self-harm and unintentional injuries in youths with and without psychiatric disorders. J Child Psychol Psychiatry. 2023;64(7):1027-36. https://doi.org/10.1111/jcpp.13785
21. Reiter RJ, Tan DX, Manchester LC, Paredes SD, Mayo JC, Sainz RM. Melatonin and reproduction revisited. Biol Reprod. 2009;81(3):445-56. https://doi.org/10.1095/biolreprod.108.075655
22. Wang F, Tian X, Zhang L, Tan D, Reiter RJ, Liu G. Melatonin promotes the in vitro development of pronuclear embryos and increases the efficiency of blastocyst implantation in murine. J Pineal Res. 2013;55(3):267-74. https://doi.org/10.1111/jpi.12069
23. Sewerynek E. Melatonin and the cardiovascular system. Neuro Endocrinol Lett. 2002;23(Suppl 1):79-83. PMID: 12019357
24. Amaral FGD, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. Arch Endocrinol Metab. 2018;62(4):472-9. https://doi.org/10.20945/2359-3997000000066
25. Pevet P, Challet E. Melatonin: both master clock output and internal time-giver in the circadian clocks network. J Physiol Paris. 2011;105(4-6):170-82. https://doi.org/10.1016/j.jphysparis.2011.07.001
26. Kostoglou-Athanassiou I, Athanassiou P, Treacher DF, Wheeler MJ, Forsling ML. Neurohypophysial hormone and melatonin secretion over the natural and suppressed menstrual cycle in premenopausal women. Clin Endocrinol (Oxf). 1998;49(2):209-16. https://doi.org/10.1046/j.1365-2265.1998.00504.x
27. Xie S, Fan W, He H, Huang F. Role of melatonin in the regulation of pain. J. Pain Res. 2020;13:331-43. https://doi.org/10.2147/JPR.S228577
28. Cummins TM, English O, Minnis H, Stahl D, O'Connor RC, Bannister K, et al. Assessment of somatosensory function and self-harm in adolescents. JAMA Netw Open. 2021;4(7):e2116853. https://doi.org/10.1001/jamanetworkopen.2021.16853
29. Leone M, Kuja-Halkola R, Lagerberg T, Bjureberg J, Butwicka A, Chang Z, et al. Melatonin use and the risk of self-harm and unintentional injuries in youths with and without psychiatric disorders. J Child Psychol Psychiatry. 2023;64(7):1027-36. https://doi.org/10.1111/jcpp.13785
30. Høier NK, Madsen T, Spira AP, Hawton K, Jennum P, Nordentoft M, et al. Associations between treatment with melatonin and suicidal behavior: a nationwide cohort study. J Clin Sleep Med. 2022;18(10):2451-8. https://doi.org/10.5664/jcsm.10118

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231673

A new insight into the pathway behind spontaneous recurrent pregnancy loss: decreased CYR61 gene expression

Fahri Burcin Firatligil[1*], Burcu Firatligil Yildirir[2,3], Ozden Yalcin-Ozuysal[3]

ABSTRACT

OBJECTIVE: Investigating the potential role of CYR61 in recurrent pregnancy loss is critical for developing diagnostic approaches and treatments for recurrent pregnancy loss.

METHODS: In this prospective case-control study, we have investigated the expression patterns of CYR61 in blood samples from participants with recurrent pregnancy loss in their medical history and control group (n=20 vs n=10). Peripheral blood mononuclear cells from study and control groups were isolated and the expression patterns of the CYR61 gene were determined by real-time semi-quantitative reverse transcriptase PCR.

RESULTS: A significant decrease in CYR61 gene expression was demonstrated in patients with two or more clinically recognized miscarriages compared with patients without miscarriages or with a history of miscarriage ($p<0.01$), which may make the CYR61 gene a potential candidate for predicting the risk of recurrent pregnancy loss.

DISCUSSION: This study provides a basis for a detailed investigation of candidate biomarkers and molecular players involved in the development of recurrent pregnancy loss and for the development of potential treatment approaches to prevent recurrent pregnancy loss.

KEYWORDS: Cytogenetics. Miscarriage. Molecular genetics.

INTRODUCTION

Spontaneous abortion is the loss of a pregnancy that occurs before 20 weeks of gestation or when the fetus weighs less than 500 g[1,2]. Recurrent pregnancy loss (RPL) is the occurrence of two or more clinically established pregnancy losses within 20 weeks of conception[3]. RPL can be seen in approximately 3–5% of pregnancies[4], which has significant negative public health implications and can be both emotionally and physically traumatizing for any couple[3]. Two types of unexplained RPLs have been identified: Type I and Type II RPL. Type I unexplained RPL is predominantly seen in women with no known underlying pathology, while Type II unexplained RPL occurs because of existing pathology that was not previously identified by routine clinical examination[5]. This type has a worse prognosis than women of similar age with unexplained type I RPL. Although the underlying cause remains unknown in approximately 50% of RPL cases, recent studies have found that most RPL cases can be associated with multiple causes, including heredity, parental age, antiphospholipid syndrome, uterine abnormalities, thrombosis, spermatogenesis, hormone metabolic or autoimmune disorders, psychological-environmental factors, and abnormalities of vascular system development and/or implantation[3,6,7].

Vascular system development and implantation play a crucial role in embryogenesis and placental formation[8,9]. The vascular system consists of two pathways: the first is vasculogenesis, which is the formation of blood vessels in situ from angioblasts, whereas the second is angiogenesis, which is the formation of blood vessels by sprouting from pre-existing vessels[8,10]. Implantation is considered the second step in embryonic development after fertilization. In this process, the adhesion of the blastocyst to the endometrial surface is followed by its invasion through the epithelial basement membrane of the endometrium to form the placenta[11]. Therefore, any problem observed in these steps may lead to various serious problems, such as preeclampsia, intrauterine fetal demise, fetal growth restriction, spontaneous abortion, and RPL[10,11].

CYR61 is cysteine-rich protein 61, which belongs to the CCN family and is an extracellular matrix-associated angiogenic inducer that acts as a ligand of integrins to promote cell adhesion, migration, and proliferation during placental development[8]. During embryogenesis, CYR61 is identified as one of the important factors secreted by various cell types, particularly trophoblasts, which form the outermost membrane of a blastocyst[12]. These cells play

[1]Ankara Etlik City Hospital, Department of Obstetrics and Gynecology, Division of Perinatology – Ankara, Turkey.
[2]Tampere University, Faculty of Engineering and Natural Sciences – Tampere, Finland.
[3]Izmir Institute of Technology, Department of Molecular Biology and Genetics – Izmir, Turkey.
*Corresponding author: md.fahri@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 15, 2024. Accepted on March 03, 2024.
This case-control study was prospectively conducted in the Perinatology Department of Etlik Zubeyde Hanim Women's Health Education and Training Hospital and the Department of Molecular Biology and Genetics of Izmir Institute of Technology between March 2019 and March 2022.

an important role in providing nutrients and regulating blood flow to the embryo, thus forming an efficient maternal–fetal vascular relationship[12]. Because CYR61 is one of the major players in trophoblast invasion during placental development, it may be a functionally important biomarker for the development of a successful pregnancy, so its downregulation may cause RPL.

In this study, we hypothesized that reduced CYR61 levels are associated with RPL. We analyzed the expression patterns of CYR61 in blood samples from participants with RPL in their medical history. Investigating the potential role of CYR61 in RPL is critical for developing diagnostic approaches and treatments for RPL.

METHODS

This case-control study was prospectively conducted in the Perinatology Department of Etlik Zubeyde Hanim Women's Health Education and Training Hospital and the Department of Molecular Biology and Genetics of Izmir Institute of Technology between March 2019 and March 2022. This study was conducted in accordance with the principles of the Declaration of Helsinki, and the local ethics committee of Dokuz Eylul University of Izmir provided its approval (May 26, 2016; no: 2016/14-39).

Inclusion–exclusion criteria

Screening for RPL was based on the consensus of The American College of Obstetricians and Gynecologists (ACOG)[13] and the European Society of Human Reproduction (ESHRE)[14].

Recurrent pregnancy loss was defined as two or more pregnancy losses before 20 weeks of gestation. The RPL group consists of RPLs with a diagnosis of missed abortion, anembryonic pregnancy, or inevitable abortion. Healthier or low-risk pregnancies (one or no abortion) were included in the control group. Pregnant women were excluded from the study if they had any of the following risk factors or conditions: uterine morphological pathologies; comorbid maternal diseases (e.g., endocrine disease, rheumatological disease, and autoimmune disorders); inflammatory conditions (presence of autoimmune disease and/or chronic inflammatory disease; the presence of infection (especially urinary tract infection, upper or lower respiratory tract infection, pelvic inflammatory disease, and active or inactive coronavirus infection, etc.); and any condition that may compromise the immune system (e.g., use of corticosteroids, antioxidants or anti-inflammatory drugs, smoking, liver and/or kidney disease, cancer, organ, or bone marrow transplant).

Data

A total of 30 participants were included in our study: 20 of them were included in the study group, while the rest were included in the control group. The data of both groups such as demographic information (age, gravidity, and parity), anthropometric parameters of the participants [height and weight to determine body mass index (BMI)], and smoking status were obtained from the medical history of patients.

Study design

Women with RPL who received outpatient or inpatient care and whose pregnancy ended at our hospital were included in the study group (group I) (n=20). Healthy pregnant women with low risk, who had one or no abortion in the past, and whose pregnancy ended in the same hospital were included in the control group (group II) (n=10).

After written informed consent was obtained from the participants, 5 mL of venous blood was collected from each patient with the anticoagulant ethylenediaminetetraacetic acid (EDTA) to prevent blood clotting. In the study group, venous blood samples were taken on the 20th day after the end of pregnancy or after puerperium. In the control group, venous blood samples were taken after the puerperium or on the 20th day of pregnancy. Transportation of the blood samples was in accordance with the regulations of the Department of Transportation (DOT) and the International Air Transport Association (IATA).

Peripheral blood mononuclear cell isolation

Peripheral blood samples from 20 patients and 10 control groups were used in the study. Mononuclear cells from human blood samples were isolated using density gradient centrifugation and Ficoll-Paque solution. Blood samples were diluted with 2–4 times the volume of cold 1× PBS buffer and mixed well by pipetting up and down. The diluted blood suspension was then carefully layered over 10–15 mL of Ficoll-Paque in a 50-mL Falcon tube and centrifuged at 400×g for 40 min at RT without brake. Later, the upper layer was aspirated leaving the cell layer undisturbed at the interphase. The mononuclear cell layer was then carefully transferred to a new 50-mL Falcon tube, and the tube was filled with cold 1× PBS buffer, mixed, and centrifuged at 300×g for 10 min at RT. After the supernatant was carefully removed completely, the cell pellet was resuspended with 1× PBS and centrifuged at 200×g for 10 min at RT. This step was repeated twice, and the cell pellet was resuspended with lysis buffer containing beta-mercaptoethanol to proceed with RNA isolation.

RNA isolation and real-time semi-quantitative reverse transcriptase PCR

RNA isolation was performed according to the protocol of the Pure Link RNA Mini Kit. cDNAs were synthesized from 1 µg total RNAs using the Revert Aid First Strand cDNA Synthesis

Kit (K1622, Thermo-Fisher Scientific, USA). The mRNA levels were analyzed by real-time semi-quantitative reverse transcriptase PCR (RT-qPCR) using the Fast Start Essential DNA Green Master Kit (06402712001, Roche) on the Light Cycler® 96 instrument. Relative expression levels of the CYR61 gene were calculated by the Delta-Ct method using human TATA box binding protein (TBP) as the housekeeping gene. Non-template controls were also included in each condition.

Statistical analysis

The demographic and clinical characteristics of patients were summarized with means, standard deviations, and median values to provide a description of the patients. Gene expression values and differences in demographic and clinical characteristics between patient groups were analyzed using a two-tailed t-test, and a $p<0.05$ was considered statistically significant.

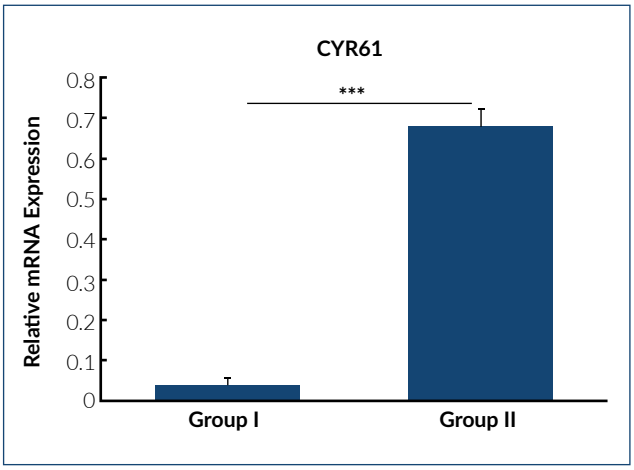

Figure 1. The expression profile of CYR61 in patients in both groups. The relative mRNA expression levels of the CYR61 gene are shown (experiments were performed in triplicate and repeated thrice). ***p<0.01.

RESULTS

The demographic and clinical characteristics of the study groups (Groups I and II) are shown in Table 1. The effects of age, gravidity and parity, and body mass index on the development of RPL were examined. No data were available on the smoking status of the patients. There were no significant differences between the groups in terms of age, number of pregnancies and births, and BMI for the development of RPL in the patients.

We hypothesized that CYR61 expression levels might be lower in patients with miscarriages compared with patients without miscarriages or one miscarriage history. This hypothesis is based on the observation that decreased expression of the CYR61 gene in mice leads to embryonic lethality because of impaired vascular integrity in embryonic arteries[15]. Therefore, we examined CYR61 expression levels in both groups of patients. As expected, CYR61 expression was significantly lower in patients with a history of RPL in their previous pregnancies than in patients who had one or no miscarriage (Figure 1).

Table 1. Demographic and clinical characteristics of the studied groups.

	Group I (mean±SD)	Group II (mean±SD)	p-value
Age (years)	29.25±4.56	32±3.53	0.9738
Gravidity (numbers)	3.58±1.55	3±0.70	0.875
Parity (numbers)	1.08±1.18	3.25±1.08	0.189
Abortion (numbers)	2.5±0.86	0.75±0.43	0.0195
BMI (kg/m^2)	24.9±4.64	28.4±2.67	0.266
Smoking status	0	0	NA

BMI: body mass index; SD: standard deviation; p<0.05.

DISCUSSION

Recurrent pregnancy loss is the condition in which there are two or more clinically recognized miscarriages before the gestation of 20 weeks. RPL is a complex and usually ill-defined situation in reproductive medicine. The etiology underlying approximately 50% of RPL patients remains unclear, causing frustration and concern for patients and their families.

Zhang et al.[16] attempted to uncover the molecular mechanism of preeclampsia caused by miR-155. Their findings provide new evidence that abnormally expressed miR-155 may play an important role in the development of preeclampsia through the downregulation of CYR61[16]. CYR61 is highly expressed in the human placenta[16]. It is mainly expressed in endothelial cells, villous stromal cells, and interstitial extravillous trophoblast giant cells and is involved in angiogenic processes and in the migratory properties of extravillous trophoblast cells in the developing human placenta[8,15]. In severe preeclampsia, Zhang et al.[16] showed that pregnant women have reduced expression of the CYR61 gene.

CYR61 gene expression in the context of RPL has not yet been investigated in English-language medical research. In this study, we wanted to show the relationship between downregulation of the CYR61 gene and RPL. Our results showed that a significant downregulation of CYR61 expression was observed in patients with a history of RPL, indicating the importance of CYR61 for the development of a successful pregnancy. Therefore, the CYR61 gene can be considered one of the most important biomarkers for the assessment of RPL risk in human patients,

especially in those who have a history of abortion. However, the causative role or functional significance of the CYR61 gene should be investigated with a view to developing new treatment and/or prevention approaches. Although the expression levels obtained from the patients' PBMCs give an idea of the importance of the CYR61 gene in a successful pregnancy, the tissue-specific expression of CYR61 should be examined to gain a deeper understanding of its role in pregnancy and RPL.

Ethics

All patients and controls provided written informed consents, and this study was approved by the Committee on Ethics of Dokuz Eylul University, Faculty of Medicine, Izmir, Turkey.

ACKNOWLEDGMENTS

We are grateful to all participants and their families who spent their precious time and participated in this research program. We are also thankful for the tireless efforts of the research team members.

AUTHORS' CONTRIBUTIONS

FBF: Conceptualization, Data curation, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **BFY:** Data curation, Formal Analysis, Investigation, Software, Writing – original draft. **OYO:** Project administration, Supervision, Visualization, Writing – review & editing.

REFERENCES

1. Liu Y, Zhang F, Dai L. C677T polymorphism increases the risk of early spontaneous abortion. J Assist Reprod Genet. 2019;36(8):1737-41. https://doi.org/10.1007/s10815-019-01500-2
2. Rouse CE, Eckert LO, Babarinsa I, Fay E, Gupta M, Harrison MS, et al. Spontaneous abortion and ectopic pregnancy: Case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. Vaccine. 2017;35(48 Pt A):6563-74. https://doi.org/10.1016/j.vaccine.2017.01.047
3. Xu Y, Ban Y, Ran L, Yu Y, Zhai S, Sun Z, et al. Relationship between unexplained recurrent pregnancy loss and 5,10-methylenetetrahydrofolate reductase) polymorphisms. Fertil Steril. 2019;111(3):597-603. https://doi.org/10.1016/j.fertnstert.2018.11.011
4. Abu-Asab NS, Ayesh SK, Ateeq RO, Nassar SM, El-Sharif WA. Association of inherited thrombophilia with recurrent pregnancy loss in palestinian women. Obstet Gynecol Int. 2011;2011:689684. https://doi.org/10.1155/2011/689684
5. Saravelos SH, Li TC. Unexplained recurrent miscarriage: how can we explain it? Hum Reprod. 2012;27(7):1882-6. https://doi.org/10.1093/humrep/des102
6. Practice Committee of the American Society for Reproductive Medicine [PCASRM]. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103-11. https://doi.org/10.1016/j.fertnstert.2012.06.048
7. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage: aetiology, management and prognosis. Hum Reprod Update. 2002;8(5):463-81. https://doi.org/10.1093/humupd/8.5.463
8. Mo FE, Muntean AG, Chen CC, Stolz DB, Watkins SC, Lau LF. CYR61 (CCN1) is essential for placental development and vascular integrity. Mol Cell Biol. 2002;22(24):8709-20. https://doi.org/10.1128/MCB.22.24.8709-8720.2002
9. Zhang X, Ding L, Diao Z, Yan G, Sun H, Hu Y. CYR61 modulates the vascular endothelial growth factor C expression of decidual NK cells via PI3K/AKT pathway. Am J Reprod Immunol. 2012;67(3):216-23. https://doi.org/10.1111/j.1600-0897.2011.01080.x
10. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature. 2000;407(6801):242-8. https://doi.org/10.1038/35025215
11. Kim SM, Kim JS. A review of mechanisms of implantation. Dev Reprod. 2017;21(4):351-9. https://doi.org/10.12717/DR.2017.21.4.351
12. Díaz P, Powell TL, Jansson T. The role of placental nutrient sensing in maternal-fetal resource allocation. Biol Reprod. 2014;91(4):82. https://doi.org/10.1095/biolreprod.114.121798
13. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology [ACOGCPB-G]. ACOG Practice Bulletin No. 200: early pregnancy loss. Obstet Gynecol. 2018;132(5):e197-207. https://doi.org/10.1097/AOG.0000000000002899
14. European Society of Human Reproduction and Embryology [ESHRE]. Recurrent pregnancy loss. Strombeek-Bever: ESHRE; 2017.
15. Gellhaus A, Schmidt M, Dunk C, Lye SJ, Kimmig R, Winterhager E. Decreased expression of the angiogenic regulators CYR61 (CCN1) and NOV (CCN3) in human placenta is associated with pre-eclampsia. Mol Hum Reprod. 2006;12(6):389-99. https://doi.org/10.1093/molehr/gal044
16. Zhang Y, Diao Z, Su L, Sun H, Li R, Cui H, et al. MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. Am J Obstet Gynecol. 2010;202(5):466.e1-7. https://doi.org/10.1016/j.ajog.2010.01.057

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231689

Portal thrombosis after surgical treatment of schistosomatic portal hypertension

Leonardo de Souza Vasconcellos[1], Vivian Resende[1], João Bernardo Sancio Rocha Rodrigues[1*], Andy Petroianu[1*]

SUMMARY

OBJECTIVE: Several studies have investigated the correlation between the effects of different surgical treatments and laboratory exams for schistosomal portal hypertension, especially concerning portal system thrombosis. The etiopathogenic factors of this thrombosis are not fully understood. In this study, the correlation between surgical treatment for schistosomal portal hypertension and the occurrence of postoperative portal system thrombosis was investigated.

METHODS: A total of 61 patients who underwent surgical treatment for schistosomal portal hypertension were distributed into four groups: Patients in Group 1 (n=12) underwent portal variceal disconnection associated with splenic artery ligation and spleen preservation. Patients in Group 2 (n=20) underwent portal variceal disconnection and total splenectomy. Patients in Group 3 (n=20) underwent portal variceal disconnection with subtotal splenectomy, preserving the upper splenic pole supplied by the splenogastric vessels. Patients in Group 4 (n=9) underwent portal variceal disconnection with total splenectomy and autogenous splenic implants on the greater omentum. Late postoperative portal vein thrombosis was diagnosed using Doppler ultrasound.

RESULTS: Over the 10-year follow-up, portal vein thrombosis occurred in 26 operated patients (42.6%), with no significant difference observed among the four surgical groups (p=0.217). Most of the thrombi only partially occluded the portal system veins. All the patients presented with a thrombus inside the portal vein. There was no difference in hematological and biochemical tests between groups with or without portal vein thrombosis.

CONCLUSIONS: Portal vein thrombosis is often observed in the late postoperative period, irrespective of the surgical treatment employed, and is not associated with patient characteristics or any hematological and biochemical tests.

KEYWORDS: Schistosomiasis mansoni. Portal hypertension. Surgical procedure. Splenectomy. Postoperative complications. Portal vein. Thrombosis.

INTRODUCTION

Schistosomiasis is an endemic disease in 52 countries, including those in South America, the Caribbean, Africa, and Asia. According to the World Health Organization, more than 200 million people are infected by one of the *Schistosoma* species (*Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma haematobium*, and *Schistosoma mekongi*), and in 10% of them, the disease progresses to the hepatosplenic form[1]. Pre-sinusoidal hepatosplenic schistosomal portal hypertension is a primary complication of *S. mansoni*[2].

In advanced stages, the rupture of gastroesophageal varices is frequent, and its morbimortality varies according to the hemorrhagic intensity[3]. Given its benign nature that preserves the liver function, the treatment of variceal bleeding and its prevention must be effective, minimizing the risk of recurrence[4]. A well-performed portal variceal disconnection (PVD) with partial or subtotal splenectomy appears to be the most effective long-term treatment for reducing the pressure in the esophagogastric veins and alleviating splenomegaly discomfort without compromising the spleen defensive function[5]. Despite the absence of a perfect operation for treating complicated portal hypertension, various techniques have been applied based on the preferences of the surgeon. However, there are no significant differences among the results of these techniques, and they generally provide satisfactory outcomes despite potential adversities[4,5].

The most common postoperative complication is portal vein thrombosis, occurring in up to half of all patients, and is typically transient[6-8]. The size and formation of the thrombus are unpredictable, and it is always spontaneous, transient, and recurrent[9,10]. Although the splenoportal thrombus may extend to other veins, such as the superior and inferior mesenteric veins and the left gastric vein, no motility or absorptive or metabolic digestive disorders have been associated with this event. Mesenteric ischemia with intestinal necrosis occurs in less than 1% of all cases[10,11].

[1]Universidade Federal de Minas Gerais, School of Medicine – Belo Horizonte (MG), Brazil.
*Corresponding author: petroian@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 11, 2024. Accepted on February 12, 2024.

The purpose of this study was to investigate the correlation between four different types of surgical treatments for schistosomal portal hypertension and postoperative portal system thrombosis, as well as to assess the impact of hematological and biochemical blood tests on this association.

METHODS

Study design and selection of patients

This research is part of a series of studies related to portal hypertension treatment[4,8,12-14]. This prospective study involved 61 consecutive adult patients who were followed up over a 10-year period at the Clinical Hospital of Universidade Federal de Minas Gerais, Brazil. All patients had severe esophageal and gastric variceal bleeding resulting from hepatosplenic *S. mansoni* associated with splenomegaly[3,4]. Before the surgical procedure, they underwent treatment with oxamniquine or praziquantel. Patients requiring emergency surgical procedures were not included[8].

All patients gave their informed consent for inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee at the Universidade Federal de Minas Gerais, Brazil, under registration number ETIC 006/08.

SURGICAL PROCEDURES

The PVD was performed by ligating all veins of the gastric lesser curvature and posterior wall, including the left gastric vein, as well as the veins surrounding the abdominal esophagus. The anterior wall of the stomach was longitudinally opened, and all the major gastric varices were ligated with running sutures up to the lower esophagus, using polyglycolic acid thread. The gastrotomy was closed in two layers with absorbable running sutures[4,5]. A liver biopsy was performed during the surgical procedure to confirm schistosomiasis and exclude other disorders[4,13-15].

The patients were distributed into four groups based on the four surgical procedures recommended in the literature for treating portal hypertension without vascular shunt[4-7,11,16] (Figure 1):

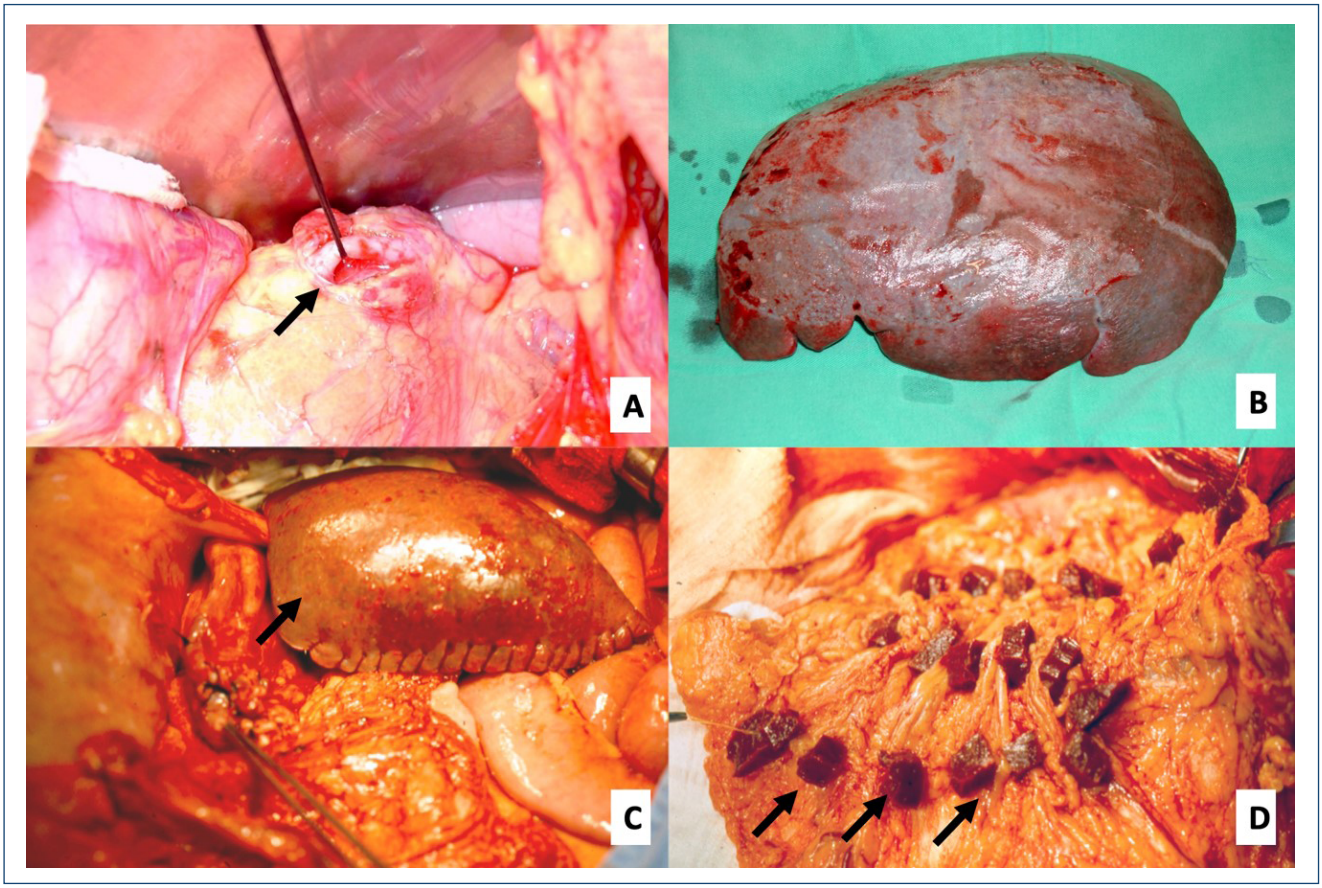

Figure 1. Surgical procedures for the treatment of schistosomal portal hypertension. (A) Tying the splenic artery (arrow). (B) Total splenectomy. (C) Subtotal splenectomy with preservation of the upper splenic pole (arrow). (D) Total splenectomy followed by autogenous splenic implants (arrows) on the greater omentum

- Group 1 (n=12): PVD associated with splenic artery ligature and spleen preservation;
- Group 2 (n=20): PVD associated with total splenectomy;
- Group 3 (n=20): PVD associated with subtotal splenectomy, maintaining the upper splenic pole supplied by the splenogastric vessels;
- Group 4 (n=9): PVD associated with total splenectomy and 20 autogenous splenic implants on the greater omentum.

VARIABLES AND OUTCOMES

The clinicodemographic and laboratory exams considered in this study included: age, sex, complete blood count, prothrombin activity, international standardized ratio (INR), urea, creatinine, glucose, total and fractionated cholesterol, triglycerides, albumin, globulins, alkaline phosphatase, gamma-glutamyltransferase, aminotransferases, and bilirubins[13,14]. Throughout the 10-year follow-up period, all patients underwent annual laboratory exams and a two-dimensional Doppler ultrasound study, or as needed. The primary outcome aimed to investigate thrombosis in the portal venous system, including portal, splenic, superior mesenteric, inferior mesenteric, and left gastric veins. Thrombi were classified as partial, cavernous (undergoing recanalization), and total[7,15]. The second outcome was to verify the association between the laboratory variables and the presence of a thrombus in the portal venous system.

STATISTICAL ANALYSES

The power calculation accepted samples higher than eight patients in each group. Descriptive statistics were expressed as median values (interquartile range [IQR]) for continuous measures and frequency (percentage) for categorical measures. The incidence of thrombosis was compared using the chi-squared test or Fisher's exact test, as appropriate. Laboratory test results were compared by analyzing patients with and without thrombosis, using Student's t-test, with a significance of $p<0.05$.

RESULTS

The vast majority of patients were male (n=40, 65.6%) with an average age of 43±11 years, while 21 were female (34.4%), with an average age of 45±12 years. Cirrhosis or liver tumors were not recorded during the 10-year follow-up. All patients exhibited fibrotic splenomegaly and hepatic fibrosis characteristic of *S. mansoni*.

Portal system thrombosis was identified in 26 cases (42.6%) with no difference in age, sex, surgical procedure, or post-operative laboratory exams (Table 1). Partial thrombus was found in 20 (77%) patients, while cavernous thrombus was observed only in 6 (23%) patients. Most of the thrombi only partially occluded the portal system veins; no total portal vein obstruction was found in this series. All patients with thrombosis presented a thrombus inside the portal vein, either isolated (n=13, 50.0%)

Table 1. Presence of thrombus in the portal vein system during the late postoperative period of 61 patients with schistosomal portal hypertension.

	Group 1 (n=12)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=9)	Total (n=61)
Portal vein system thrombus*					
Negative	7 (58.3%)	9 (45%)	15 (75%)	4 (44.4%)	35 (57.4%)
Positive	5 (41.7%)	11 (55%)	5 (25%)	5 (55.6%)	26 (42.6%)*
Thrombus classification with percentages related only to the positive cases					
Partial	4 (80%)	8 (72.7%)	4 (80%)	4 (80%)	20 (77%)
Cavernous	1 (20%)	3 (27.3%)	1 (20%)	1 (20%)	6 (23%)
Thrombus location with percentages related only to the positive cases					
Portal vein	5 (100%)	11 (100%)	5 (100%)	5 (100%)	26 (100%)
Splenic vein	1 (20%)	4 (36.4%)	3 (60%)	2 (40%)	10 (38.5%)
Superior mesenteric vein	0	1 (9.1%)	0	1 (20%)	2 (7.7%)
Inferior mesenteric vein	0	1 (9.1%)	0	0	1 (3.8%)

Data are expressed as n (%). Group 1 (n=12): portal variceal disconnection associated with splenic artery ligature and spleen preservation; Group 2 (n=20): portal variceal disconnection associated with total splenectomy; Group 3 (n=20): portal variceal disconnection associated with subtotal splenectomy, maintaining the upper splenic pole supplied by the splenogastric vessels; Group 4 (n=9): portal variceal disconnection associated with total splenectomy and 20 autogenous splenic implants on the greater omentum. *Portal thrombosis: p=0.217.

or associated with other veins of the portal system: splenic vein (n=10, 38.5%), superior mesenteric vein (n=2, 7.7%), and inferior mesenteric vein (n=1, 3.8%) (Table 1).

No differences were observed in hematological and biochemical results when comparing patients of all groups with and without thrombosis during the 10-year follow-up (Table 2).

DISCUSSION

Schistosomiasis has become a focal point in various lines of research within the literature. This disease causes peritoneal inflammation with several consequences, including chronic iliac pain due to abdominal and pelvic adhesions[15]. Therefore, the accuracy of treatment is important for practical clinics. The increased availability of diagnostic resources, notably Doppler ultrasonography, has enabled more effective follow-up of patients treated for schistosomiasis. These resources have disclosed the high incidence of thrombosis in the portal system during the postoperative period as an additional post-operative adverse event of the surgical treatment for complicated schistosomal portal hypertension[6]. According to Widman et al., these rates range from 19% after PVD with total splenectomy to 50%, after distal splenorenal shunt[6]. Cleva et al. observed an incidence of up to 55% of post-portal-variceal disconnection thrombosis[7].

The first report of portal thrombosis was in 1956, with the necropsy of a cirrhotic patient[17]. In schistosomiasis, portal thrombosis was described by Bogliolo[17]. Since the 1970s, with the advent of ultrasound, which has been associated with Doppler in the 1980s, many reports of postoperative portal thrombosis have been published[18-21], generally without clinical consequences[22-25]. In this study, the prevalence of thrombosis was 42.6% during the 10-year follow-up period, and no patient presented symptoms related to this event. The assessment of portal thrombosis took place during the yearly general examination of each patient.

Table 2. Association between portal vein thrombosis and laboratory exams (mean ± standard deviation of the mean) in 61 patients with schistosomal portal hypertension 1 year after the surgical procedure.

Exams	Portal thrombosis		p-value
	Positive	Negative	
Red blood cells (×10⁶/mm³)	4.72±0.60	4.71±0.47	0.815
Hemoglobin (g/dL)	12.7±1.7	13.6±1.8	0.104
Hematocrit (%)	38.0±5.3	40.5±4.7	0.112
Total white blood cells (cells/mm³)	6570±2347	7085±2650	0.426
Eosinophils (%)	7±7	5±4	0.213
Platelets (×10³/mm³)	302±137	254±136	0.177
Prothrombin activity (%)	74±15	80±16	0.156
International normalized ratio–INR	1.21±0.18	1.17±0.16	0.308
Albumin (mg/dL)	4.1±0.5	4.2±0.4	0.353
Globulins (mg/dL)	3.4±0.3	3.3±0.4	0.149
Alkaline phosphatase (U/l)	117±37	111±45	0.495
Glutamyl transferase range (U/l)	80±29	68±35	0.128
Aspartate aminotransferase (U/l)	40±8	39±10	0.731
Alanine aminotransferase (U/l)	40±8	36±12	0.180
Direct bilirubin (mg/dL)	0.2±0.1	0.2±0.2	0.940
Indirect bilirubin (mg/dL)	0.6±0.3	0.7±0.3	0.360
Total cholesterol (mg/dL)	179±26	186±31	0.373
High density cholesterol (mg/dL)	47±8	50±10	0.253
Low density cholesterol (mg/dL)	110±25	117±30	0.327
Very low density cholesterol (mg/dL)	22±7	19±6	0.122
Triglycerides (mg/dL)	108±37	95±30	0.136
Glucose (mg/dL)	89±9	86±7	0.256
Urea (mg/dL)	28±9	30±11	0.301
Creatinine (mg/dL)	0.8±0.2	0.7±0.2	0.346

Some patients experienced more than one episode of portal thrombosis during the total follow-up, and typically, the thrombi resolved within different periods, with none persisting for more than 6 months. Importantly, there was no difference in thrombosis among the patients who underwent different surgical procedures to treat portal hypertension.

According to the literature, reduced blood flow and consequent decreased vein pressure after PVD are associated with thrombogenesis[6,7,10-12]. In fact, the purpose of this surgical procedure was to reduce the blood pressure in the portal system to prevent variceal bleeding[5]. PVD leads to a partial stop in the hepatofugal portal flow to the esophagogastric area. It is important to consider that this blockage increases portal flow and pressure in the rest of the portal-venous system[10,21]. The ligature of the splenic artery diminished blood flow to the spleen, and patients in Group 1 showed a reduction in spleen size during the post-operative follow-up[12,22,25].

The etiopathogenesis of the portal system thrombosis is still not adequately understood in the literature[3,6,7]. In most cases, it is transient and recurrent without symptoms. No drugs, such as heparins, salicylic acid, or other antithrombotics, have proven effective in preventing or treating specific portal thrombosis. All drugs have been prescribed based on their systemic effects, which differ from their effects on the portal system[10,11]. Considering that portal thrombi are transient and self-limited, the presumed efficacy of these drugs may not align with reality[4,5,11].

No patient in this series presented total portal thrombosis during the follow-up period. All thrombi reduced in size or disappeared at different periods, and there was no association between thrombi growth and disappearance with age, sex, or surgical procedure. These data are in accordance with the literature, which reports that partial thrombi occur in most cases without clinical manifestation[13,22].

In this study, no differences were observed in the comparative results of hematological and biochemical exams between patients with and without portal thrombosis. Post-splenectomy thrombocytosis was not found to be related to the formation of a thrombus in the portal system[4,14,22,25]. Thus, the pathophysiology of postoperative thrombosis in patients with schistosomatic portal hypertension remains unknown, despite the numerous studies conducted[1,4,8,13,23,24].

Patients with splenorenal shunts, another acceptable surgical procedure to treat schistosomatic portal hypertension, were not included in this study to maintain the uniformity of this series on PVD. The patients were randomly assigned to each surgical procedure according to the surgeons' choice in each case. No patient experienced variceal bleeding, and all patients are still alive. Hepatosplenic schistosomiasis is a benign process that does not impair the functions of the liver or spleen. In this study, most of the patients were peasants, and all of them returned to their normal lives after the surgical procedure.

A limitation of this study is the small sample size. Surgical treatment of schistosomiasis is rare, and this study shows the experience of a single institution with a long-term follow-up involving four different types of surgical approaches. Very few studies comparing post-operative portal system thrombosis with liver function must be emphasized. Most studies refer only to the presence of portal thrombosis as a frequent complication of total splenectomy.

CONCLUSION

Portal vein thrombosis is a common postoperative complication in patients with portal hypertension, irrespective of surgical treatment, and it is not associated with patient characteristics or any hematological and biochemical tests.

ETHICAL ASPECTS

This research is part of a series of studies related to portal hypertension treatment. All patients gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Committee of the Research Ethics of the Federal University of Minas Gerais (UFMG), Brazil, registered under the protocol number ETIC 006/08.

ACKNOWLEDGMENTS

The authors gratefully thank the Research Support Foundation of the State of Minas Gerais (FAPEMIG), the National Council for Scientific and Technological Development (CNPq), and the Dean's Office for Research (Pró-reitoria de Pesquisa) at the Universidade Federal de Minas Gerais for their financial support.

AUTHORS' CONTRIBUTIONS

LSV: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **VR:** Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **JBSRR:** Data curation, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. **AP:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Silva LFD, Nunes BEBR, Leal TC, Paiva JPS, Lemos AMS, Araújo LMM, et al. Schistosomiasis mansoni in the northeast region of brazil: temporal modeling of positivity, hospitalization, and mortality rates. Rev Soc Bras Med Trop. 2019;52:e20180458. https://doi.org/10.1590/0037-8682-0458-2018
2. Ferrer E, Villegas B, Mughini-Gras L, Hernández D, Jiménez V, Catalano E, et al. Diagnostic performance of parasitological, immunological and molecular tests for the diagnosis of Schistosoma mansoni infection in a community of low transmission in Venezuela. Acta Trop. 2020;204:105360. https://doi.org/10.1016/j.actatropica.2020.105360
3. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383(9936):2253-64. https://doi.org/10.1016/S0140-6736(13)61949-2
4. Petroianu A. Surgical treatment of portal hypertension in schistosomiasis mansoni. Rev Soc Bras Med Trop. 2003;36(2):253-65. https://doi.org/10.1590/s0037-86822003000200010
5. Henderson JM, Anderson CD. The surgical treatment of portal hypertension. Clin Liver Dis (Hoboken). 2020;15(Suppl 1):S52-63. https://doi.org/10.1002/cld.877
6. Widman A, Oliveira IR, Speranzini MB, Cerri GG, Saad WA, Gama-Rodrigues J. Portal thrombosis: late postoperative prevalence in Mansoni's schistosomiasis. Hepatogastroenterology. 2003;50(53):1463-6. PMID: 14571764
7. Cleva R, Pugliese V, Zilberstein B, Saad WA, Pinotti HW, Laudanna AA. Systemic hemodynamic changes in mansonic schistosomiasis with portal hypertension treated by azygoportal disconnection and splenectomy. Am J Gastroenterol. 1999;94(6):1632-7. https://doi.org/10.1111/j.1572-0241.1999.01086.x
8. Petroianu A, Oliveira AE, Alberti LR. Hypersplenism in schistosomatic portal hypertension. Arch Med Res. 2005;36(5):496-501. https://doi.org/10.1016/j.arcmed.2005.04.003
9. Webb LJ, Sherlock S. The aetiology, presentation and natural history of extra-hepatic portal venous obstruction. Q J Med. 1979;48(192):627-39. PMID: 538223
10. Santos JC, Pereira CLD, Domingues ALC, Lopes EP. Noninvasive diagnosis of periportal fibrosis in schistosomiasis mansoni: a comprehensive review. World J Hepatol. 2022;14(4):696-707. https://doi.org/10.4254/wjh.v14.i4.696
11. Cançado GGL, Nardelli MJ, Barbosa FA, Silva CF, Osório FMF, Ferrari TCA, et al. Portal vein thrombosis in patients with hepatosplenic schistosomiasis who underwent oesophagogastric devascularization combined with splenectomy. Trans R Soc Trop Med Hyg. 2021;115(9):1004-9. https://doi.org/10.1093/trstmh/trab013
12. Voieta I, Queiroz LC, Andrade LM, Silva LC, Fontes VF, Barbosa A, et al. Imaging techniques and histology in the evaluation of liver fibrosis in hepatosplenic schistosomiasis mansoni in Brazil: a comparative study. Mem Inst Oswaldo Cruz. 2010;105(4):414-21. https://doi.org/10.1590/s0074-02762010000400011
13. Vasconcellos LS, Petroianu A, Romeiro JR, Tavares Junior WC, Resende V. Correlation between the values of circulating blood elements with the size of spleen in the presence of schistosomal splenomegaly. Acta Cir Bras. 2018;33(12):1103-9. https://doi.org/10.1590/s0102-865020180120000008
14. Petroianu A, Buzatti KC, Resende V, Sabino KR. Haematological and biochemical characteristics of the splenic effluent blood in schistosomal patients undergoing splenectomy. Rev Col Bras Cir. 2014;41(3):176-80. https://doi.org/10.1590/s0100-69912014000300007
15. Nicolau SM, Soares JM, Schor E, Gonçalves WJ, Freitas V, Baracat EC. Schistosoma mansoni pseudo-cyst as a cause of chronic pelvic pain. J Obstet Gynaecol Res. 2003;29(6):392-4. https://doi.org/10.1111/j.1341-8076.2003.00134.x
16. Petroianu A, Petroianu LP. Splenic autotransplantation for treatment of portal hypertension. Can J Surg. 2005;48(5):382-6. PMID: 16248137
17. Bogliolo L. Splenoportography in hepato-splenic Manson's schistosomiasis of the Symmers type. Rev Assoc Med Bras. 1957;3(3):263-9. PMID: 13506130
18. Andrianah GEP, Rakotomena D, Rakotondrainibe A, Rajaonarison Ny Ony LHN, Ranoharison HD, Ratsimba HR, et al. Contribution of ultrasonography in the diagnosis of periportal fibrosis caused by schistosomiasis. J Med Ultrasound. 2020;28(1):41-3. https://doi.org/10.4103/JMU.JMU_16_19
19. Swinson B, Waters PS, Webber L, Nathanson L, Cavallucci DJ, O'Rourke N, et al. Portal vein thrombosis following elective laparoscopic splenectomy: incidence and analysis of risk factors. Surg Endosc. 2022;36(5):3332-9. https://doi.org/10.1007/s00464-021-08649-x
20. Abdel Wahab MF, Esmat G. The value of ultrasonography in assessment of portal hypertension in hepatosplenic schistosomiasis. Mem Inst Oswaldo Cruz. 1992;87(Suppl 4):143-7. https://doi.org/10.1590/s0074-02761992000800021
21. Tan R, Daneshmand A, Parys S, Watanabe Y, Sieunarine K. Splanchnic venous thrombosis: aetiologies and a review of the literature. ANZ J Surg. 2022;92(9):2224-8. https://doi.org/10.1111/ans.17863
22. Petroianu A, Resende V, Silva RG. Late follow-up of patients submitted to subtotal splenectomy. Int J Surg. 2006;4(3):172-8. https://doi.org/10.1016/j.ijsu.2005.12.006
23. Petroianu A, Cabezas-Andrade MA, Neto RB. Laparoscopic splenic autotransplantation. Surg Laparosc Endosc Percutan Tech. 2006;16(4):259-62. https://doi.org/10.1097/00129689-200608000-00014
24. Petroianu A. Subtotal splenectomy and portal variceal disconnection in the treatment of portal hypertension. Can J Surg. 1993;36(3):251-4. PMID: 8324672
25. Eyayu T, Zeleke AJ, Seyoum M, Worku L. Basic coagulation profiles and platelet count among schistosoma mansoni-infected adults attending Sanja Primary Hospital, northwest Ethiopia. Res Rep Trop Med. 2020;11:27-36. https://doi.org/10.2147/RRTM.S244912

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231742

Knowledge and awareness level of health undergraduate students on child abuse: a cross-sectional study

Alev Üstündağ[1*], Ayşe Göktaş[2], Çiğdem Aytekin[3]

SUMMARY

OBJECTIVE: The aim of the study was to investigate the level of knowledge of symptoms and risks of child abuse among undergraduate health science students according to their socio-demographic characteristics.

METHODS: This is a cross-sectional study involving 485 student volunteers. The data collection tools used in the study were the Demographic Data Collection Form and the Scale for Diagnosing Symptoms and Risks of Child Abuse and Neglect.

RESULTS: It was found that health students' knowledge of the symptoms and risks of child abuse was moderate. It was also found that knowledge of diagnosing the symptoms and risks of child abuse was higher among women than among men, higher among those who had received education on child abuse, and increased with grade level.

CONCLUSION: Child abuse is an important public health issue, and there is a need to raise awareness of this issue among health students.

KEYWORDS: Child abuse. Child neglect. Students, public health.

INTRODUCTION

Child abuse, which is a significant social and health threat today, is defined by the World Health Organization (WHO)[1] as "*any physical and/or emotional maltreatment, sexual abuse, neglect and commercial exploitation of children under the age of 18 that results in actual or potential harm to the health, development or dignity of the child*." According to the WHO report, 23% of children were subjected to physical abuse, 16% were subjected to physical neglect, 36% were subjected to emotional abuse, and 18% of girls and 8% of boys were subjected to sexual abuse[1]. In Turkey, although the studies on child abuse are limited, it can be observed that the rates are at a considerable level[2].

Child abuse is associated with a wide range of long-term negative health and developmental outcomes, extending into adolescence and young adulthood[3]. Early identification of child abuse is therefore important. Health professionals who encounter children and are likely to encounter children have an important role to play in the early identification of child abuse[4]. Health professionals have responsibilities not only in the area of child health but also in the area of child protection[5]. The most important of these responsibilities is the early recognition of symptoms of abuse or endangerment in terms of child welfare[5]. For this reason, all health professionals, regardless of their field of work, need to recognize the early signs and risks of child abuse[6]. In the hospital environment, healthcare professionals frequently encounter abused children. However, a lack of awareness of the early signs and risks of child abuse and inadequate knowledge on this topic may lead to cases that come to the hospital environment being missed[7].

Given that all children are at risk of child abuse, it is important and necessary for undergraduate health students to have knowledge about diagnosing the symptoms and risks of child maltreatment, especially as future health professionals. Although the studies on the level of knowledge about child abuse among students studying in undergraduate programs related to health sciences are quite limited, it has been found that students' knowledge about this topic is insufficient, their level of identification of symptoms and risks is not at the desired level, and they need information about this topic[8,9]. In addition, according to the results of the research, public students know very less about sexual infection disease and male adolescents are less interested in health services[10]. Based on these findings, our study aimed to investigate the level of knowledge of the symptoms and risks of child maltreatment

[1]University of Health Sciences, Gülhane Faculty of Health Sciences, Department of Child Development – Ankara, Turkey.
[2]University of Health Sciences, Gülhane Faculty of Health Sciences, Department of Occupational Therapy – Ankara, Turkey.
[3]Hacettepe University, Faculty of Health Sciences, Department of Child Development – Ankara, Turkey.
*Corresponding author: alev.ustundag@sbu.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 17, 2024. Accepted on February 19, 2024.

among undergraduate health science students according to their socio-demographic characteristics.

METHODS

Research model

This is a cross-sectional study.

Participants

In this research, the study group comprised 485 volunteer students at Ankara, who are continuing their undergraduate programs related to health sciences. The sample size was determined with the G*POWER 3.1.9.4 statistics software used in cases where the population was known. Of the 485 students included in the study, 88% (n=427) were female, 12% (n=58) were male, and the mean age was 21.46±2.22 years.

Measurement

The data regarding the research were collected using the Demographic Data Collection Form and the Scale for Diagnosing Symptoms and Risks of Child Abuse and Neglect forms through the face-to-face interview method.

Demographic Data Collection Form

The information form was prepared by the researchers. It consists of 11 questions, including the socio-demographic characteristics of the students.

Scale for Diagnosing Symptoms and Risks of Child Abuse and Neglect

The scale developed by Uysal[11] was designed to assess the competence of health professionals in recognizing the symptoms and risks of child abuse and neglect and in differentiating between the risks. The 5-point Likert scale consists of 67 questions. The scale has six sub-dimensions: Physical Symptoms of Child Abuse (PSCA), Behavioral Symptoms of Child Abuse And Neglect (BSCAN), Symptoms of Child Neglect (SCN), Characteristics of Parents Prone to Abuse and Neglect (CPPAN), Characteristics of Children Prone To Abuse and Neglect (CCPAN), and Family Characteristics in Child Abuse and Neglect (FCCAN). In this study, the Cronbach's alpha value of the scale was found to be 0.83.

Data analysis

Mean and standard deviation were used as descriptive statistics for continuous data. The Kolmogorov-Smirnov test was used to test for normality, and the Student's t-test in independent groups was used to assess the distribution of continuous variables that fit the normal distribution in two groups, and one-way ANOVA was used to assess the distribution in three or more groups. The distribution of non-normally distributed variables in two groups was compared by the Mann-Whitney U test, and the distribution in three or more groups was compared by the Kruskal-Wallis test. The Dunn-Bonferroni test was used as a multiple comparison test for the Kruskal-Wallis test to find the source of the difference.

The relationship between the variables was assessed by point bi-serial and Spearman correlation analysis. The cut-off points used for the interpretation of the correlation coefficients obtained are as follows: 0.00–0.19 very weak, 0.20–0.39 weak, 0.40–0.69 moderate, 0.70–0.89 high, and 0.90–1.00 very high. The Statistical Package for the Social Sciences Statistics for Macintosh (version 21.0; IBM Corp., Armonk, NY, USA) was used for all statistical analyses performed within the scope of the study. The statistical significance level was set at 0.05.

Ethics Committee approval of the study was obtained with the meeting decision of the University of Health Sciences, Gülhane Scientific Research Ethics Committee (dated 05.12.2023 and No. 2023/359).

RESULTS

According to the results of the research, the mean total score of the female participants was 3.61±0.30 and the mean total score of the male participants was 3.51±0.35. Accordingly, the students' level of diagnosing the symptoms and risks of child neglect and abuse is at a moderate level. Table 1 shows the results of the comparison of the scores of students according to some demographic information.

The results of the comparison of the scores of students on the SDSRCAN scale according to the departments they attend are presented in Table 2, and the results of the correlation analysis are presented in Table 3.

DISCUSSION

When the results of the study were analyzed, it was found that the total score of the SDSRCAN scale and the subscale scores of the PSCA and SCN differed significantly according to gender and that the mean scores of women were higher. In other words, women are better at diagnosing risk symptoms of child abuse and neglect, physical symptoms of abuse in the child, and behavioral symptoms in the child. Similarly, in the study conducted by Güdek-Seferoğlu et al.[12], they

Table 1. Comparison of total scale scores and sub-dimension total scores according to some demographic information.

		PSCA (Mean±SS)	BSCAN (Mean±SS)	SCN (Mean± SS)	CPPAN (Mean±SS)	CCPAN (Mean ±SS)	FCCAN (Mean ±SS)	SDSRCAN (Mean±SS)
Gender	Women n=427	3.85±0.40	3.94±0.57	3.73±0.39	3.61±0.48	3.26±0.42	2.79±0.33	3.61±0.30
	Men n=58	3.67±0.51	3.80±0.76	3.59±0.49	3.65±0.44	3.19±0.47	2.76±0.46	3.51±0.35
Test statistics**		z=-2.573 p=0.010	z=-1.199 p=0.231	z=-2.270 p=0.023	z=0.542 p=0.588	z=-1.526 p=0.127	z=0.002 p=0.999	z=-2.178 p=0.029
Grade level	1 n=122	3.75±0.40	3.86±0.60	3.62±0.40	3.47±0.43	3.20±0.39	2.81±0.36	3.52±0.27
	2 n=107	3.81±0.40	3.86±0.60	3.63±0.40	3.50±0.46	3.20±0.42	2.85±0.35	3.56±0.31
	3 n=97	3.81±0.42	3.95±0.60	3.73±0.35	3.68±0.43	3.31±0.38	2.76±0.36	3.62±0.29
	4 n=146	3.93±0.43	4.00±0.59	3.80±0.45	3.78±0.51	3.30±0.47	2.75±0.34	3.68±0.33
Test statistics*		χ^2=11.077 p=0.011 4>1	χ^2=3.410 p=0.333	χ^2=9.502 p=0.023 4>1	χ^2=33.879 p<0.001 3>1,2 4>1,2	χ^2=8.527 p=0.036	χ^2=2.607 p=0.456	χ^2=17.048 p=0.001 4>1
Relatives living at home during childhood	Yes n=103	3.88±0.40	4.00±0.52	3.77±0.42	3.70±0.48	3.30±0.41	2.72±0.33	3.64±0.29
	No n=382	3.81±0.42	3.90±0.62	3.69±0.41	3.60±0.47	3.24±0.43	2.81±0.35	3.59±0.31
Test statistics**		z=-1.283 p=0.200	z=-1.483 p=0.138	z=-0.922 p=0.357	z=-1.661 p=0.097	z=-1.500 p=0.134	Z=2.354 p=0.019	t=1.689 p=0.092
Status of education about child abuse	Yes n=136	3.87±0.39	3.98±0.56	3.74±0.40	3.72±0.49	3.25±0.43	2.83±0.38	3.65±0.29
	No n=349	3.81±0.43	3.90±0.61	3.70±0.41	3.57±0.46	3.25±0.42	2.78±0.34	3.58±0.31
Test statistics**		z=-1.237 p=0.216	z=-1.332 p=0.183	z=-1.004 p=0.316	z=-2.830 p=0.005	z=-0.097 p=0.922	z=-0.699 p=0.484	t=2.196 p=0.029
Status of believing that knowledge about child abuse is adequate	Yes n=107	3.91±0.46	3.98±0.63	3.80±0.45	3.67±0.52	3.27±0.50	2.86±0.37	3.67±0.33
	No n=378	3.80±0.40	3.91±0.59	3.69±0.39	3.60±0.46	3.24±0.40	2.77±0.34	3.58±0.30
Test statistics**		z=-2.202 p=0.028	z=-0.820 p=0.412	z=-1.875 p=0.061	z=-0.896 p=0.370	z=-0.611 p=0.541	z=-1.804 p=0.071	t=2.612 p=0.009

*Kruskal-Wallis test statistics. **Mann-Whitney U test statistical value. Bold indicates p<0.05.

investigated the level of SDSRCAN of nursing students, and in the study conducted by Pesen and Epçaçan[13] with pre-service teachers, the mean score of women was found to be higher than that of men. According to the results of the study conducted by Türk et al.[14] which aimed to investigate the level of SDSRCAN among university students, the total mean score of the scale and the mean scores of the subscales of PSCA, BSCAN, SCN, and FCCAN are higher among females. In the studies of Tek and Karakaş[15] and Ozbey et al.[16] which investigated the level of knowledge and awareness of child neglect and abuse among nursing students, and in the study of Jeong et al.[17] which investigated the awareness and intention to report child neglect and abuse among nursing and education students, the mean scores of female students were higher than male students.

According to the results obtained, it was found that the grade level of the students made a significant difference in the level of the SDSRCAN. Accordingly, the total score of the SDSRCAN and the subscale scores of the PSCA, SCN, CPPAN, and CCPAN increased as the grade level increased

Table 2. Comparison of the total and sub-dimension total scores of the Scale for Diagnosing Symptoms and Risks of Child Abuse and Neglect scale according to the departments attended by the students.

		PSCA (Mean±SS)	BSCAN (Mean±SS)	SCN (Mean±SS)	CPPAN (Mean±SS)	CCPAN (Mean±SS)	FCCAN (Mean±SS)	SDSRCAN (Mean±SS)
Programs	Nutrition and Dietetics[1] n=34	3.96±0.36	3.93±0.56	3.76±0.35	3.66±0.43	3.34±0.41	2.86±0.25	3.67±0.25
	Child Development[2] n=127	3.76±0.38	3.87±0.59	3.69±0.40	3.71±0.47	3.21±0.41	2.79±0.36	3.58±0.28
	Speech and Language Therapy[3] n=17	3.73±0.36	3.83±0.52	3.61±0.28	3.63±0.36	3.27±0.31	2.76±0.28	3.54±0.27
	Dental Medicine[4] n=16	3.45±0.37	3.55±0.52	3.49±0.37	3.36±0.47	3.04±0.51	2.83±0.35	3.35±0.25
	Midwifery[5] (n=15)	3.87±0.45	3.86±0.60	3.59±0.38	3.60±0.51	3.37±0.48	2.81±0.44	3.59±0.27
	Pharmaceutics[6] n=25	3.86±0.40	3.90±0.66	3.73±0.40	3.53±0.41	3.37±0.39	2.76±0.35	3.60±0.29
	Ergotherapy[7] n=31	3.78±0.28	3.97±0.56	3.74±0.30	3.64±0.45	3.32±0.43	2.67±0.39	3.59±0.24
	Physiotherapy and Rehabilitation[8] n=28	3.98±0.39	4.12±0.68	3.95±0.45	3.76±0.48	3.26±0.43	2.60±0.37	3.72±0.35
	Nursing[9] n=81	3.94±0.45	4.09±0.61	3.80±0.44	3.63±0.51	3.29±0.41	2.87±0.30	3.68±0.34
	Audiology[10] n=21	3.65±0.40	3.63±0.45	3.38±0.39	3.40±0.46	2.93±0.41	2.98±0.43	3.40±0.26
	Health Administratio[11] n=22	3.72±0.34	3.95±0.53	3.63±0.34	3.39±0.53	3.14±0.37	2.77±0.29	3.50±0.30
	Social Services[12] n=33	3.83±0.6	4.05±0.63	3.65±0.36	3.45±0.45	3.23±0.36	2.79±0.40	3.56±0.29
	Medicine[13] n=35	3.97±0.52	3.87±0.59	3.85±0.49	3.66±0.43	3.38±0.48	2.74±0.27	3.68±0.37
Test statistics*		χ^2=41.073 p<0.001	χ^2=27.115 p=0.007	χ^2=39.140 p<0.001	χ^2=24.650 p=0.017	χ^2=26.687 p=0.009	χ^2=24.428 p=0.018	χ^2=37.383 p<0.001
Source of difference**		4<1 4<8 4<9 4<13	9>4 9>10	10<1 10<8 10<9 10<13		10<6 10<9 10)<13	8<9 8<10	4<1 4<8 4<9 4<13 10<8 1<9

*Kruskal-Wallis test statistics. **Dunn-Bonferroni post hoc test results. Bold indicates p<0.05.

Table 3. Analyses of the relationship between some variables and scale total and subscale.

		PSCA	BSCAN	SCN	CPPAN	CCPAN	FCCAN	SDSRCAN
Gender	r_{pb}	-0.131	-0.078	-0.109	0.027	-0.054	-0.028	-0.101
	p	0.004	0.088	0.016	0.558	0.238	0.538	0.026
Status of education about child abuse	r_{pb}	-0.068	-0.063	-0.048	-0.137	0.002	-0.065	-0.099
	p	0.135	0.169	0.294	0.002	0.969	0.155	0.029
Status of believing that knowledge about child abuse is adequate	r_{pb}	-0.103	-0.045	-0.119	-0.058	-0.027	-0.107	-0.118
	p	0.023	0.319	0.009	0.203	0.554	0.019	0.009
Grade level	rho	0.160	0.096	0.170	0.269	0.114	-0.084	0.208
	p	0.000	0.036	0.000	0.000	0.013	0.069	0.000
Programs	r_{pb}	0.103	0.092	0.074	-0.098	0.016	-0.014	0.053
	p	0.025	0.046	0.107	0.033	0.724	0.756	0.249

r_{pb}: Point bi-serial correlation. rho: Spearman correlation.
Bold indicates p<0.05.

and the score of the fourth-grade students was significantly higher than that of the first-grade students. In line with the findings of this study, Poreddi et al.[18] examined the level of knowledge of nursing students on child abuse and neglect and found that the students' knowledge and attitudes toward child abuse increased as they progressed through the academic year.

Another finding from the study was that the mean scores of students who had received training on child abuse, and those who felt their knowledge was sufficient, were significantly higher. Looking at the literature, some studies conclude that the level of awareness is higher among those who receive child abuse training[15,19,20]. The results of the research and the literature are consistent. Increasing the knowledge of individuals is important not only for their future health but also for preventing the negative consequences of violence and abuse[21].

CONCLUSION

The study found that undergraduate health students' knowledge of recognizing the symptoms and risks of child abuse and neglect was average. In this context, our study makes a general contribution to the literature on international health education. Therefore, in an international context, it is recommended that students should be given more detailed information on this subject in the undergraduate curriculum and that scientific activities should be carried out to increase students' awareness.

In addition, the strengths of this study are as follows. The findings were obtained through adolescents' self-reports, and it has a rigorous approach to data analysis. Although this study produced important results, it is important to mention its limitations. Our relatively small sample and one-off design limit the broad applicability of our findings. Therefore, further studies are needed to make the findings more generalizable. Future research should be conducted at regular intervals in larger sample groups. However, it should be noted that this study is only a preliminary investigation of a topic that requires further research. Furthermore, as this is a cross-sectional study, it is not possible to make a causal inference from our results. A potential limitation of the research is that it relied on self-reported data. Self-reported measures may not always accurately reflect actual behavior or awareness levels of participants. Hence, future studies may consider including observational or behavioral measures to supplement self-report data.

AUTHORS' CONTRIBUTIONS

AÜ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration. **AG:** Formal Analysis. **ÇA:** Conceptualization, Investigation.

REFERENCES

1. World Health Organization. Child maltreatment. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/child-maltreatment
2. Polat O. Child abuse report in Turkey-2. 2018. Available from: http://imdat.org/wp-content/uploads/2018/05/RAPOR%C3%87OCUK-%C4%B0ST%C4%B0SMARI-tam.pdf
3. Strathearn L, Giannotti M, Mills R, Kisely S, Najman J, Abajobir A. Long-term cognitive, psychological, and health outcomes associated with child abuse and neglect. Pediatrics. 2020;146(4):e20200438. https://doi.org/10.1542/peds.2020-0438
4. Harris NB. The deepest well. Boston (MA): Houghton Mifflin Harcourt; 2018.
5. Thiekötter L, Schmidt P, Scheiderer ML, Wunram HL, Paulussen M, Reis D, et al. How stressful ıs examining children with symptoms of child abuse?-Measurement of stress appraisal (SAM) in German physicians with key expertise in pediatrics. Children (Basel). 2022;9(10):1578. https://doi.org/10.3390/children9101578
6. Işık Metinyurt HA, Yıldırım Sarı H. Awareness of health professionals about child abuse and neglect. Çocuk ve Medeniyet. 2016;1(1):101-21.
7. Dessena B, Mullan PC. A cross-sectional survey of child abuse management knowledge among emergency medicine personnel in Cape Town, South Africa. Afr J Emerg Med. 2018;8(2):59-63. https://doi.org/10.1016/j.afjem.2018.01.005
8. Topçu E, Kazan EE, Küçük S, Murat Y, Alpaslan B, Molozoğlu H, et al. The levels of knowledge of nursing students related to the identification to the symptom and risks of child abuse and neglect. J Higher Educ Sci. 2022;12(2):264-73.
9. Türkkan T, Çakıcı AB, Bülbül K. Investigation of the knowledge level of social work students about child abuse and neglect: the case of Gumushane University. Toplum ve Sosyal Hizmet. 2020;31(2):368-88.
10. Soares Junior JM, Oliveira HMC, Luquetti CM, Zuchelo LTS, Arruda Veiga EC, Raimundo JZ, et al. Adolescents' knowledge of HPV and sexually transmitted infections at public high schools in São Paulo: a cross-sectional study. Clinics (Sao Paulo). 2022;77:100138. https://doi.org/10.1016/j.clinsp.2022.100138
11. Uysal A. Determining the knowledge levels of nurses and midwives in diagnosing the symptoms and risks of child abuse and neglect. Unpublished Master's Thesis. Izmir: Ege University; 1998.
12. Güdek Seferoğlu E, Sezici E, Yiğit D. Nursing students' level of recognition of the symptoms and risks of child abuse and neglect. OPUS Int J Soc Res. 2019;10(17):257-76.
13. Pesen A, Epçaçan U. The relationship between teacher candidates' knowledge and risk recognition levels about child abuse and neglect and their decision-making styles. Toplum ve Sosyal Hizmet. 2021;32(1):121-40.
14. Türk B, Hamzaoğlu N, Yayak A, Şenyuva G. Analysis of the level of knowledge and awareness about child abuse and neglect: cross-sectional research. Turkiye Klinikleri J Forensic Med Forensic Sci. 2021;18(3):205-14.

15. Tek S, Karakaş G. Awareness levels of child neglect and abuse in nursing students. International J Soc Res. 2021;18(43):6162-76.
16. Ozbey H, Ozcelep GA, Gul U, Kahriman I. Knowledge and awareness of nursing students about child abuse and neglect. J Nurs Res Pract. 2018;2(3):21-5.
17. Jeong Y, Jang SY, Song MK, Kum DJ, Park SE, Bang KS. Child abuse awareness and reporting intention among nursing and education students. J Korean Acad Soc Nurs Educ. 2019;25(2):216-26.
18. Poreddi V, Pashapu DR, Kathyayani BV, Gandhi S, El-Arousy W, Math SB. Nursing students' knowledge of child abuse and neglect in India. Br J Nurs. 2016;25(5):264-8. https://doi.org/10.12968/bjon.2016.25.5.264
19. Uysal G, Bozkurt G, Sönmez-Düzkaya D. Awareness of child abuse and neglect among students. J Psychiatric Nurs. 2022;13(1):43-8.
20. Altan H, Şahin-Dağlı F, Budakoğlu II. The impact of child abuse and neglect training on knowledge and awareness in university students. Marmara Med J. 2020;33(2):48-53.
21. Moraes SD, Fonseca AM, Soares JM, Bagnoli VR, Souza MA, Ariê WM, et al. Construction and validation of an instrument that breaks the silence: the impact of domestic and/or sexual violence on women's health, as shown during climacterium. Menopause. 2012;19(1):16-22. https://doi.org/10.1097/gme.0b013e3182214e12

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231788

Effect of *Cymbopogon* olivieri-based herbal vaginal product on bacterial vaginosis

Mitra Esmaili[1], Zarrin Sarhadynejad[2], Zohreh Salari[3], Tania Dehesh[4], Mahdiyeh Lashkarizadeh[5], Haleh Tajadini[6], Mohadese Kamali[1]*

SUMMARY

OBJECTIVE: Bacterial vaginosis is the most common vaginal infection in reproductive-age women. If it is not treated, the quality of life will be reduced. In this study, the herbal medicine product *Cymbopogon olivieri* was used for its treatment.

METHODS: This study was conducted with 90 women. The patients were randomly divided into two groups of 45: *Cymbopogon olivieri* and metronidazole. The treatment period was 7 days for each group. Improvement status was determined by eliminating at least three out of four of Amsel's criteria. A new variable with two order levels (negative and positive) was constructed. This new variable shows the status of the treatment process. Chi-square and Fisher's exact tests were used to examine the relationship between the new variable and treatment status.

RESULTS: The results demonstrate that *Cymbopogon olivieri* and metronidazole significantly reduced the burning, itching, malodor, abnormal vaginal discharge, pH, clue cell, and positive whiff test ($p<0.05$). The findings also demonstrate that neither treatment was statistically different from the other for at least three of Amsel's criteria.

CONCLUSION: *This study shows that the effect of Cymbopogon olivieri on bacterial vaginosis is similar to that of metronidazole. Hence, Cymbopogon olivieri* is a suitable option to treat bacterial vaginosis.

KEYWORDS: Bacterial vaginitis. Metronidazole. Medicine, Persian. Herbal medicine. Lemon grass.

INTRODUCTION

Bacterial vaginosis (BV) is characterized by the loss of normal vaginal flora and the overgrowth of facultative anaerobic bacteria[1]. BV is the most common form of vaginal infection in women of reproductive age worldwide[2], and it has been reported from 20 to 60% in different populations[3]. The most common symptom of BV is a bad odor from vaginal discharge[4]. Vaginal examination shows a gray, thin, and homogeneous discharge covering the vaginal walls[5]. Risk factors include low socioeconomic status, poor hygiene, early sexual activity, multiple sexual partners, psychological stress, and biogenetic factors[3]. Metronidazole (orally or vaginally) is the first line of therapy[6]. However, its side effects (nausea, vomiting, abdominal pain, or diarrhea)[7-9] and traditional use of herbal medicines have encouraged researchers to investigate the effectiveness and safety of herbal medicines. Due to its better cultural acceptability, greater adaptability to the human body, and fewer side effects, herbal therapy is the main treatment method for primary care for about 75% of the world's population, especially in developing and developed countries[10]. However, in evidence-based medicine, the traditional use of herbs and expert opinion are considered the lowest level of evidence for the safety and efficacy of drugs. The highest level of evidence comes from randomized clinical trials and unbiased systematic reviews, with or without meta-analysis[11]. One of the plants whose antimicrobial and antioxidant effects have been shown in non-clinical studies is Lemongrass [*Cymbopogon olivieri (Boiss.)*][12]. *Cymbopogon* genus is a member of the family of *Gramineae*[13]. Mahboubi and Kazempour[13] and Tibenda et al.[14] showed these effects for

[1]Kerman University of Medical Sciences, Faculty of Persian Medicine, Herbal and Traditional Medicines Research Center, Department of Traditional Medicine – Kerman, Iran.
[2]Kerman University of Medical Sciences, Faculty of Persian Medicine, Herbal and Traditional Medicines Research Center, Department of Traditional Pharmacy – Kerman, Iran.
[3]Kerman University of Medical Sciences, Afzalipour School of Medicine, Obstetrics and Gynecology Center – Kerman, Iran.
[4]Kerman University of Medical Sciences, School of Public Health, Department of Biostatistics and Epidemiology – Kerman, Iran.
[5]Kerman University of Medical Sciences, School of Medicine, Department of Pathology and Stem Cell Research Center – Kerman, Iran.
[6]Kerman University of Medical Sciences, Faculty of Persian Medicine, Medical Mycology and Bacteriology Research Center, Department of Traditional Medicine – Kerman, Iran.
*Corresponding author: mohadesekamali0912@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on March 11, 2024. Accepted on March 17, 2024.

this plant. As no clinical study has investigated the effect of this plant on BV, *C. olivieri* was selected for this clinical trial.

METHODS

Study design

This study was a clinical trial using the double-blind, randomized, quadraplexes one-to-one block method, which was designed by the epidemiologist and conducted on married women aged 18–50 years in Kerman, Iran, from December 2021 to November 2022. The trial participants were divided into two groups: *C. olivieri* and metronidazole. It was performed following Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Ethical consideration

The trial protocol adhered to the Declaration of Helsinki criteria and was approved by the Medical Ethics Committee of Kerman University (No. IR.KMU.REC.1400.547). It was also registered with the Iranian Registry of Clinical Trials (No. IRCT20211111053036N1).

Preparation of herbal medicine

Cymbopogon olivieri was collected from farms in Kerman (30.3°N, 57.0°E) from March to April 2021. An herbalist verified the authenticity of the plant. Herbarium number in the Natural Resources Research Center in Kerman, Iran, is 5662. A dried hydroalcoholic extract of *C. olivieri* (ethanol 70%) was utilized to make the formulation. Samples underwent standardization testing and microbiological contamination research. Similar dose forms, hard gelatin capsules containing powdered metronidazole, were utilized to blind the medications. The final preparations were labeled and coded. Researchers and patients did not know the contents of the packages.

Assessment of microbial contamination

The microbial contamination was evaluated with the total number of live microorganisms[15].

Determining the amount of essential oil and plant extract

The essential oil was prepared from the dried leaves and stem of *C. olivieri* by the method of hydro distillation with a Clevenger apparatus.

Total polyphenol content determination

This test was performed on plant extract by the Folin-Ciocalteu method, and the standard solution of gallic acid (in concentrations of 12.5, 25, 50, 100, and 200 μg/mL) was used for calibration curve[15].

Determination of total ash and moisture content and pH

The usual methods of The United States Pharmacopeia were used for determining of total ash and moisture content of the prepared formulation. Also, a pH meter was used to measure the acidity or alkalinity of the aqueous solution of final preparation[15].

GC/MS analysis

The gas chromatography device used in the study was an Agilent 6890 type with specific column specifications. The essential oil sample was diluted and injected into the GC/MS machine, and the temperature was controlled in a specific manner during the analysis. The mass spectrometer used was an Agilent 5973 model with specific settings. The spectra obtained were compared with reference books and articles to identify the components of the essential oil[13].

Sample size

A pilot study with 60 patients was conducted (30 patients in each group). The final sample size was calculated based on the results of the pilot study, with 90 patients (45 patients in each group). The outcome of the sample size calculation was the number of improved patients according to at least three symptoms. In fact, the patients who have an improvement in at least three symptoms (burning, itching, malodor, abnormal vaginal discharge, pH, clue cell, and positive whiff test) were considered improved. The number of improved patients is the outcome of the calculation of the final sample size. The sample size was calculated with the PASS software version 15.

Randomization and allocation concealment

The mechanism for implementing the allocation sequence was performed with sealed envelopes. The doctor visited the patient, opened the envelope, and allocated the code to the patient. Then, the patient was referred to the nurse and received the drug according to the code. The patient's name and code were registered. The doctor and the nurse did not know the content of the codes and drugs. Drugs had exactly a similar shape in both codes. We tried to remind the patient about the importance of treatment by phone every day.

Inclusion and exclusion criteria

Inclusion criteria were as follows: married women between 18 and 50 years old, ready to go for examinations, consent to participate in the study, and not using antibiotics and other vaginal creams or suppositories in the last 2 weeks before entering the study. Exclusion criteria were as follows: any complications during treatment, pregnancy, or breastfeeding; having a complex

and recurrent BV infection diagnosed by a gynecologist; the presence of chronic diseases; use of immunosuppressants or immunodeficiency disease; and use of oral contraceptive pill (OCP).

Intervention

The patients in the herbal product group were advised to use one hard gelatin capsule, size 00, containing 500 mg hydroalcoholic extract of *C. olivieri* vaginally with an applicator at bedtime for seven nights. The other group received 500 mg metronidazole powder capsules like the herbal product group. The demographic data of patients were entered into a premade checklist. After history-taking, vaginal samples were taken in a lithotomy position with a disposable, sterile specimen without lubricant. The vagina and cervix of each patient were examined by a gynecologist and any abnormal evidence was noted in the checklist. Using a sterile swab, samples were taken from the top portion of the vagina's lateral wall, and the discharge specimen was then put on two slides. The pathology lab received one slide with a fixative on it for microscopic examination. The second specimen was mixed with one drop of potassium hydroxide (KOH) at 10%. The pH meter paper with a range of 1–14 made by Merck Germany Company was used to measure the pH. The researcher tested the vaginal pH 1 min after the pH-meter strip contacted the vaginal wall. Clinical criteria were entered in the checklist too. The researcher changed the color and then compared it with the box's regular color. A gynecologist confirmed the uniformity of the vaginal samples. Every day, a phone call was used to inquire about the patients' symptom intensity, which was then noted in the checklist. The patients returned 10 days after the completion of the treatment. The clinical criteria and the patient's complaints were re-evaluated.

Outcome measures

Clinical diagnosis of BV was based on Amsel's criteria, which include pH>4.5, a positive whiff test, grayish-white homogeneous discharge, and the presence of Clue cells >20%[16]. Abnormal evidence, any inflammation, and vaginal discharge were examined in terms of color, texture, and malodor. Outcome measures were burning, itching, malodor, abnormal vaginal discharge, pH, clue cell, and positive whiff test. The absence of at least three of Amsel's criteria after the end of treatment was considered treatment improvement, and any other result was considered treatment failure.

Statistical analysis

The outcome variable was categorical with two levels (yes and no), the postcode was subtracted from the previous code, and a new variable with two order levels (negative and positive) was constructed. This new variable shows the status of the treatment process. Chi-square and Fisher's exact tests were used to examine the relationship between the new variable and treatment status.

RESULTS

Evaluation of herbal medicine microbial contamination

Microbial tests were run on the final formulation after extracting and preparing the final sample. The findings revealed that *C. olivieri*'s fungal and microbial contamination levels were within The United States Pharmacopeia's permissible limit. Also, the absence of pathogenic pathogens, especially *Candida albicans, Pseudomonas aeruginosa, and Staphylococcus aureus*, in the final product of *C. olivieri* was confirmed.

Amount of essential oil and extractable matter

Notably, 2.5 mL of essential oil was obtained per 100 g of the dried plant (2.5% w/v) and 11 g of hydroalcoholic extract was obtained per 100 g of the dry mass of the plant (11% w/w).

Physicochemical evaluation of the final product of Cymbopogon olivieri

pH is 5.33, total ash is 13.25%, and the moisture content in the final product of *C. olivieri* is 9.59% w/w.

Determination of the total amount of phenolic compounds based on gallic acid in the final product of Cymbopogon olivieri

The total amount of phenols in the final product of *C. olivieri* was 88/86±6/50 mg gallic acid/g.

Analysis of essential oil of Cymbopogon olivieri

Analysis was performed by gas chromatography and GC/MS mass spectrometer. The analysis is shown in Table 1. The most components are in order: β-Eudesmol (14.42%), ρ-2-menthen (11.84%), Elemol (9.81%), and Agarospirol (6.49%).

Evaluation of capsule disintegration time

The disintegration time of *C. olivieri* in water is 7 min, and in pH 4.6, it is 7 min.

Baseline and demographic characteristics

A total of 116 patients were assessed for eligibility and 26 of them were excluded. At the end of the study, 90 women with BV, 45 of whom were in the *C. olivieri* group and 45 in the

Table 1. Gas chromatography-mass spectrometry (GC/MS) analysis

No.	RT	%	Components	KI	Type
1	10.75	0.14	Tricyclene	926	MH
2	11.29	0.25	α-Pinene	939	MH
3	12.17	0.58	Camphene	954	MH
4	14.67	2.8	δ-2-Carene	1002	MH
5	15.13	0.73	α-Phellandrene	1003	MH
6	15.66	0.29	α-Terpinene	1017	MH
7	16.16	0.95	ρ-Cymene	1024	MH
8	16.32	1.12	Limonene	1029	MH
9	16.44	1.69	β-Phellandrene	1029	MH
10	16.66	0 16	(E)-β-Ocimene	1050	MH
11	19.72	0.07	ρ-Cymenene	1091	MH
12	20.08	0.14	Linalool	1096	MO
13	21.21	0.06	Fenchol	1119	MO
14	21.44	11.84	ρ-2-Menthen-l-ol	1119	MO
15	22.07	0.1	trans-ρ-Mentha-2,8-dien-1 -ol	1122	MO
16	22.38	7.41	1-Terpineol	1133	MO
17	23.93	0.93	ρ-Mentha-1,5-dien-8-ol	1170	MO
18	24.25	0.09	Terpinen-4-ol	1177	MO
19	24.75	0.62	ρ-Cymen-8-ol	1182	MO
20	25.06	3.89	cis-Piperitol	1198	MO
21	25.69	4.48	trans-Piperitol	1208	MO
22	27.94	1.11	Piperitone	1252	MO
23	31.68	0.05	α-Cubebene	1351	SH
24	33.02	0.22	α-Copaene	1376	SH
25	33.38	0.16	β-Bourbonene	1388	SH
26	33.62	1.03	β-Elemene	1390	SH
27	34.97	0.08	(E)-Caryophyllene	1419	SH
28	36.38	0.05	β-Barbatene	1442	SH
29	37.32	0.38	γ-Muurolene	1479	SH
30	37.61	0.73	Germacrene D	1481	SH
31	37.73	0.67	4, 1 1-selinadiene	1485	SH
32	37.99	0.66	β-Selinene	1490	SH
33	38.08	0.66	Valencene	1496	SH
34	38.26	0.77	α-Selinene	1498	SH
35	38.72	0.12	α-Chamigrene	1503	SH
36	38.87	0.25	Cuparene	1504	SH
37	38.94	0.25	γ-Cadinene	1513	SH
38	39.08	0.92	δ-Cadinene	1523	SH
39	39.23	0.35	7-epi-α-Sclinene	1526	SH
40	39.35	0.21	cis-Calamenene	1532	SH
41	40.43	9.81	Elemol	1549	SO

Continue...

Table 1. Continuation.

No.	RT	%	Components	KI	Type
42	41.83	0.13	Caryophyllene oxide	1583	SO
43	42.79	2.17	Eudesmol <5-epi-7-epi-α->	1607	SO
44	43.75	2.84	γ-Eudesmol	1632	SO
45	43.89	6.49	Agarospirol	1642	SO
46	44.25	1.34	Hinesol	1641	SO
47	44.72	14.42	β-Eudesmol	1650	SO
48	44.92	0.97	7-epi-α-Eudesmol	1663	SO
49	45.12	9.49	β-Maaliene	1671	SH
		94.67	Total Identified		

MH: monoterpene hydrocarbons; MO: oxygenated monoterpenes; SH: sesquiterpene hydrocarbons; SO: oxygenated sesquiterpene.

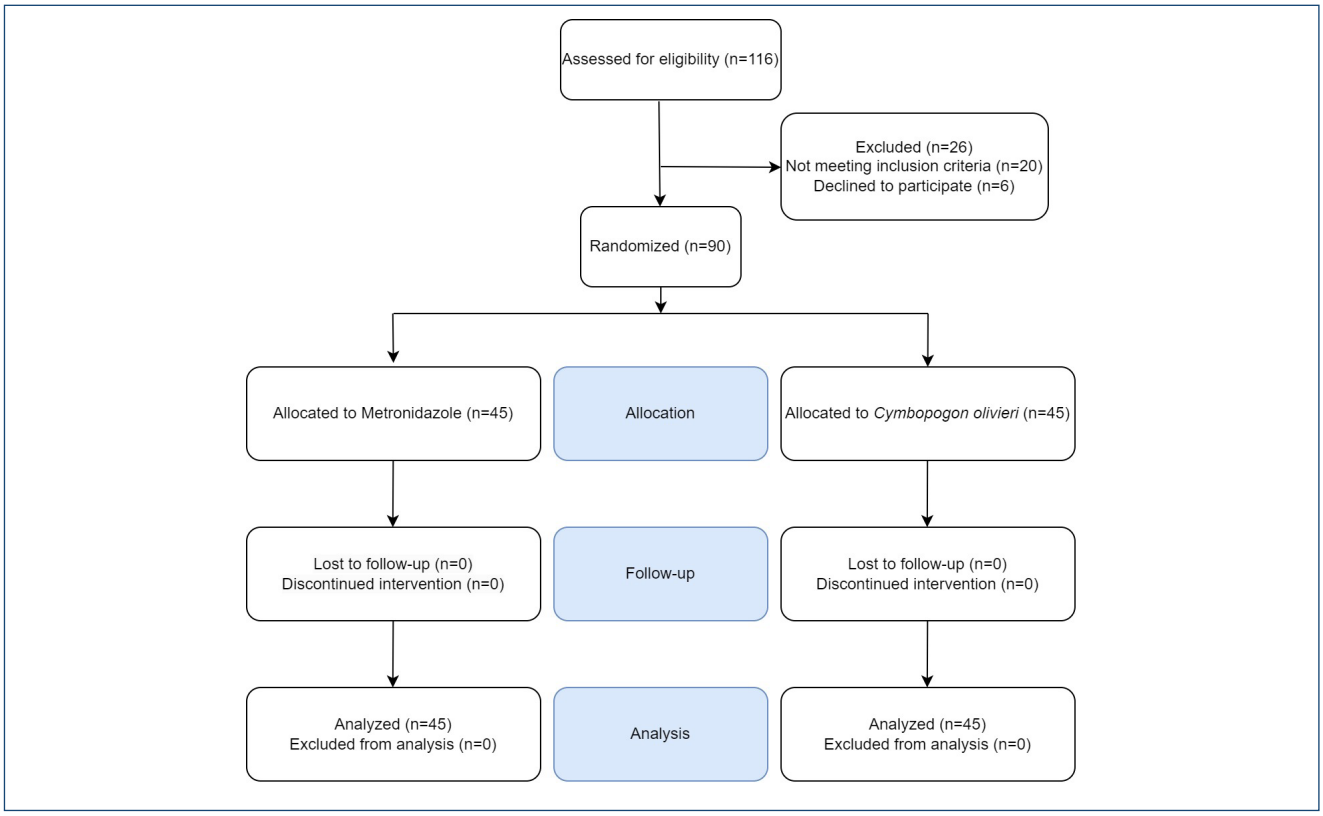

Figure 1. Flow diagram of the study.

metronidazole group, completed the study. The study process is illustrated in Figure 1.

The findings indicate that there is no difference in any of the demographic factors between the metronidazole and *C. olivieri* groups. Patients who received *C. olivieri* had an average age and marriage average of 30.86 and 18.73 years, respectively, while patients who received metronidazole had an average age and marriage average of 32.60 and 18.93 years, respectively. Patients who received *C. olivieri* had 4.09, 3.35, and 0.73 pregnancies, children, and abortions, respectively. Patients who received metronidazole had 4.82, 4.3, and 0.57 pregnancies, children, and abortions, respectively. Table 2 shows the outcomes.

Clinical outcomes

Clinical results are shown in Table 3. According to the McNemar test, the use of *C. olivieri* and metronidazole has

Table 2. Comparison of baseline demographic characteristics of the *Cymbopogon olivieri* and metronidazole groups before intervention.

Demographic characteristics	*Cymbopogon olivieri* Mean±SD	Metronidazole Mean±SD	p-value
Age	30.86±7.56	32.60±6.74	0.645
Age of marriage	18.73±3.21	18.93±2.81	0.438
Number of pregnancies	4.09±2.87	4.82±2.86	0.445
Number of children	3.35±2.52	4.3±2.68	0.138
Number of abortions	0.73±1.17	0.57±0.86	0.742

Table 3. Comparison of signs, symptoms, and Amsel's criteria before and after the test in *Cymbopogon olivieri* and metronidazole groups.

Variables		*Cymbopogon olivieri*			Metronidazole			p-values* (comparison of drugs after treatment)
		Before n (%)	After n (%)	p-value	Before n (%)	After n (%)	p-value	
Vaginal burning	No	0 (0)	42 (93.3)	<0.001	2 (4.4)	38 (84.4)	<0.001	0.180
	Yes	45 (100)	3 (6.7)		43 (95.6)	7 (15.6)		
Itching	No	0 (0)	43 (95.6)	<0.001	8 (17.8)	39 (86.7)	<0.001	0.138
	Yes	45 (100)	2 (4.4)		37 (82.2)	6 (13.3)		
Malodor	No	3 (6.7)	40 (88.9)	<0.001	9 (20)	41 (91.1)	<0.001	0.725
	Yes	42 (93.3)	5 (11.1)		36 (80)	4 (8.9)		
Abundant vaginal discharge	No	0 (0)	38 (84.4)	<0.001	0 (0)	36 (80)	<0.001	0.581
	Yes	45 (100)	7 (15.6)		45 (100)	9 (20)		
pH	<4.5	4 (8.9)	42 (93.3)	<0.001	0 (0)	40 (88.9)	<0.001	0.459
	>4.5	41 (91.1)	3 (6.7)		45 (100)	5 (11.1)		
Whiff test	Negative	3 (6.7)	40 (88.9)	<0.001	4 (8.9)	42 (93.3)	<0.001	0.459
	Positive	42 (93.3)	5 (11.1)		41 (91.1)	3 (6.7)		
Clue cell	Negative	5 (11.1)	40 (88.9)	<0.001	5 (11.1)	42 (93.3)	<0.001	0.459
	Positive	40 (88.9)	5 (11.1)		40 (88.9)	3 (6.7)		

*Chi-square test.

significantly reduced the burning, itching, malodor, abnormal vaginal discharge, pH, clue cell, and positive whiff test (significance level less than 0.05). The results show that both treatments were effective in eliminating at least three out of four Amsel's criteria, and no statistically significant difference was found among them. The chi-square test shows that after taking the drugs *C. olivieri* and metronidazole, the number of improved patients in terms of burning, itching, malodor, abnormal vaginal discharge, pH, whiff test, and clue cell is not significantly different.

The results show that after taking *C. olivieri* product and metronidazole, variables of burning, itching, malodor, abnormal vaginal discharge, pH, whiff test, and clue cell are not significantly different from each other. Therefore, the effect of the herbal medicine of *C. olivieri* on the problems of patients with BV is the same as the effect of metronidazole.

DISCUSSION

This study showed that *C. olivieri* is as effective as metronidazole in the treatment of BV. Based on the study of Azizian Sharme et al., *C. olivieri* methanolic extract had the highest amount of phenolic and flavonoid compounds and the essential oil analysis shows the main compounds were piperitone, 2-carene, and D-limonene. Phenolic, flavonoid, and terpenoid compounds have a high inhibitory effect against free radicals (antioxidant activity) and pathogenic pathogens (bacteria and fungi)[17]. The antibacterial impact of essential

oils on Gram-positive bacteria is most likely due to the cytoplasm of the microbe releasing from the cell walls, which in effect causes the bacterium to become inactive. The basic mechanism of the effect of essential oils can be considered the inhibition of the synthesis of DNA, RNA, proteins, and polysaccharides[18]. Mahboubi M. et al.'s study showed that *C. olivieri* had an antibacterial effect on *Acinetobacter* sp., and even the antimicrobial effect of this plant was greater than that of other plants in that research[19]. The good effect of the plant on the symptoms and clinical criteria of Amsel seems to be due to its antibacterial activity. Some studies show that *Cymbopogon schoenanthu* has anti-inflammatory properties, which also confirm the improvement of the clinical symptoms of patients, including burning and itching[20]. Other similar studies have investigated the effect of other herbs or probiotics[21,22] on BV. In the study of Baery et al., vaginal suppositories comprising the plants of *Tribulus terrestris, Myrtus communis, Foeniculum vulgare*, and *Tamarindus indica* were used for treatment. The amount of abnormal vaginal discharge, Amsel criteria, pelvic pain, and cervical inflammation were significantly reduced in the group of herbal medicine and metronidazole (p=0.001). There was no statistically significant difference between the two groups of metronidazole and herbal medicine in any of the clinical symptoms or laboratory evaluations[23]. In the study of Alizadeh et al., the effect of *Hypericum perforatum L.* on BV was investigated and compared with metronidazole. In 10–12 days, the recovery rate was 82% in the *H. perforatum* group and 85% in the metronidazole group[24]. These studies show that plants can be used to treat BV along with antibiotics. Also, in this study, *C. olivieri* was good at treating BV. As a result, the treatment of BV by herbal medicine such as *C. olivieri* can alternatively be recommended. The limitations of this study included the limited time for follow-up, the low sample size, and the use of a single dose of the herbal formulation. It is suggested to conduct studies with larger sample sizes in this field and to follow up the patients after the study.

CONCLUSION

This study demonstrates that *C. olivieri* is effective in lowering the clinical symptoms of BV. Its effect was comparable to that of metronidazole. These results are obtained due to its antimicrobial, anti-inflammatory, and antioxidant effects.

ETHICAL COMMITTEE

The study was approved by the Medical Ethics Committee of Kerman University (No. IR.KMU.REC.1400.547).

ACKNOWLEDGMENTS

This study was based on a research proposal approved by Kerman University of Medical Sciences (400000711). We would like to thank them for their support.

AUTHORS' CONTRIBUTIONS

MK: Conceptualization, Investigation, Validation, Writing – original draft, Writing – review & editing. **ME:** Data curation, Investigation, Methodology, Project administration, Validation, Writing – original draft. **TD:** Formal Analysis. **ZaS:** Investigation. **ML:** Investigation. **ZoS:** Resources, Writing – review & editing. **HT:** Writing – review & editing.

REFERENCES

1. Afzali E, Siahposh A, Haghighi-Zadeh MH, Farajzadeh A, Abbaspoor Z. The effect of Quercus (Oak Gal) vaginal cream versus metronidazole vaginal gel on bacterial vaginosis: a double-blind randomized controlled trial. Complement Ther Med. 2020;52:102497. https://doi.org/10.1016/j.ctim.2020.102497
2. Peebles K, Velloza J, Balkus JE, McClelland RS, Barnabas RV. High global burden and costs of bacterial vaginosis: a systematic review and meta-analysis. Sex Transm Dis. 2019;46(5):304-11. https://doi.org/10.1097/OLQ.0000000000000972
3. Coudray MS, Madhivanan P. Bacterial vaginosis-A brief synopsis of the literature. Eur J Obstet Gynecol Reprod Biol. 2020;245:143-8. https://doi.org/10.1016/j.ejogrb.2019.12.035
4. Abou Chacra L, Fenollar F, Diop K. Bacterial vaginosis: what do we currently know? Front Cell Infect Microbiol. 2022;11:672429. https://doi.org/10.3389/fcimb.2021.672429
5. Sultana A, Baig K, Rahman K, Mehdi S, Heyat BB, Akhtar F, et al. Contemporary overview of bacterial vaginosis in conventional and complementary and alternative medicine. Comput Intell Healthc Appl. 2022;33-53. https://doi.org/10.1016/B978-0-323-99031-8.00024-7
6. Cerca N. Addressing the challenges with bacterial vaginosis pharmacotherapy. Expert Opin Pharmacother. 2023;24(1):11-3. https://doi.org/10.1080/14656566.2022.2082285
7. Ayinde O, Ross JD. The frequency and duration of side-effects associated with the use of oral metronidazole; a prospective study of VITA trial participants. Int J STD AIDS. 2023;34(12):897-902. https://doi.org/10.1177/09564624231179505
8. Brandt M, Abels C, May T, Lohmann K, Schmidts-Winkler I, Hoyme UB. Intravaginally applied metronidazole is as effective as orally applied in the treatment of bacterial vaginosis, but exhibits significantly less side effects. Eur J Obstet Gynecol Reprod Biol. 2008;141(2):158-62. https://doi.org/10.1016/j.ejogrb.2008.07.022

9. Reiter S, Kellogg Spadt S. Bacterial vaginosis: a primer for clinicians. Postgrad Med. 2019;131(1):8-18. https://doi.org/10.1080/00325481.2019.1546534
10. Wachtel-Galor S, Benzie IF. Herbal medicine. In: Herbal medicine: biomolecular and clinical aspects. 2nd ed. CRC Press/Taylor & Francis; 2011.
11. Moreira DL, Teixeira SS, Monteiro MHD, Oliveira ACAX, Paumgartten FJR. Traditional use and safety of herbal medicines. Rev Bras Farmacogn. 2014;24(2):248-57. https://doi.org/10.1016/j.bjp.2014.03.006
12. Kahnouji Z, Sharifi-Sirchi GR, Sassan HA. The genetic and phytochemical diversity of four populations of lemongrass (*Cymbopogon olivieri*) from Southeast Iran. J Appl Biotechnol Rep. 2017;4(3):657-60.
13. Mahboubi M, Kazempour N. Biochemical activities of Iranian *Cymbopogon olivieri* (Boiss) Bor. essential oil. Indian J Pharm Sci. 2012;74(4):356-60. https://doi.org/10.4103/0250-474X.107071
14. Tibenda JJ, Yi Q, Wang X, Zhao Q. Review of phytomedicine, phytochemistry, ethnopharmacology, toxicology, and pharmacological activities of *Cymbopogon* genus. Front Pharmacol. 2022;13:997918. https://doi.org/10.3389/fphar.2022.997918
15. United States Pharmacopeial Convention (editor). The United States Pharmacopeia, USP 41/The National Formulary. Rockville (MD): US Pharmacopeial Convention; 2018.
16. Sha BE, Chen HY, Wang QJ, Zariffard MR, Cohen MH, Spear GT. Utility of Amsel criteria, Nugent score, and quantitative PCR for *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Lactobacillus* spp. for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. J Clin Microbiol. 2005;43(9):4607-12. https://doi.org/10.1128/JCM.43.9.4607-4612.2005
17. Azizian Shermeh O, Taherizadeh M, Valizadeh M, Zaboli A. Investigation of antioxidant and antimicrobial activities and phytochemical Compounds of essential oil and different extracts of *Cymbopogon olivieri (Boiss.) Bor.* from Sistan and Baluchestan province. JSUMS 2018;25(2):279-92.
18. Hashim GM, Almasaudi SB, Azhar E, Al Jaouni SK, Harakeh S. Biological activity of Cymbopogon schoenanthus essential oil. Saudi J Biol Sci. 2017;24(7):1458-64. https://doi.org/10.1016/j.sjbs.2016.06.001
19. Mahboubi M, Feizabadi MM. Antimicrobial activity of some essential oils alone and combination with amikacin against *Acinetobacter* sp. J Microbiol Biotechnol Food Sci. 2021;2021:412-5. https://doi.org/10.1016/j.sjbs.2016.06.001
20. Gomes E, Bernardo J, Barbosa M, Andrade PB, Valentão P, Lopes G. Ethnopharmacological use of *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon schoenanthus* (L.) Spreng.: anti-inflammatory potential of phenol-rich extracts: PS129. Porto Biomed J. 2017;2(5):216-7. https://doi.org/10.1016/j.pbj.2017.07.099
21. Silva VF, Refinetti P, Vicariotto F, Baracat EC, Soares Junior JM. Oral probiotics and vaginal microbiome in post-menopause women: an opinion for the improvement of natural therapies in gynecology. Rev Assoc Med Bras (1992). 2024;70(2):e702EDIT. https://doi.org/10.1590/1806-9282.702EDIT
22. Silva VF, Refinetti P, Junior JMS, Baracat EC, Pacchetti B. Vaginal microbiome and a prospective analysis of vaginal microbiome testing and personalized probiotics supplementation as a beneficial approach to unlock optimal health for women: a narrative review. Eur Gynecol Obstetr. 2023;5(2):49-54.
23. Baery N, Ghasemi Nejad A, Amin M, Mahroozade S, Mokaberinejad R, Bioos S, et al. Effect of vaginal suppository on bacterial vaginitis based on Persian medicine (Iranian traditional medicine): a randomised double blind clinical study. J Obstet Gynaecol. 2018;38(8):1110-4. https://doi.org/10.1080/01443615.2018.1445706
24. Mohammad-Alizadeh-Charandabi S, Mohammadzadeh Z, Farshbaf-Khalili A, Javadzadeh Y. Effect of *Hypericum perforatum* L. compared with metronidazole in bacterial vaginosis: a double-blind randomized trial. Asian Pac J Trop Biomed 2014;4(11):896-902. https://doi.org/10.12980/APJTB.4.201414B160

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240025

Biochanin A restored the blood–brain barrier in cerebral ischemia-reperfusion in rats

Abdulmutalip Karaaslanli[1], Fırat Aşir[2], Görkem Tutal Gürsoy[3], Mehmet Cudi Tuncer[4]*

SUMMARY

OBJECTIVE: Blood–brain barrier is a protective layer that regulates the influx and efflux of biological materials for cerebral tissue. The aim of this study was to investigate the effects of Biochanin A on cerebral histopathology and blood–brain barrier immunohistochemically.

METHODS: A total of 24 rats were assigned to three groups: sham, ischemia-reperfusion, and ischemia-reperfusion+Biochanin A. Ischemia-reperfusion was performed by occluding the left carotid artery for 2/24 h. Notably, 20 mg/kg Biochanin A was administered to rats for 7 days after ischemia-reperfusion. Blood was collected for malondialdehyde and total oxidant/antioxidant status analysis. Cerebral tissues were processed for histopathology and further for immunohistochemical analysis.

RESULTS: Malondialdehyde content with total oxidant status value was significantly increased and total antioxidant status values were significantly decreased in the ischemia-reperfusion group compared with the sham group. Biochanin A treatment significantly improved scores in the ischemia-reperfusion+Biochanin A group. The normal histological appearance was recorded in the cerebral sections of the sham group. Degenerated neurons and vascular structures with disrupted integrity of the cerebral cortex were observed after ischemia-reperfusion. Biochanin A alleviated the histopathology in the cerebrum in the ischemia-reperfusion+Biochanin A group. Ischemia-reperfusion injury decreased the expression of blood–brain barrier in the ischemia-reperfusion group compared to the sham group. Administration of Biochanin A upregulated the blood–brain barrier immunoreactivity in the cerebrum by restoring blood–brain barrier.

CONCLUSION: Cerebral ischemia-reperfusion caused an increase in oxidative stress and pathological lesions in the cerebrum. Biochanin A treatment restored the adverse effects of ischemia-reperfusion injury by restoring blood–brain barrier.

KEYWORDS: Blood–brain barrier. Artery occlusion. Antioxidant. Cerebrum. Endothelium.

INTRODUCTION

Brain is an element of the central nervous system that contains numerous nerve cells. Homeostasis and function of the brain are important to elucidate brain damage[1,2]. The cross-ancestry genetic risk score has been reported to predict ischemic stroke independently of clinical risk factors and outperform previous genetic risk assessment[3]. It has been stated that Biochanin A (BCA) shows protective effects in angiotensin II-induced model rats and may cause an increase in endophilin A2 expression and a decrease in angiotensin II type 1 receptor expression due to the inhibition of inflammatory responses[4]. BCA (C16H12O5) is an O-methylated natural flavonoid found in red clover, chickpeas, and other legumes, belonging to the phytoestrogen family[5]. Recent studies showed that BCA has various pharmacological properties, including anti-tumorigenesis, anti-oxidation, anti-inflammatory, and hypoglycemic effects[6-8]. BCA was reported to be effective in the treatment of cerebral Ischemia-reperfusion (IR) injury in rats[9,10]. BCA was also specifically shown to prevent the initiation of inflammatory response and downregulate the expression of pro-inflammatory factors in rats[9,11].

Cerebral IR can clinically cause vasogenic edema and hemorrhagic transformation and may result in mortality if not treated in the acute phase[12]. SMI71 is a specific marker to show rat blood–brain barrier (BBB). Many studies showed that SMI71 could be used to investigate BBB integrity[13]. SMI 71 is an antibody designed for detecting a rat endothelial protein localized in regions containing BBB. SMI71 does not react with endothelial cells in periventricular and peripheral tissues such as the liver, heart, adrenal glands, skeletal muscle, intestine, thymus, lymph nodes, pancreas, thyroid, and skin. Notably, the reactivity with this antibody emerges in newborn rats concurrently with the maturation of the BBB[14,15].

This study aimed to investigate the effectiveness of BCA on the histology of BBB after cerebral IR by examining the expression level of components of the BBB.

[1]Ergani State Hospital, Department of Brain and Neurosurgery – Diyarbakır, Turkey.
[2]Dicle University, Faculty of Medicine, Department of Histology and Embryology – Diyarbkır, Turkey.
[3]Ankara City Hospital, Department of Neurology – Ankara, Turkey.
[4]Dicle University, Faculty of Medicine, Department of Anatomy – Diyarbakir, Turkey.
*Corresponding author: drcudi@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 11, 2024. Accepted on February 12, 2024.

METHODS

Ethical approval and animal housing

All animal experiments were approved by the Animal Experimentation Local Ethics Committee of Dicle University (2023/04). Animals were allowed access to water and food *ad libitum* and housed in cages (12 h/12 h dark/light period, 23±1°C). BCA was purchased from Merck (catalog no: D2016, Germany).

Surgical procedures

All procedures were performed under anesthesia. A total of 24 Wistar albino female rats were assigned to three categories: sham, IR, and IR+ BCA (n=8 per group). The rats were fixed on the operating table in the supine position, and the neckline was cleaned with povidone iodine. Using surgical scissors, a midline incision was made from the upper edge of the sternum to the hyoid bone. The incision area was enlarged using a tissue retractor through the trachea. Then, the paratracheal muscles were dissected, and the common carotid artery (CCA) was observed. CCA was occluded for 2 h via a micro bulldog clamp on the left CCA approximately 1 cm proximal to the carotid bifurcation. After cerebral Ischemia, the clamp was removed, the tissues were placed back to their anatomical location, and the skin and subcutaneous fascia were sutured. Cerebral reperfusion was allowed for 24 h. A 200 mM stock solution was prepared by dissolving BCA in DMSO solution.

1. **Sham group:** Cerebral artery occlusion was not performed. Only the left CCA was isolated and placed back to anatomical location. Animals were given 1 mL of DMSO intraperitoneally for 7 days.
2. **IR group:** Cerebral IR procedure was performed. Animals were given 1 mL of DMSO intraperitoneally for 7 days.
3. **IR+BCA group:** After IR treatment, 20 mg/kg BCA was administered to rats intraperitoneally for 7 days.

Malondialdehyde and total antioxidant status/ total oxidant status

At the end of the experimental protocol (at the end of the seventh day), all animals were sacrificed under anesthesia. Malondialdehyde (MDA, MERCK, catalog no: MAK085), total antioxidant (TAS, mmol Trolox Equiv./L), and oxidant status (TOS, μmol H_2O_2 Equiv./L) kits were commercially purchased (Rel Assay Diagnostics, Turkey). Blood samples of each rat were centrifuged at 2000 rpm for 10 min, and the supernatant was collected. The measurement of MDA, TAS, and TOS was done. Serum plasma of blood samples were further analyzed for MDA, TAS, and TOS levels that were determined according to Durgun et al.[16]

Histological tissue processing

Cerebral tissues were excised for histological sampling. Dissected cerebral samples were further analyzed for histological evaluation. Samples were immersed in zinc-formalin, dehydrated through grading alcohol series, and incubated in paraffin wax. Sections of 5 μm were cut from paraffin blocks and stained for hematoxylin–eosin dye and immunostaining[17].

Immunohistochemical examination

Cerebral sections were dewaxed, hydrated in grading alcohol series, and washed in distilled water. Hydrogen peroxide (H_2O_2; 3%) was dropped on slides to block endogen peroxidase activity. After washing in PBS, sections were incubated with anti-BBB (catalog no: 836804, Biolegend, California, USA), overnight at+4°C. Sections were biotinylated and allowed to react with streptavidin peroxidase solution (Thermo Fischer, USA) for 15 min. After PBS washing, diaminobenzidine (DAB) chromogen was used as a chromogen to observe color change. The reactions were stopped with PBS solution, and sections were counter-stained with hematoxylin dye. Slides were mounted and imaged with Zeiss Imager A2 light microscope. All images were processed and quantified using the ImageJ software. Negative control staining was done similar to the same protocol, but only sections were incubated with PBS instead of an antibody of interest.

Image J analysis

The staining intensity of BBB expression was measured by the Image J software (version 1.53, http://imagej. nih.gov/ij). Measurement was performed by the method of Crowe et al.[18]. Quantification was recorded by analyzing 10 fields from each specimen per group[19]. In specimens, the brown color stands for the positive expression of the antibody of interest, while the blue color represents a negative expression of the antibody of interest. Signal intensity (expression) from a field was calculated by dividing the intensity of the antibody of interest by the whole area of the specimen. A value for staining area/whole area was calculated for each specimen from ten fields. An average value was measured for groups and analyzed for semi-quantitative immunohistochemistry scoring.

Statistical analysis

Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, USA). Data distribution was done by the Shapiro-Wilk test. The data were recorded as median (IQR). The non-parametric Kruskal-Wallis test was used for analyses between more than two groups, and

the post-hoc Dunn test was used due to the small number of animals in the groups. Statistical significance was accepted for values $p<0.05$.

RESULTS

Oxidative stress findings

Statistical analysis of biochemical and histopathologic scores is shown in Table 1. MDA and TOS values were significantly increased in the IR group compared with the sham group. TAS value was statistically decreased in the IR group compared with the sham group. After BCA treatment, MDA and TOS levels statistically decreased and TAS content statistically increased in the IR+BCA group compared with the IR group.

Histopathologic findings

Hematoxylin–eosin staining of cerebral sections is shown in Figures 1A–C. The sham group showed no pathological lesions in the cerebrum. Neurons were histologically normal along with normal vessels (Figure 1A). In the IR group, cerebral cortex integrity was disrupted with degenerated neurons and vascular structures. A high number of cells were with pyknotic nucleus (Figure 1B). Compared with the IR group, BCA treatment restored the cerebral pathologies after IR in the IR+BCA group (Figure 1C). BBB immunoreactivity is shown in Figures 1D–F. High expression of BBB was recorded in the sham group around the blood vessels where the nerve–blood barrier existed (Figure 1D). BBB immunoreactivity was decreased in IR due to the disruption of BBB (Figure 1E). Post-BCA treatment increased the BBB immune activity by restoring the BBB in the cerebral cortex. BBB immune reactivity was intensely observed around the regions where the barrier existed compared with the IR group (Figure 1F). Negative and positive control immunostaining of the cerebral section of the healthy rat is shown in Figures 1G and H, respectively.

Image J analysis

The staining intensity of BBB expression is shown in Figure 2. BBB expression was downregulated after cerebral IR injury. However, BCA treatment upregulated BBB expression with its antioxidant properties.

DISCUSSION

IR injury is listed as one of the causes of tissue damage in clinics such as myocardial infarction, stroke, and organ transplantation. After re-blooding of tissue, more damage occurs in IR as the paradox of IR injury. This process is quite complex and not fully understood yet[20]. During IR injury, the production of reactive oxygen species (ROS) increases, and alterations in mitochondrial homeostasis lead to oxidative damage in tissues and eventually induce the proinflammatory response[21,22]. Medicinal plants with antioxidant activity alleviate the IR injury[23-25]. BCA is a medicinal plant that has a similar action to melatonin[26]. BCA exhibits antioxidant properties, which can help neutralize ROS generated during reperfusion. By scavenging ROS, BCA may reduce oxidative stress and prevent cellular damage[27]. Additionally, IR injury triggers an inflammatory response, leading to tissue damage. BCA with its anti-inflammatory properties may reduce inflammation and favor the production of pro-inflammatory cytokines, attenuating tissue inflammation[28]. BCA has also exerted vasodilatory effects, which may help improve blood flow and tissue perfusion during reperfusion following Ischemia. Enhanced vasodilation can deliver more oxygen and

Table 1. Evaluation of biochemical parameters and Image J analysis of blood–brain barrier signal in groups.

Groups	MDA	TAS	TOS
Sham	1.05 (0.93–1.19)	4.47 (4.20–6.33)	4.63 (3.94–6.02)
IR	7.18 (6.11–7.95)	1.23 (1.12–1.31)	16.71 (15.25–18.37)
IR+BCA	3.33 (2.33–4.43)	1.42 (1.38–1.50)	8.57 (7.43–9.66)
Dunn's test	<0.01*	<0.01*	<0.01*
	0.022**	0.024**	0.022**
Groups	BBB signal	Kruskal-Wallis	Dunn's test
Sham	39.41 (35.20–42.98)	0.025	0.028*
IR	32.91 (26.39–35.33)		0.048**
IR+BCA	37.86 (34.21–40.68)		

Data were presented as median (IQR), *sham vs. IR, **IR vs. IR+BCA.

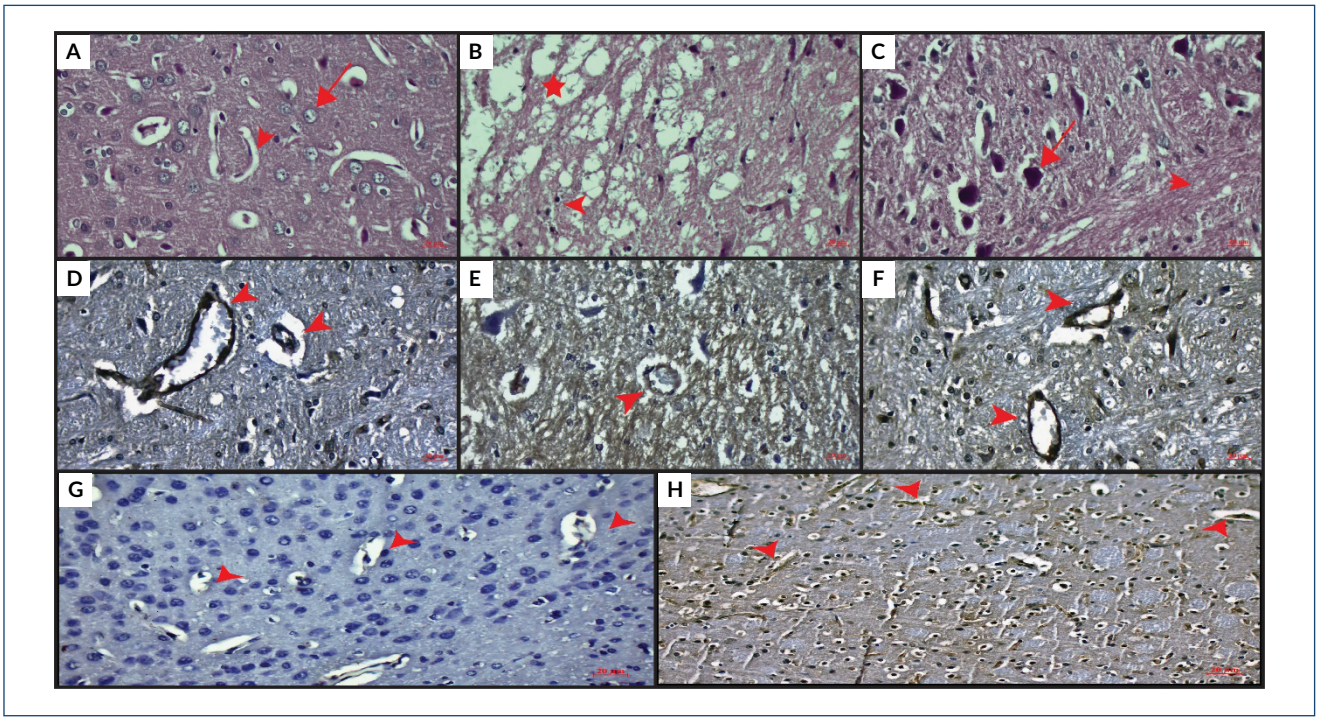

Figure 1. Hematoxylin–eosin staining of the cerebrum. (A) Histologically normal neurons (arrow) and capillaries (arrowhead) in the sham group. (B) Cerebral pathologies in the IR group. Disrupted cortex integrity (star) and pyknotic neurons (arrow). (C) Histologically normal neurons (arrow) and axon fibers (arrowhead). Immunoreactivity of blood–brain barrier in cerebral section (D) Sham group, (E) Ischemia-reperfusion group, and (F) Ischemia-reperfusion+Biochanin A group. Arrowheads: capillaries. (G) Negative control. (H) Positive control of the cerebral section of the healthy rat. Scale bar: 20 µm; Original magnification: 40×.

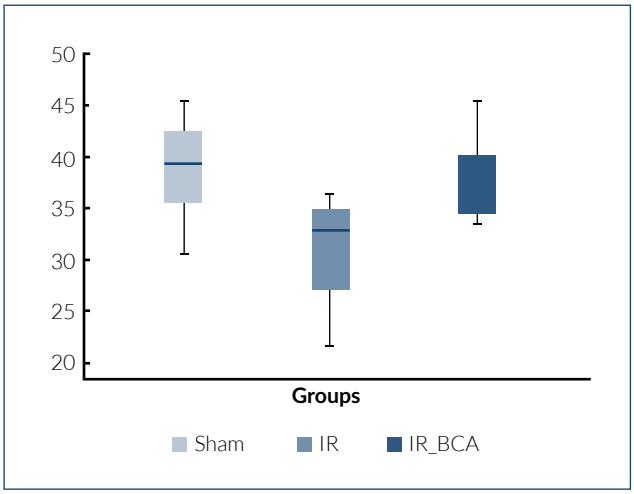

Figure 2. Box plot of blood–brain barrier signal (expression) per group.

nutrient transport to the ischemic area, potentially reducing the extent of injury[29].

This study showed that IR injury increased the MDA content and TOS value and lowered the TAS value. BCA treatment improved the scores because it has many biological activities (Table 1). BCA is a good free radical scavenger, and it induces the antioxidant system after IR, especially its antioxidant properties. IR causes the disruption of the cerebral cortex and degeneration of neurons. Pathological alterations are restored after BCA treatment (Figures 1A–C). Due to the neuroprotective effects of BCA, the cerebral cortex is histologically improved by BCA treatment after IR injury.

BBB protects the delicate nervous tissue from pathogens and microbes and its maintenance is quite vital for cerebral homeostasis. BBB endothelial cells help the regulation of BBB by induction of mechanical induction[30]. BECs are different from other peripheral endothelial cells such as possessing low adhesion molecules, high number of mitochondria, and high polarization[2]. Impairment of BBB causes alteration in the semi-selective permeability of BBB, leading to numerous neurological disorders. IR injury deteriorates the BBB and causes the upregulation of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6)[31]. BCA is a medicinal plant with many pharmacological activities such as anti-inflammatory and antioxidant properties. Guo et al., showed that BCA protected the neural tissue against the cerebral IR via oxidative stress and inflammation pathway[32]. El-Sayed et al.[33] showed that BCA had neuroprotective effects in an epileptic animal model via modulation of inflammatory and autophagy pathways.

In this study, cerebral IR injury caused the disruption of BBB and reduced immunoreactivity of BBB. Administration of BCA upregulated the BBB expression and restored the BBB because its expression was increased compared with the IR group (Table 1, Figures 1D–F).

Although phytotherapy is acknowledged as a healing approach endorsed by national health authorities, it is still not officially recognized as a medical specialty[34]. However, we suggest that BCA treatment may modulate the components of BBB via induction of inflammation pathway and anti-oxidative stress mechanism.

CONCLUSION

Cerebral IR injury causes the generation of free radicals and deteriorates the cerebral histology and BBB. With its antioxidant, BCA treatment reduced the ROS generated during IR injury and promoted the cellular scavenging system. Additionally, with its anti-inflammatory properties, BCA restored the BBB by modulating the inflammatory response pathway after cerebral IR injury.

ETHICS APPROVAL

All animal experiments were approved by the Animal Experimentation Local Ethics Committee of Dicle University (2023/04).

AUTHORS' CONTRIBUTIONS

AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **FA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GTG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MCT:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Firląg M, Kamaszewski M, Gaca K, Adamek D, Bałasińska B. The neuroprotective effect of long-term n-3 polyunsaturated fatty acids supplementation in the cerebral cortex and hippocampus of aging rats. Folia Neuropathol. 2013;51(3):235-42. https://doi.org/10.5114/fn.2013.37708
2. Kadry H, Noorani B, Cucullo L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids Barriers CNS. 2020;17(1):69. https://doi.org/10.1186/s12987-020-00230-3
3. Mishra A, Malik R, Hachiya T, Jürgenson T, Namba S, Posner DC, et al. Stroke genetics informs drug discovery and risk prediction across ancestries. Nature. 2022;611(7934):115-23. https://doi.org/10.1038/s41586-022-05165-3
4. Xue HX, Kong H, Yu YG, Zhou JW, Chen HQ, Yin YY. Biochanin A protects against angiotensin II-induced damage of dopaminergic neurons in rats associated with the increased endophilin A2 expression. Behav Pharmacol. 2019;30(8):700-11. https://doi.org/10.1097/FBP.0000000000000515
5. Biradar SM, Joshi H, Chheda TK. Biochanin-A ameliorates behavioural and neurochemical derangements in cognitive-deficit mice for the betterment of Alzheimer's disease. Hum Exp Toxicol. 2014;33(4):369-82.https://doi.org/10.1177/0960327113497772
6. Mishra P, Kale RK, Kar A. Chemoprevention of mammary tumorigenesis and chemomodulation of the antioxidative enzymes and peroxidative damage in prepubertal Sprague Dawley rats by Biochanin A. Mol Cell Biochem. 2008;312(1-2):1-9. https://doi.org/10.1007/s11010-008-9714-8
7. Zhang S, Morris ME. Effect of the flavonoids Biochanin A and silymarin on the P-glycoprotein-mediated transport of digoxin and vinblastine in human intestinal Caco-2 cells. Pharm Res. 2003;20(8):1184-91.https://doi.org/10.1023/a:1025044913766
8. Harini R, Ezhumalai M, Pugalendi KV. Antihyperglycemic effect of Biochanin A, a soy isoflavone, on streptozotocin-diabetic rats. Eur J Pharmacol. 2012;676(1-3):89-94. https://doi.org/10.1016/j.ejphar.2011.11.051
9. Wang W, Tang L, Li Y, Wang Y. Biochanin A protects against focal cerebral ischemia/reperfusion in rats via inhibition of p38-mediated inflammatory responses. J Neurol Sci. 2015;348(1-2):121-5.https://doi.org/10.1016/j.jns.2014.11.018
10. Chen HQ, Jin ZY, Li GH. Biochanin A protects dopaminergic neurons against lipopolysaccharide-induced damage through inhibition of microglia activation and proinflammatory factors generation. Neurosci Lett. 2007;417(2):112-7. https://doi.org/10.1016/j.neulet.2006.11.045
11. Wu WY, Wu YY, Huang H, He C, Li WZ, Wang HL, et al. Biochanin A attenuates LPS-induced pro-inflammatory responses and inhibits the activation of the MAPK pathway in BV2 microglial cells. Int J Mol Med. 2015;35(2):391-8. https://doi.org/10.3892/ijmm.2014.2020
12. Kapanova G, Tashenova G, Akhenbekova A, Tokpınar A, Yılmaz S. Cerebral ischemia reperfusion injury: from public health perspectives to mechanisms. Folia Neuropathol. 2022;60(4):384-9. https://doi.org/10.5114/fn.2022.120101
13. Freeman LR, Granholm AC. Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. J Cereb Blood Flow Metab. 2012;32(4):643-53. https://doi.org/10.1038/jcbfm.2011.168
14. Lu H, Demny S, Zuo Y, Rea W, Wang L, Chefer SI, et al. Temporary disruption of the rat blood-brain barrier with a monoclonal antibody: a novel method for dynamic manganese-enhanced MRI. Neuroimage. 2010;50(1):7-14.https://doi.org/10.1016/j.neuroimage.2009.12.053
15. Freeman LR, Granholm AC. Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. J Cereb Blood Flow Metab. 2012;32(4):643-53. https://doi.org/10.1038/jcbfm.2011.168

16. Durgun C, Aşır F. Daidzein alleviated the pathologies in intestinal tissue against ischemia-reperfusion. Eur Rev Med Pharmacol Sci. 2023;27(4):1487-93. https://doi.org/10.26355/eurrev_202302_31389
17. Ermiş IS, Deveci E, Aşır F. Effects of quince gel and hesperidin mixture on experimental endometriosis. Molecules. 2023;28(16):5945. https://doi.org/10.3390/molecules28165945
18. Crowe AR, Yue W. Semi-quantitative determination of protein expression using Immunohistochemistry staining and analysis: an integrated protocol. Bio Protoc. 2019;9(24):e3465. https://doi.org/10.21769/BioProtoc.3465
19. Panzan MQ, Mattar R, Maganhin CC, Simões RS, Rossi AG, Motta EL, et al. Evaluation of FAS and caspase-3 in the endometrial tissue of patients with idiopathic infertility and recurrent pregnancy loss. Eur J Obstet Gynecol Reprod Biol. 2013;167(1):47-52. https://doi.org/10.1016/j.ejogrb.2012.10.021
20. Vries DK, Kortekaas KA, Tsikas D, Wijermars LG, Noorden CJ, Suchy MT, et al. Oxidative damage in clinical ischemia/reperfusion injury: a reappraisal. Antioxid Redox Signal. 2013;19(6):535-45. https://doi.org/10.1089/ars.2012.4580
21. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: the evolution of a concept. Redox Biol. 2015;6:524-51. https://doi.org/10.1016/j.redox.2015.08.020
22. Cadenas S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. Free Radic Biol Med. 2018;117:76-89. https://doi.org/10.1016/j.freeradbiomed.2018.01.024
23. Guo MM, Qu SB, Lu HL, Wang WB, He ML, Su JL, et al. Biochanin A alleviates cerebral ischemia/reperfusion injury by suppressing endoplasmic reticulum stress-induced apoptosis and p38MAPK signaling pathway in vivo and in vitro. Front Endocrinol (Lausanne). 2021;12:646720. https://doi.org/10.3389/fendo.2021.646720
24. Bai Y, Li Z, Liu W, Gao D, Liu M, Zhang P. Biochanin A attenuates myocardial ischemia/reperfusion injury through the TLR4/NF-κB/NLRP3 signaling pathway. Acta Cir Bras. 2019;34(11):e201901104. https://doi.org/10.1590/s0102-865020190110000004
25. Wang W, Tang L, Li Y, Wang Y. Biochanin A protects against focal cerebral ischemia/reperfusion in rats via inhibition of p38-mediated inflammatory responses. J Neurol Sci. 2015;348(1-2):121-5. https://doi.org/10.1016/j.jns.2014.11.018
26. Lima E, Soares JM, Carmen Sanabria Garrido Y, Gomes Valente S, Priel MR, Chada Baracat E, et al. Effects of pinealectomy and the treatment with melatonin on the temporal lobe epilepsy in rats. Brain Res. 2005;1043(1-2):24-31. https://doi.org/10.1016/j.brainres.2005.02.027
27. Amri J, Alaee M, Babaei R, Salemi Z, Meshkani R, Ghazavi A, et al. Biochanin-A has antidiabetic, antihyperlipidemic, antioxidant, and protective effects on diabetic nephropathy via suppression of TGF-β1 and PAR-2 genes expression in kidney tissues of STZ-induced diabetic rats. Biotechnol Appl Biochem. 2022;69(5):2112-121. https://doi.org/10.1002/bab.2272
28. Sarfraz A, Javeed M, Shah MA, Hussain G, Shafiq N, Sarfraz I, et al. Biochanin A: a novel bioactive multifunctional compound from nature. Sci Total Environ. 2020;722:722:137907. https://doi.org/10.1016/j.scitotenv.2020.137907
29. Anuranjana PV, Beegum F, K P D, George KT, Viswanatha GL, Nayak PG, et al. Mechanisms behind the pharmacological application of Biochanin-A: a review. F1000Res. 2023;12:107. https://doi.org/10.12688/f1000research.126059.3
30. Nian K, Harding IC, Herman IM, Ebong EE. Blood-brain barrier damage in ischemic stroke and its regulation by endothelial mechanotransduction. Front Physiol. 2020;11:605398. https://doi.org/10.3389/fphys.2020.605398
31. Guo X, Liu R, Jia M, Wang Q, Wu J. Ischemia reperfusion injury induced blood brain barrier dysfunction and the involved molecular mechanism. Neurochem Res. 2023;48(8):2320-34. https://doi.org/10.1007/s11064-023-03923-x
32. Guo M, Lu H, Qin J, Qu S, Wang W, Guo Y, et al. Biochanin A provides neuroprotection against cerebral ischemia/reperfusion injury by Nrf2-mediated inhibition of oxidative stress and inflammation signaling pathway in rats. Med Sci Monit. 2019;25:8975-83. https://doi.org/10.12659/MSM.918665
33. El-Sayed RM, Fawzy MN, Zaki HF, Abd El-Haleim EA. Neuroprotection impact of Biochanin A against pentylenetetrazol-kindled mice: targeting NLRP3 inflammasome/TXNIP pathway and autophagy modulation. Int Immunopharmacol. 2023;115:109711. https://doi.org/10.1016/j.intimp.2023.109711
34. Lopes CMC, Lima SMRR, Veiga ECA, Soares-Jr JM, Baracat EC. Phytotherapeutic medicines: reality or myth? Rev Assoc Med Bras (1992). 2019;65(3):292-4. https://doi.org/10.1590/1806-9282.65.3.292

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240045

Clinical and uterine cervix characteristics of women with *Mycoplasma* and *Ureaplasma* in genital discharge

Margarida Santos Matos[1,2*], Maria Clara Andrade Teles da Silva[1], Milena Bastos Brito[1,2], Ana Katherine Gonçalves[3,4,5]

SUMMARY

OBJECTIVE: The objective of this study was to assess the clinical and uterine cervix characteristics of patients displaying vaginal discharge with positive results for *Mycoplasma* sp. and/or *Ureaplasma* spp.

METHODS: An analytical cross-sectional study involving women aged 18–45 years was conducted. Microbiological assessments included *Ureaplasma* and *Mycoplasma* cultures, as well as human papillomavirus hybrid capture using ecto and endocervix swabs. All tests were two-tailed, and significance was set at $p<0.05$.

RESULTS: Among 324 women, *Ureaplasma* prevalence was 17.9%, and *Mycoplasma* prevalence was 3.1%. The *Ureaplasma*-positive group exhibited a higher frequency of urinary tract infections (39.1 vs. 19%, $p=0.002$) and human papillomavirus (39.7 vs. 12.8%, $p\leq0.001$) compared with controls. The *Mycoplasma*-positive group showed a higher frequency of non-contraceptive use compared with controls (66.2 vs. 30.0%, $p=0.036$). Abnormal colposcopic findings were more prevalent in the *Mycoplasma/Ureaplasma*-positive group than in controls (positive: 65% vs. control: 35%, $p=0.001$). Pap smear findings did not differ between the groups.

CONCLUSION: *Ureaplasma* spp. was associated with urinary tract infections and human papillomavirus, while the presence of *Mycoplasma* sp. was linked to reduced contraceptive use. When analyzing both pathogens together, a higher frequency of abnormal colposcopic findings was observed, with no difference in cytological findings in the positive group.

KEYWORDS: *Mycoplasma*. Uterine cervicitis. HPV. Cervix.

INTRODUCTION

The prevalence of vaginal colonization by *Mycoplasma* sp. and *Ureaplasma* spp. among women tends to increase post-puberty, correlating with the number of sexual partners over their lifetime. While some authors characterize these microorganisms as commensal residents, they are also linked to various pathological conditions, including premature birth, vaginal discharge, urethritis, pelvic inflammatory disease, and infertility[1-3].

Exposure of the cervicovaginal epithelium to *Mycoplasma* sp. and *Ureaplasma* spp. may give rise to a persistent intracellular infection, potentially leading to tissue damage mediated by inflammatory cytokines. Although the relationship between human papillomavirus (HPV) and these microorganisms is not conclusively established, the nature of the infection they cause allows for both direct interaction with HPV during co-infection of a single cell and indirect interaction through cytokine responses[4].

Several studies indicate that the presence of *Mycoplasma* bacteria heightens the risk of more severe cervical lesions, such as low- and high-grade intraepithelial lesions[5]. In addition, women with abnormal cervical cytologies exhibit a 17.6 times greater risk for co-infection with *Mycoplasma hominis* and *Ureaplasma urealyticum*[6].

However, studies assessing the relationship between *Ureaplasma*/*Mycoplasma* and cervical cell changes are controversial. In 2018, a study examined the association between *M. hominis* infection and abnormal cervical cells but found no correlation between bacterial infections and abnormal cervical cytology[7]. Another study investigated the relationship between *Mycoplasma*, *Ureaplasma*, and HPV infections in sex workers,

[1]Bahiana School of Medicine and Public Health, Department of Women's Health – Salvador (BA), Brazil.
[2]Universidade Federal da Bahia, School of Medicine of Bahia, Department of Gynecology and Obstetrics – Salvador (BA), Brazil.
[3]Universidade Federal do Rio Grande do Norte, Postgraduate Program in Health Sciences – Natal (RN), Brazil.
[4]Universidade Federal do Rio Grande do Norte, Postgraduate Program in Applied Sciences to Women's Health – Natal (RN), Brazil.
[5]Universidade Federal do Rio Grande do Norte, Department of Gynecology and Obstetrics – Natal (RN), Brazil.
*Corresponding author: margaridamatos@bahiana.edu.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 08, 2024. Accepted on March 02, 2024.

also failing to identify a correlation between *M. hominis*, *U. urealyticum*, and HPV infection[8].

Contrary to these findings, it has been observed that high-risk HPV (hr-HPV) infection is a necessary cause of cervical cancer. However, other common microbes in the lower genital tract may enhance hr-HPV infection and cervical cytopathy[9]. The association of co-infection between HPV and sexually transmitted infections was compared using cervical samples from women with cervical dysplasia. Significant correlations were found between HPV, sexually transmitted infections, abnormal cervical cytology, HPV status, types of sexually transmitted infections, and the presence of *Ureaplasma* spp. and *M. hominis*[10].

To clarify the importance of *Mycoplasma*/*Ureaplasma* infection in the uterine cervix, this study aimed to describe the gynecological clinical data and uterine cervix alterations in patients presenting with vaginal discharge and positive results for *Mycoplasma* sp. and *Ureaplasma* spp.

METHODS

An analytical cross-sectional study was conducted following the guidelines outlined in the STROBE statement[11]. The study took place between 2022 and 2025 in a private health service located in a region of northeastern Brazil with a Human Development Index (HDI) of 0.63.

The inclusion criteria encompassed women aged 18–45 years, with an active sexual life and complaints of non-physiological vaginal discharge. Exclusion criteria comprised menopausal status, genital bleeding during examination, immunosuppression, pregnancy, incomplete medical records, and hysterectomy.

Clinical data were collected by reviewing patient medical records and documented on a study-specific form. Variables included age, menarche age, number of sexual partners, obstetric history, parity, abortions, contraceptive method, urinary tract infections (UTIs), HPV status, characteristics of vaginal discharge (odor and itching), and cultures for *Ureaplasma* spp. and *Mycoplasma* sp. Reports related to colposcopic, cytological, and microbiological examinations were also consulted.

Colposcopic findings were categorized as normal, abnormal, or miscellaneous. Cytological findings were classified as unsatisfactory, normal (including normal smears and inflammation/cytolysis), and abnormal (ASC-US, ASC-H, ACG, LIEBG, and LIEAG).

The sample selection involved individuals classified as "Positive for *Ureaplasma*" and/or "Positive for *Mycoplasma*" constituting the case group, and those labeled "Negative for *Ureaplasma*" and "Negative for *Mycoplasma*" forming the control group. In addition, the presence of *Candida* sp., *Gardnerella*, and HPV was also investigated.

Diagnosis of *Ureaplasma* and *Mycoplasma* was obtained through microbiological culture using semi-liquid medium A/3 and A/7 specific to these microorganisms. The Sabouraud-Agar culture was utilized for diagnosing fungi and hybrid capture for HPV. The presence of *Gardnerella* was indicated by the identification of clue cells in the Pap smear and the presence of an odor. Patients with dysuria or hematuria underwent a urine culture and antibiogram to evaluate urinary infection.

Numerical and categorical data from the collected information were tabulated and statistically analyzed using the SPSS (Statistical Package for the Social Sciences) program version 14.0 (SPSS Inc., Chicago, IL, USA). An inductive/inferential analysis was conducted to describe the population and compare the groups. The Student's t-test was employed for quantitative variables with a normal distribution, the Mann-Whitney test was used for non-normally distributed quantitative variables, and the chi-square test was used for variables with n>5, with Fisher's exact test applied when n<5 for qualitative variables. All tests were two-tailed, with a significance level set at 5% ($p<0.05$) and a confidence interval of 95%.

Ethics

The study adhered to the ethical and legal standards outlined in Resolution 466/12 of the National Health Council and received approval from the research ethics committee of the Fundação Bahiana para Desenvolvimento das Ciências, under CAAE number 6333520.5.0000.5544. Furthermore, the study was conducted by the Declaration of Helsinki and its subsequent revisions.

RESULTS

Initially, 404 patients were enrolled, with 80 subsequently excluded based on pre-established exclusion criteria. Ultimately, 324 women of reproductive age were selected, among whom 58 tested positive for *Ureaplasma* spp., 10 tested positive for *Mycoplasma* sp., and 256 had negative cultures for these microorganisms.

The prevalence rates were 3.1% (10/324) for *Mycoplasma* sp. and 17.9% (58/324) for *Ureaplasma* spp. Coital activity was more common among individuals aged 10–20 years (81.01%). Most reported having one to five sexual partners, with 67.1% having never been pregnant and 81.8% having no history of abortion. The majority (65.1%) used some form of

contraceptive method. Notably, white discharge without odor or itching was prevalent in the sample (56.8%).

In the bivariate analysis, only the presence of HPV was associated with *Ureaplasma* infection, even after adjusting for confounding variables (OR: 17.42, 95%CI: 3.08–161.2, p=0.004). Regarding *Mycoplasma* infection, only the use of contraceptives proved to be a protective factor (OR: 0.23, 95%CI: 0.005–0.86, p=0.038). Among the patients studied, 211 (65.1%) were using a contraceptive method. The most used method was hormonal contraceptives (56.4%), in both its oral and injectable versions, followed by the variable of patients not using contraceptive methods (34.9%). The male condom was used for 52 (24.6%) of the patients' partners.

Analysis of co-infections revealed that the presence of *Ureaplasma* spp. occurred simultaneously with HPV infection in 39.7% (n=23) of cases, showing a significant difference (p=0.001) and a moderate strength of association (contingency coefficient=0.261). However, there is a higher likelihood of patients with non-physiological genital flow being negative for both infections, accounting for 87.2% (n=232) in our sample. Further examination of HPV positivity within groups revealed an uneven distribution (p=0.001), with a higher frequency of oncogenic HPV. In the *Ureaplasma*-positive group, the frequency of oncogenic HPV was 22.4% (n=13). Other infections did not show differences (Table 1).

Regarding co-infections involving HPV and *Mycoplasma*, it was noted that most cases tested negative for both HPV and *Mycoplasma*, comprising 83.1% (n=261) of the total cases. Among those cases that tested positive for *Mycoplasma*, 60% (n=6) were negative for HPV (OR=1.38). However, this association demonstrated a weak correlation (contingency coefficient=0.104) and lacked statistical significance between the groups (p=0.079). In the case of Fungi and *Gardnerella*, both exhibited higher percentages of negative cases in both groups, those with and without *Mycoplasma*. The analysis of the two infections revealed a greater occurrence of patients in the sample but did not indicate simultaneous infection with *Mycoplasma* (OR Fungi=1.14; OR *Gardnerella*=1.0). Nevertheless, no differences were identified in the studied groups for both fungi (p=0.235) and *Gardnerella* (p=0.403) (Table 2).

DISCUSSION

We observed a prevalence of 3.08% for *Mycoplasma* sp. and 17.9% for *Ureaplasma* spp. among women reporting non-physiological vaginal discharge. The higher prevalence of *Ureaplasma* compared with *Mycoplasma* aligns with previous studies where *Ureaplasma* spp. values ranged from 4.8 to 48.07%, while *Mycoplasma* sp. values varied between 0.8 and 23.4%[12-15].

Contrary to this pattern, some studies reported a higher prevalence of *Mycoplasma* than *Ureaplasma*. For instance, Cardillo found 35.89% for *Mycoplasma* spp. and 25.54% for *U. urealyticum*[15], and Christofolini et al.[16] found 11.3% for *M. hominis* and 0.94% for *U. urealyticum*. Such discrepancies in frequency among studies may result from variations in the populations studied and the techniques used to detect microorganisms[12,17].

Table 1. Description of data on the presence and absence of infections in the group of patients positive or negative for *Ureaplasma* sp.

Variables	n	*Ureaplasma* sp. n (%)		p-value	OR
		Negative	Positive		
HPV				0.001	
Negative	267	87.2% (232)	60.3% (35)		1.44
Positive	57	12.8% (34)	39.7% (23)		0.32
HPV				0.001	
Non-oncogenic	11	3% (8)	5.2% (3)		
Oncogenic	30	6.4% (17)	22.4% (13)		
Non-oncogenic and Oncogenic	16	3.4% (9)	12.1% (7)		
Fungus				0.847	
Negative	293	90% (240)	91.4% (53)		0.99
Positive	30	9.4% (25)	8.6% (5)		1.0
Gardnerella				0.573	
Positive	16	4.9% (13)	5.2% (3)		0.94
Negative	308	93.6% (300)	2.4% (8)		1.0

Table 2. Description of data on the presence and absence of infections in the group of patients positive or negative for *Mycoplasma* sp.

Variables	n	*Mycoplasma* sp. n (%)		p-value	OR
		Negative	Positive		
HPV				0.079	
Negative	267	83.1% (261)	60% (6)		1.38
Positive	57	16.9% (53)	40% (4)		0.42
HPV				0.001	
Non-oncogenic	11	2.5% (8)	30% (3)		
Oncogenic	30	9.2% (29)	10% (1)		
Non-oncogenic and oncogenic	16	5.1% (16)	0% (0)		
Fungus				0.235	
Negative	293	91.1% (285)	80% (8)		1.14
Positive	30	8.9% (28)	20% (2)		0.45
Gardnerella				0.403	
Negative	307	95.2% (298)	90% (9)		1.0
Positive	16	4.8% (15)	10% (1)		0.48

Furthermore, we identified an association between *Ureaplasma* infection and UTIs, consistent with a 2020 meta-analysis by Moridi et al.[17] evaluating the prevalence of *M. hominis*, *Mycoplasma genitalium*, and *U. urealyticum* among Iranian couples. They reported a *U. urealyticum* prevalence of 17.53% and an *M. hominis* prevalence of 9.68%, noting higher infection rates in women with symptoms of genito-UTI compared with men with UTI (7.67% vs. 5.88 and 21.04% vs. 12.13%, respectively).

Recent studies propose a potential interference of *M. hominis*, *U. urealyticum*, and *Ureaplasma parvum* with HPV infection, leading to virus persistence. Some studies found a positive relationship between *U. urealyticum* and HPV, while others reported an overall correlation between *Ureaplasma* spp. and *M. hominis* with HPV9[10,12,14,18]. Our study aligns with these findings, showing a significant relationship between *Ureaplasma* spp. and the presence of HPV. However, contradicting these results, a 2018 Indonesian study concluded no connection between *Ureaplasma* and *Mycoplasma* sp. and HPV[8].

Additionally, a study by Zdrodowska-Stefanow et al.[14] demonstrated that the risk of HPV infection doubled when a woman was infected with any of the four species of *Mycoplasma*. In cases of concomitant *U. urealyticum* infection, the risk of HPV infection was 4.7 times higher. In contrast, another study from 2018 concluded that *Ureaplasma* spp. and *Mycoplasma* sp. were not linked to HPV[12]. The complexity of these relationships underscores the need for further research and genotyping of *Ureaplasma* spp. species[14].

Regarding colposcopic findings, we noted a higher prevalence of abnormal results in positive patients, contrasting with a study reporting inconclusive colposcopy outcomes in patients with *U. urealyticum* and *M. hominis*[15]. However, concerning cytological findings in our study, no significant association was observed. This aligns with the study by Effiana et al., which found no relationship between *M. hominis* and altered Pap smear results[7]. Yet, earlier studies demonstrated that *U. urealyticum*, *U. parvum*, and *M. hominis* may increase the risk of cytological changes in the uterine cervix[8,9,10].

The influence of the vaginal microbiome on the development of neoplastic lesions in the uterine cervix has been documented in previous studies. While some reported the relevance of *Mycoplasma* sp. and *Ureaplasma* spp. in the context of cervical cancer[8,14,16], others did not find a clear relationship with the onset and progression of CIN[8,17]. The varied findings emphasize the intricacies of these interactions and the need for future investigation in this field.

CONCLUSION

Ureaplasma spp. was more prevalent and associated with UTI and HPV, whereas *Mycoplasma* sp. was linked to reduced contraceptive use. In addition, abnormal colposcopic findings were more prevalent in patients positive for *Ureaplasma* spp. and/or *Mycoplasma* sp.

However, more robust studies are needed to explore the interrelationship of *Ureaplasma* and *Mycoplasma* with HPV and preneoplastic lesions.

AUTHORS' CONTRIBUTIONS

MSM: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MCATS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **MBB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **AKG:** Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing.

REFERENCES

1. Albig J, Micevska M, Jovchevski S, Georgiveska J, Cekovska S, Stankov A. Incidence and prevalence of vaginal infections in women of reproductive age in North Macedonia. Pril (Makedon Akad Nauk Umet Odd Med Nauki). 2023;44(2):73-80. https://doi.org/10.2478/prilozi-2023-0027
2. Park JH, Kim N, Shin S, Roh EY, Yoon JH, Park H. Prevalence and correlated factors of sexually transmitted infections among women attending a Korean sexual assault center. J Forensic Leg Med. 2020;71:101935. https://doi.org/10.1016/j.jflm.2020.101935
3. Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, et al. Prevalence of cervical colonization by *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium* in childbearing age women by a commercially available multiplex real-time PCR: an Italian observational multicentre study. J Microbiol Immunol Infect. 2018;51(2):220-5. https://doi.org/10.1016/j.jmii.2017.05.004
4. Audirac-Chalifour A, Torres-Poveda K, Bahena-Román M, Téllez-Sosa J, Martínez-Barnetche J, Cortina-Ceballos B, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. PLoS One. 2016;11(4):e0153274. https://doi.org/10.1371/journal.pone.0153274
5. Klein C, Samwel K, Kahesa C, Mwaiselage J, West JT, Wood C, et al. *Mycoplasma* co-infection is associated with cervical cancer risk. Cancers (Basel). 2020;12(5):1093. https://doi.org/10.3390/cancers12051093
6. Adamopoulou M, Avgoustidis D, Voyiatjaki C, Beloukas A, Yapijakis C, Tsiambas E, et al. Impact of combined mycoplasmataceae and HPV co-infection on females with cervical intraepithelial neoplasia and carcinoma. J BUON. 2021;26(4):1313-9. PMID: 34564986
7. Effiana M, Irsan A, Natalia D, Rahmayanti S, Indarti J, Rachmadi L, et al. Infections of Chlamydia trachomatis and *Mycoplasma hominis* as risk factors for abnormal cervical cells. Makara J Health Res. 2018;22(1):53-7. https://doi.org/10.7454/msk.v22i1.7965
8. Feriyawati L, Anggraini DR, Nasution TA. Co-infection of *Human Papillomavirus* with *Mycoplasma Hominis/Ureaplasma Urealyticum* among female sex workers in Medan, Indonesia. Open Access Maced J Med Sci. 2019;7(20):3425-8. https://doi.org/10.3889/oamjms.2019.438
9. Lv P, Zhao F, Xu X, Xu J, Wang Q, Zhao Z. Correlation between common lower genital tract microbes and high-risk human papillomavirus infection. Can J Infect Dis Med Microbiol. 2019;2019:9678104. https://doi.org/10.1155/2019/9678104
10. Alotaibi HJ, Almajhdi FN, Alsaleh AN, Obeid DA, Khayat HH, Muammer TA, et al. Association of sexually transmitted infections and human papillomavirus co-infection with abnormal cervical cytology among women in Saudi Arabia. Saudi J Biol Sci. 2020;27(6):1587-95. https://doi.org/10.1016/j.sjbs.2020.03.021
11. Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61(4):344-9. https://doi.org/10.1016/j.jclinepi.2007.11.008
12. Xie L, Li Q, Dong X, Kong Q, Duan Y, Chen X, et al. Cervical cytopathological findings in Korean women with *Chlamydia* trachomatis, *Mycoplasma hominis*, and *Ureaplasma urealyticum* infections. Mol Clin Oncol. 2021;15(1):132.
13. Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, et al. Prevalence of cervical colonization by *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium* in childbearing age women by a commercially available multiplex real-time PCR: an Italian observational multicentre study. J Microbiol Immunol Infect. 2018;51(2):220-5. https://doi.org/10.1016/j.jmii.2017.05.004
14. Zdrodowska-Stefanow B, Kłosowska WM, Ostaszewska-Puchalska I, Bułhak-Kozioł V, Kotowicz B. *Ureaplasma urealyticum* and *Mycoplasma hominis* infection in women with urogenital diseases. Adv Med Sci. 2006;51:250-3. PMID: 17357319
15. Christofolini DM, Leuzzi L, Mafra FA, Rodart I, Kayaki EA, Bianco B, et al. Prevalence of cases of *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum* and *Chlamydia trachomatis* in women with no gynecologic complaints. Reprod Med Biol. 2012;11(4):201-5. https://doi.org/10.1007/s12522-012-0132-y
16. Rar VA, Maksimova TG, Trukhina AV, Rempel' EG, Klimashevskaia NI, Riabova EN, et al. [Level of colonization by *Ureaplasma urealyticum* of definite biovars in a group of women with different clinical symptoms]. Zh Mikrobiol Epidemiol Immunobiol. 2004;(4):(4):12-7. PMID: 15481912
17. Moridi K, Hemmaty M, Azimian A, Fallah MH, Khaneghahi Abyaneh H, Ghazvini K. Epidemiology of genital infections caused by *Mycoplasma hominis*, M. *genitalium* and *Ureaplasma urealyticum* in Iran; a systematic review and meta-analysis study (2000-2019). BMC Public Health. 2020;20(1):1020. https://doi.org/10.1186/s12889-020-08962-5
18. Kletzel HH, Rotem R, Barg M, Michaeli J, Reichman O. *Ureaplasma urealyticum*: the role as a pathogen in women's health, a systematic review. Curr Infect Dis Rep. 2018;20(9):33. https://doi.org/10.1007/s11908-018-0640-y

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240049

Accuracy of neck circumference in the diagnosis of overweight in children

Guilherme de Azevedo Traebert [1], André Legat Albino[1], Milcia Almeida Zaidan [1], Franciane Bobinski [2], Francieli Pereira Ribeiro [2], Eliane Traebert [1,2], Jefferson Traebert [1,2*],

SUMMARY

OBJECTIVE: The objective of this study was to estimate the accuracy of measuring neck circumference as a diagnostic method for overweight in 10-year-old children.

METHODS: A cross-sectional diagnostic accuracy study was performed in 2019. The population was composed of 942 school children from the municipality of Palhoça, SC, Brazil. For each measurement of the neck circumference, sensitivity, specificity, positive and negative predictive values, likelihood ratio for a positive test, and accuracy were estimated using the receiver operator characteristic curve, with body mass index as a reference.

RESULTS: The estimated overall accuracy was 88.9%. For males, the accuracy was 90.1%, and for females, 88.5%. A 30.0 cm neck circumference had a sensitivity of 22.8%, a specificity of 95.4%, a positive predictive value of 76.6%, a negative predictive value of 65.3%, a likelihood ratio for a positive test of 5.0, and an accuracy of 66.7% for all students.

CONCLUSION: Neck circumference showed a global accuracy of 88.9% as a method for diagnosing overweight in 10-year-old children. Predictive values showed high values, mainly starting with a neck circumference of 30 cm.

KEYWORDS: Pediatric obesity. Body mass index. Diagnosis.

INTRODUCTION

Childhood obesity is a global problem. The prevalence of obesity in Brazil has been increasing gradually, with epidemic behavior in both adults and children[1]. However, a recent systematic review[2] has shown data on childhood obesity in Brazil that cannot be generalized due to the large methodological differences between the studies.

Several parameters are used to classify overweight and obesity, but the body mass index (BMI) is more commonly used in adults, even though it does not differentiate adipose tissue from lean mass, thus not being fully correlated with body fat[1,3]. This index is calculated by the ratio between the individual's weight and height squared (kg/m^2)[3].

In children and adolescents aged 5–19 years, overweight and obesity are characterized by BMI percentile curves or z-scores. The World Health Organization[3] defines overweight as a BMI situated on z-score curves between values 1 and 2 for age. The obesity classification corresponds to the BMI located on the curve above the value 2. Such values differ with age according to the variation in corpulence, which is understood as different dimensions that the body assumes at different ages during growth[1,3].

On the contrary, other diagnostic methods have been proposed to help measure childhood obesity, such as waist circumference[4] and neck circumference[5]. The latter showed an important correlation with metabolic syndrome parameters and has a very low cost[6].

Studies[4,5,7] have shown a relationship between childhood overweight and obesity and neck circumference, being potentially as effective as waist circumference and BMI in measuring overweight and obesity. In 6-year-old children, neck circumference presented an accuracy of 77.2% as a diagnostic method for overweight. A positive linear correlation of 0.57 was observed between neck circumference and BMI. Sensitivity and specificity values were low, but high positive predictive values were observed, particularly in 30- and 31-cm neck circumference measurements[6].

[1]Universidade do Sul de Santa Catarina, School of Medicine – Palhoça (SC), Brazil
[2]Universidade do Sul de Santa Catarina, Post-Graduation Program in Health Sciences – Palhoça (SC), Brazil.
*Corresponding author: jefferson.traebert@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: Foundation for Research and Innovation Support of the State of Santa Catarina-FAPESC/Brazil (Grant No. 09/2015).
Received on January 16, 2024. Accepted on February 19, 2024.

Although there is some evidence about the accuracy of neck circumference as a diagnostic measure of obesity in 6-year-old children, no references were found about such indicators beyond that age. Therefore, the objective of this study was to estimate the accuracy of the neck circumference measurement as a method for diagnosing overweight in 10-year-old children.

METHODS

This is a cross-sectional diagnostic accuracy study. The information was obtained from the database of the *Coorte Brasil Sul* study[8] involving 10-year-old school children from 37 public and 19 private schools in Palhoça/SC, Brazil. The study population consisted of data from 942 children. The parameters used to calculate the sample size were as follows: population of 1,270 10-year-old children, expected prevalence of unknown outcome (p=50%), 95% confidence level, and 2% relative error, which generated a minimum sample of 831 children. That number was beefed up by 10% to compensate for refusals, which generated a final sample of 942 children randomly selected from all schools.

Data collection was carried out directly in the schools by two surveyors who participated in the training and calibration process for anthropometric data collection. The training of the surveyors was carried out based on joint training, observing the variation in anthropometric data obtained simultaneously by both surveyors. In the second step, calibration was carried out by collecting data from 30 children of 10 years old to check inter-examiner and intra-examiner reproducibility. The agreement of weight, height, and neck circumference measurements was evaluated using the kappa test. All values were greater than 0.7, which was considered an adequate agreement.

Weight and height were collected following the norms proposed by the Brazilian Ministry of Health[9]. The anthropometric assessment was performed using BMI obtained by dividing the weight in kilograms by height in meters squared[10]. The cutoff points in the BMI z-score were as follows: normal weight (≥ -2 and <+1), overweight (≥ +1 and <+2), and obesity (≥ +2)[10]. Neck circumference was measured in centimeters using a measuring tape. The child remained upright with the head positioned in the horizontal plane. The upper edge of the measurement tape was positioned just below the cricothyroid cartilage and surrounded perpendicularly the neck.

Specific data for this investigation (gender, BMI, and neck circumference measurements) were entered into an Excel spreadsheet and subsequently exported to the Statistical Package for Social Sciences (SPSS) 18.0 software, which was used for data analysis.

For each measurement of the neck circumference, sensitivity, specificity, and positive and negative predictive values were estimated, as well as the likelihood ratio for a positive test and accuracy. BMI was used as a reference. Accuracy was calculated by the ratio between the sum of true positives and true negatives in the total sample. Accuracy was expressed by the receiver operator characteristic (ROC) curve and its relevant confidence interval. Additionally, the correlation between neck circumference and BMI was reviewed using Pearson's correlation test. All measures were estimated for the population as a whole and by gender. Measures that had p-values <0.05 were considered statistically significant.

The study was approved by the Ethics Committee for Research in Humans of the Universidade do Sul de Santa Catarina under number 3.362.267.

RESULTS

The prevalence of excess weight was 39.6% (95%CI 36.5–42.5). Neck circumference ranged from 21 to 38 cm, with a mean of 28.8 cm (SD=2.2), a median of 28.0 cm, and a mode of 29.0 cm.

The relationship between neck circumference and BMI is shown in Figure 1. A positive and statistically significant correlation was observed (p<0.001; Pearson's correlation coefficient, r=0.751; and determination coefficient, R^2=0.564).

The neck circumference values, as well as the sensitivity, specificity, positive and negative predictive values, the likelihood ratio for the positive test, and data accuracy according to sex are presented in Table 1. A neck circumference of 29.0 cm had a sensitivity of 19.3%, a specificity of 87.5%, and an accuracy of 60.5% for all students. The positive and negative predictive values were 50.3 and 62.3%, respectively. The likelihood ratio for the positive test showed that it is 1.6 times more likely to find a 29 cm neck circumference among overweight children when compared with children without excess weight (Table 1).

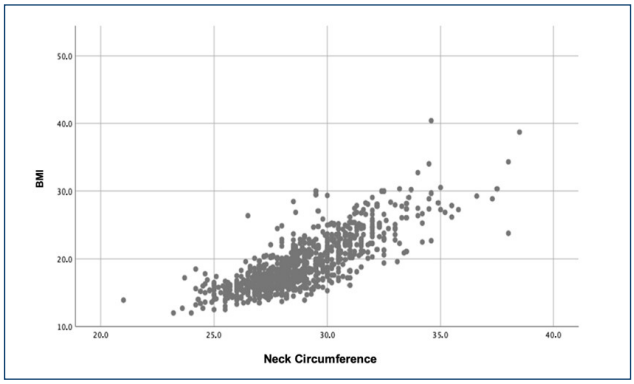

Figure 1. Correlation between measures of neck circumference (cm) and body mass index. Palhoça (SC), Brazil (n=942).

The neck circumference value of 30.0 cm had a sensitivity of 22.8%, a specificity of 95.4%, a positive predictive value of 76.6%, a negative predictive value of 65.3%, a likelihood ratio for the positive test of 5.0, and an accuracy of 66.7% for the entire group of school children. The sensitivity and specificity values for males were 24.1 and 94.3%, and the accuracy was 65.1%. The positive and negative predictive values were 75.0 and 63.6%, respectively. The likelihood ratio for the positive test showed that it is 4.2 times more likely to find an overweight male child with a neck circumference of 30 cm when compared with those without excess weight (Table 1). On the contrary, in females, the sensitivity and specificity values were 21.5 and 96.4%, and the accuracy was 68.1%. The likelihood ratio for the positive test showed that it is 6.0 times more likely to find an overweight female child with a neck circumference of 30 cm when compared with those without excess weight (Table 1).

The ROC curve is shown in Figure 1. The area under the curve corresponds to the accuracy of neck circumference as a method for diagnosing overweight. The accuracy value of the global ROC curve was 88.9% (95%CI 86.7–90.9), p<0.001 (Figure 2). The accuracy for males was 90.1% (95%CI 87.2–92.9), p<0.001 (Figure 2), and it was 88.5% (95%CI 85.6–91.5), p<0.001 in females.

DISCUSSION

As a representative measure of fat deposition in the upper body, neck circumference is a new, pathogenic, and independent fat deposit, which is related to the rate of visceral fat and may be associated with greater cardiovascular risks compared with fat in the central region of the body[11]. This is because subcutaneous fat in the upper body region supplies most of the free fatty acids to the systemic circulation in post-absorptive and postprandial conditions and can cause disorders such as hypertriglyceridemia[12].

A Brazilian study[5] carried out with 6-year-old children demonstrated an accuracy of 77.2% as a diagnostic measure to identify overweight and obesity, while this study showed a greater accuracy of 88.9%, albeit with 10-year-old children. Regarding sensitivity, both studies showed low values; at 6 years of age, sensitivity reached a maximum of 23% in children with a neck circumference of 27 cm. On the contrary, this study detected a maximum sensitivity value of 24.1% when the neck circumference value was 30 cm in males. Regarding specificity, both studies showed high values. When comparing this variable in children with a neck circumference of 31 cm, our study presented 98.6% sensitivity, while the study with 6-year-old children presented 99.0% sensitivity. In the aforementioned study, the positive predictive values in young girls with a neck

Table 1. Sensitivity, specificity, predictive values, likelihood ratio for the positive test, and accuracy of neck circumference measurements as a diagnostic method for overweight by gender in 10-year-old school children, Palhoça (SC), Brazil (n=942).

Accuracy measures for the male						Accuracy measures for the female						
NC (cm)	S (%)	Sp (%)	PPV (%)	NPV (%)	LR+	A (%)	S (%)	Sp (%)	PPV (%)	NPV (%)	LR+	A (%)
23.0	–	99.2	–	58.3	–	58.0	–	99.3	–	62.0	–	61.2
24.0	–	99.6	–	58.4	–	58.2	–	94.8	–	60.9	–	58.9
25.0	–	98.1	–	58.0	–	57.3	–	89.5	–	59.6	–	55.7
26.0	–	84.8	2.4	54.5	–	49.8	2.2	80.4	6.3	57.5	0.1	50.8
27.0	2.1	74.5	5.6	51.7	–	44.4	6.5	68.0	10.9	54.5	0.2	44.7
28.0	10.7	67.7	19.0	57.6	0.3	44.0	17.2	81.7	36.4	61.9	0.9	57.3
29.0	15.0	83.7	39.4	58.0	0.9	55.1	23.7	90.8	61.1	66.2	2.6	65.4
30.0	24.1	94.3	75.0	63.6	4.2	65.1	21.5	96.4	78.4	66.9	6.0	68.1
31.0	18.2	98.1	87.2	62.8	9.6	64.9	13.4	99.0	89.3	65.3	13.7	66.7
32.0	13.9	100.0	100.0	62.0	–	64.2	8.1	100.0	100.0	64.2	–	65.2
33.0	7.0	100.0	100.0	60.2	–	61.3	2.7	100.0	100.0	62.8	–	63.2
34.0	8.6	100.0	100.0	59.4	–	60.9	3.2	100.0	100.0	63.0	–	63.4
35.0	2.1	100.0	100.0	59.0	–	59.3	1.1	100.0	100.0	62.4	–	62.6
36.0	0.5	100.0	100.0	58.6	–	58.7		–	–	–	–	–
37.0	0.5	100.0	100.0	58.6	–	58.7	0.5	100.0	100.0	62.3	–	62.4
38.0	1.6	100.0	100.0	58.8	–	59.1	–	–	–	–	–	–

NC: neck circumference; S: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; LR+: likelihood ratio for the positive test; A: accuracy.

circumference of 30 and 31 cm presented values of 77 and 85% sensitivity, respectively. On the contrary, for males, the values were 85 and 93% for boys having 30 and 31 cm neck circumference. Despite evaluating students of a different age, this study presented positive predictive values of 75.0 and 87.2% with 30 and 31 cm in males, respectively. In females, the values were 78.4% with 30 cm and 89.3% with a neck circumference of 31 cm.

A similar Chinese study[13] evaluated 3,719 children aged 7–10 years. It reported a significant positive correlation between neck circumference and obesity at all ages and in both genders. Accuracy was 70% and the optimal neck circumference to diagnose overweight/obesity in boys was between 24.75 and 27.25 cm, while for girls, it was between 23.75 and 26.25 cm. According to the authors, the best measurement to estimate excess weight was 29 cm neck circumference, which had a sensitivity of 19.3%, a specificity of 87.5%, a positive predictive value of 50.3%, a negative predictive value of 62.3%, and an accuracy of 60.5% for all children.

Another study[14] in the United States involving 1,102 children aged 6–18 years showed a positive correlation in both boys and girls, with a higher number of older children. In that study, a neck circumference of 28.5–39 cm indicated high BMI in boys and 27–34.6 cm high BMI in girls. In 10-year-old boys, overweight could be diagnosed from 32 cm neck circumference onward with 94% accuracy, 85.7% sensitivity, and 95.2% specificity. On the contrary, in 10-year-old girls, overweight could be diagnosed starting with 30.5 cm with 79.9% accuracy 79.9% sensitivity, and 70.3% specificity. Pearson's correlation index between BMI and neck circumference in 10-year-old children was 0.71 and 0.78 for boys and girls, respectively. In comparison, our study presented a 0.75 Pearson global correlation index. Also, in the Michigan study[14], globally, the measure to estimate excess weight was 29 cm, with a sensitivity of 15.0%, a specificity of 83.7%, a positive predictive value of 39.4%, a negative predictive value of 58.0%, and an accuracy of 55.1% in boys and 23.7, 90.8, 61.1, 66.2, and 65.4% in girls, respectively.

A meta-analysis study[15] with children and adolescents between 6 and 18 years demonstrated moderate accuracy for diagnosing overweight and obesity in this population because the accuracy value estimated by the global ROC curve was 87.0%, which corroborates with the result of this study, in which the global accuracy value was 88.9%.

The predictive values found in this study were higher than the sensitivity and specificity values. For clinical practice, predictive values are more useful[16] as they indicate the probability of the event assessed to occur; specifically, in this case, overweight at 10 years of age, considering the results of the diagnostic test.

Thus, the proportion of female children with a positive test result who were overweight was 78.4 and 89.3% with neck circumference measurements of 30 and 31 cm, respectively. In males, the proportion was 75 and 87% with neck circumference measurements of 30 and 31 cm, respectively. Thus, it can be observed that the higher the BMI, the greater the predictive values found, which is in line with another study that confirms that neck circumference is a reliable measure for the diagnosis of overweight and obesity in children[17]. It is important to emphasize that for the same test, the greater the prevalence of the event, the greater the positive predictive value and the lower the negative predictive value, which is extremely important in the case of childhood obesity, as this is a highly prevalent event[16]. In addition, the values of the likelihood ratios for the positive test at 30 cm of neck circumference showed a good probability of finding overweight children, which were 4.2 times in males and 6.0 times in females.

A possible limitation of this study is the fact that more than one surveyor collected the data, which could, eventually, produce measurement bias. However, training exercises made it possible to measure the inter-examiner and intra-examiner reproducibility. Furthermore, the standardization and strict adherence to the collection methods ensure the reliability of the results.

It can be concluded that the neck circumference showed a global accuracy of 88.9% as a method for diagnosing overweight. Predictive values showed high values, mainly starting with a neck circumference of 30 cm.

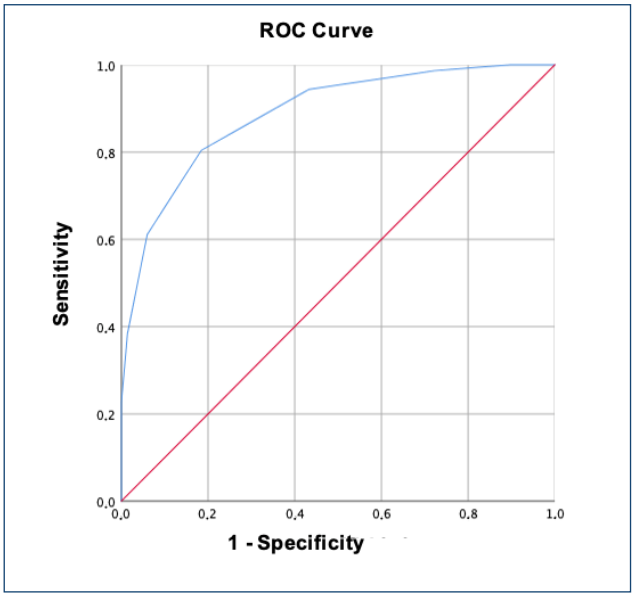

Figure 2. Receiver operator characteristic curve of neck circumference measurements (cm) as a diagnostic method for overweight in 10-year-old school children. Palhoça (SC), Brazil (n=942).

AUTHORS' CONTRIBUTIONS

GAT: Data curation, Writing – original draft, Writing – review & editing. **ALA:** Data curation, Writing – original draft, Writing – review & editing. **MAZ:** Writing – review & editing. **FB:** Writing – review & editing. **FPR:** Writing – original draft, Writing – review & editing. **ET:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **JT:** Formal Analysis, Supervision, Writing – original draft, Writing – review & editing.

REFERENCES

1. Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica. Diretrizes Brasileiras de Obesidade. 4th ed. São Paulo (SP): ABESO; 2016.
2. Heinz C, Afonso L, Traebert E, Trevisol DJ, Traebert J. Prevalência de excesso de peso corporal infantil no Brasil: uma revisão sistemática. Res Soc Develop. 2022;11(5):e4911426685.
3. World Health Organization. Obesity and overweight. 2024. [cited on 2024 Jan 08]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight
4. Traebert E, Leão G, Traebert GA, Flôres APR, Traebert J. Accuracy of abdominal circumference for diagnosing overweight in six-to-seven-years-old children. Res Soc Develop. 2022;11(1):e23511124638.
5. Mucelin E, Traebert J, Zaidan MA, Piovezan AP, Nunes RD, Traebert E. Accuracy of neck circumference for diagnosing overweight in six- and seven-year-old children. J Pediatr (Rio J). 2021;97(5):559-63. https://doi.org/10.1016/j.jped.2020.11.005
6. Mendes CG, Barbalho SM, Tofano RJ, Lopes G, Quesada KR, Detregiachi CRP, et al. Is neck circumference as reliable as waist circumference for determining metabolic syndrome? Metab Syndr Relat Disord. 2021;19(1):32-8. https://doi.org/10.1089/met.2020.0083
7. Kelishadi R, Djalalinia S, Motlagh ME, Rahimi A, Bahreynian M, Arefirad T, et al. Association of neck circumference with general and abdominal obesity in children and adolescents: the weight disorders survey of the CASPIAN-IV study. BMJ Open. 2016;6(9):e011794. https://doi.org/10.1136/bmjopen-2016-011794
8. Traebert J, Lunardelli SE, Martins LGT, Santos KD, Nunes RD, Lunardelli AN, et al. Methodological description and preliminary results of a cohort study on the influence of the first 1,000 days of life on the children's future health. An Acad Bras Cienc. 2018;90(3):3105-14. https://doi.org/10.1590/0001-3765201820170937
9. Ministério da Saúde do Brasil. Secretaria de Atenção à Saúde. Incorporação das curvas de crescimento da Organização Mundial de Saúde de 2006 e 2007 no SISVAN. 2024. [cited on 2024 Jan 08]. Available from: http://www.nutricao.saude.gov.br/docs/geral/curvas oms 2006 2007.pdf
10. World Health Organization. Body mass index (BMI). 2024. [cited on 2024 Jan 08]. Available from: https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/body-mass-index?introPage=intro_3.html
11. Preis SR, Massaro JM, Hoffmann U, D'Agostino RB, Levy D, Robins SJ, et al. Neck circumference as a novel measure of cardiometabolic risk: the Framingham Heart study. J Clin Endocrinol Metab. 2010;95(8):3701-10. https://doi.org/10.1210/jc.2009-1779
12. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab. 2008;93(11 Suppl 1):S57-63. https://doi.org/10.1210/jc.2008-1585
13. Hu NN, He M, Li YF, Wang RY, Yang J, Li WJ, et al. Correlation of neck circumference with body mass index in preschool children. Zhongguo Dang Dai Er Ke Za Zhi. 2022;24(9):1042-6. https://doi.org/10.7499/j.issn.1008-8830.2203072
14. Nafiu OO, Burke C, Lee J, Voepel-Lewis T, Malviya S, Tremper KK. Neck circumference as a screening measure for identifying children with high body mass index. Pediatrics. 2010;126(2):e306-10. https://doi.org/10.1542/peds.2010-0242
15. Ma C, Wang R, Liu Y, Lu Q, Liu X, Yin F. Diagnostic performance of neck circumference to identify overweight and obesity as defined by body mass index in children and adolescents: systematic review and meta-analysis. Ann Hum Biol. 2017;44(3):223-9. https://doi.org/10.1080/03014460.2016.1224387
16. Medronho R, Bloch KV, Luiz RR, Werneck GL. Epidemiologia. São Paulo (SP): Atheneu; 2009.
17. Taheri M, Kajbaf TZ, Taheri MR, Aminzadeh M. Neck circumference as a useful marker for screening overweight and obesity in children and adolescents. Oman Med J. 2016;31(3):170-5. https://doi.org/10.5001/omj.2016.34N

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240059

The effect of mandala coloring on anxiety and quality of life of women in the climacteric period: a randomized controlled study

Ayça Şolt Kırca[1], Elif Dağlı[2*], Efsun Derin[1], Nurettin Aka[3]

SUMMARY

OBJECTIVE: This study was conducted to determine the effect of mandala coloring on anxiety and quality of life of women in the climacteric period.
METHODS: This research was conducted as an experimental study based on a randomized controlled pre-test and post-test model (single-blind). The study was conducted with women in the climacteric period who presented to a training and research hospital in a western city of Turkey between November 1, 2022, and April 28, 2023. Participants were divided into mandala coloring (n=38) and control groups (n=38).
RESULTS: According to the women's socio-demographic and descriptive characteristics, mean age, body mass index, and frequency of menopausal symptoms were similar in both groups. The mean post-test scores of the women in the mandala coloring group on the state-trait anxiety inventory and menopause-specific quality of life questionnaire vasomotor, psychosocial, physical, and sexual subscales (29.71±5.22, 0.86±0.97, 0.53±0.61, 0.79±0.84, and 0.92±1.24, respectively) were lower than the mean post-test scores of the women in the control group on the same scales (41.02±1.20, 1.79±1.76, 1.49±1.39, 1.72±1.38, and 1.95±1.82, respectively) (p=0.000).
CONCLUSION: Mandala coloring reduces menopause-related anxiety levels and improves quality of life effectively.
KEYWORDS: Climacteric. Women. Menopause. Painting. Anxiety. Quality of life.

INTRODUCTION

Effective management of anxiety, which can aggravate menopausal symptoms, may improve the quality of life of women in the climacteric period[1]. Therefore, anxiety management may be effective in helping women cope with menopause symptoms. Many alternative methods have been used in the literature to reduce anxiety (music medicine, acupressure, or virtual reality glasses)[1-5], and the therapeutic effect of mandala coloring, which is one of the art therapy methods, has been reported in some studies[6,7]. Mandala coloring is a safe and accessible activity that does not require any special skills or training and can be used as a complementary strategy to reduce anxiety[6-8]. In a systematic review, Abbing et al. showed that art therapy had a positive effect on reducing anxiety in patients[9]. Flett et al. indicated that daily mandala coloring helped reduce anxiety[10].

Art therapy is considered therapeutic in the sense that it reconciles emotional conflicts, increases awareness, reduces anxiety, provides relief from destructive emotions and traumas, creates opportunities to solve problems, directs people to reality, improves social skills, and increases self-esteem. Art therapy is a form of expression that helps people give meaning to their inner world and reflect their unconscious emotions externally. In this respect, it also activates creative problem-solving activities[11-14].

A review of the literature indicated that there was no study about the effects of mandala coloring on menopausal symptom-related anxiety and quality of life of women in the climacteric period. In this context, this study was conducted to determine the effect of mandala coloring on the anxiety and quality of life of women in the climacteric period.

METHODS

Research design

An experimental study was conducted based on a randomized controlled pre-test and post-test model. The study was carried out according to CONSORT guidelines (Figure 1), and a clinical trial registration code was obtained (NCT05575349)[15].

Participants

The population of the study comprised women who were in the climacteric period and presented to a training and

[1]Kırklareli University, Faculty of Health Sciences, Department of Midwifery – Kırklareli, Turkey.
[2]Çukurova University, Abdi Sütcü Vocational School of Health Services, Department of Health Care Services – Adana, Turkey.
[3]Kırklareli University – Kırklareli, Turkey.
*Corresponding author: elifarik90@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 10, 2024. Accepted on February 12, 2024.

research hospital in a western city of Turkey for outpatient treatment between December 15, 2022, and April 28, 2023. The G*Power software (version 3.1.9.3) was employed to calculate the sample size of the study. A review of the literature indicated that there were no studies on whether mandala coloring reduced the anxiety of women in the climacteric period. The COHEN standard effect size was assumed to be 0.70 to determine the sample size of the study. Accordingly, it was calculated as 68 women (n=34 in each group), based on a Type I error of 0.05, a test power of 0.80 (power analysis) (α=0.05, 1–β=0.80, effect size=0.70), and a 1:1 distribution ratio. Considering some attrition, it was decided to recruit 80 women (n=40 in each group). Participants were randomly assigned to mandala coloring and control groups using the simple random number generator software. This list was recorded by the researcher.

Study inclusion criteria: Women who volunteered to participate in the research, filled out the questionnaires and scales completely, could read and understand Turkish, were in the climacteric period (42–65 years old), and had an STAI score of ≥37 were included in the study.

Data collection tools

Descriptive information form (DIF): This form was created by the researchers following a review of the literature[5-10].

The state-trait anxiety inventory (STAI): Spielberger et al. developed this inventory, and Öner and Compte conducted its Turkish adaptation. A high score indicates a high anxiety level, while a small score shows a low level of anxiety. A total score of 36 or less means no anxiety, scores between 37 and 42 indicate slight anxiety, and scores that are greater than 42 show high anxiety[16,17].

Menopause-specific quality of life questionnaire (MENQOL): Hilditch et al. developed this scale, and Kharbouch and Şahin adapted it into Turkish. High scores from the scale show increased severity of the complaint and decreased quality of life[18,19].

Data collection procedure

The first interview with the women was made when they applied to the clinic. To prevent bias in the research, a nurse/midwife who worked at the outpatient clinic that day but was not involved in the study helped the participants fill out the DIF, STAI-S, and MENQOL forms face-to-face during the pre-test phase. Contact information of the women was taken. Post-test data

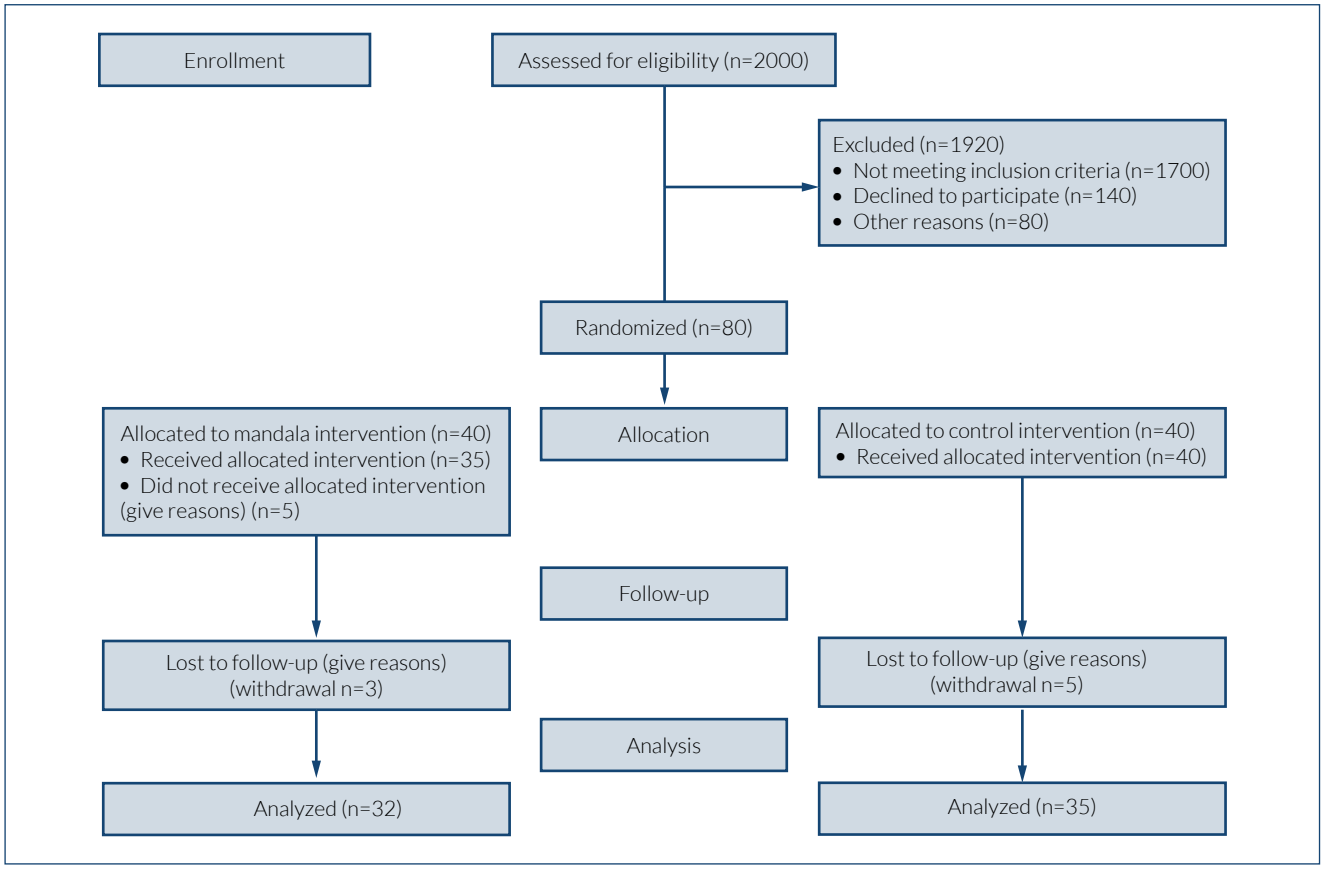

Figure 1. CONSORT flowchart.

were collected through an online form. It took approximately 20–25 min to fill out the form.

Interventions

Mandala coloring group: Women were given 12-color felt-tip pen sets and 12 mandala coloring pages by the researcher. They were asked to choose a coloring page and color it as they liked once a week, at any time of the day, for an average of 20–30 min each time. This application took 6 weeks.

Participants were asked to send a message before they started mandala coloring and to take a picture of the coloring and send it to the researcher when they finished the session. They were sent a reminder message twice a week in case they forgot to do mandala coloring.

Control group: The participants in this group comprised people who did not routinely do any practice on their own to reduce their anxiety symptoms. During the same 6 weeks, they were called by the researcher twice a week to find out whether they had taken any action to reduce their anxiety symptoms. Those who used either pharmacological or non-pharmacological practices to reduce anxiety symptoms were excluded from the study. At the end of the study, participants in the control group were informed about mandala coloring, and those who wanted to practice it were given the same chance to do it.

Data analysis

The Shapiro-Wilk test was employed to check the normality of the variables included in this study. Descriptive statistics and chi-square test were used to compare categorical data. In normally distributed data, the independent-samples t-test was employed to make inter-group comparisons, and the dependent-samples t-test was used to make intra-group comparisons. The Mann-Whitney U test was used to compare data that did not show normal distribution. To present the analysis results, median values (minimum–maximum) were used for non-normally distributed data, mean±standard deviation values were employed for normally distributed data, and frequency (percentage) values were utilized for categorical data. The confidence interval was determined to be less than 0.05.

Ethical considerations

Ethics committee approval of the research was obtained from the Non-Interventional Clinical Research Ethics Committee of a state university (approval date: October 7, 2022, and decision number: 126/38).

RESULTS

The comparison of the participants' sociodemographic and descriptive characteristics indicated that mean age, body mass index (BMI), and frequency of menopausal symptoms were similar in both groups. The education level of the women in the experimental group was higher, and the majority of them were in their postmenopausal period for 5 years or more (Table 1).

The anxiety and quality of life levels of women in the mandala coloring and control groups were analyzed using their mean scores on the pre-test and post-test applications of the STAI and MENQOL scales, and intra-and inter-group comparisons were made.

Table 1. Socio-demographic and descriptive characteristics.

	Mandala coloring group (n=32)		Control group (n=35)	
Age	54.31±5.78 (43–65)		53.08±3.92 (44–60)	
	n	%	n	%
BMI				
Normal	8	25	10	28.6
Overweight	9	28.1	10	28.6
Obese	15	46.9	15	42.9
Education status				
Secondary education	15	46.9	20	57.1
High school and above	17	53.1	15	42.8
Menopausal period				
Less than 5 years postmenopause	13	40.6	18	51.4
5 years or more postmenopause	19	59.3	17	48.5
Frequency of experiencing menopause symptoms				
Weekly	17	53.1	24	68.5
Monthly	15	46.9	11	31.4

The intra-group comparisons of STAI-S scores of the participants in the mandala coloring group indicated that their mean post-test STAI-S score (29.71±5.22) was lower than their mean pre-test score (42.90±4.29) and that the difference was significant (p<0.001). There was no statistical difference between the mean scores of the participants in the control group from the pre-test and post-test applications of the STAI-S (41.22±1.47 and 41.02±1.20, p>0.05).

The inter-group comparisons of the mean pre-test STAI-S scores indicated that there was no significant difference between mandala coloring (42.90±4.29) and control groups (41.22±1.47) (p>0.05). However, the mean post-test STAI-S score of the participants in the mandala coloring group (29.71±5.22) was significantly lower than that of the participants in the control group (41.02±1.20) (p=0.000) (Table 2).

The intra-group comparison of MENQOL subscale scores indicated that the mean post-test scores of the mandala coloring group from the vasomotor, psychosocial, physical, and sexual subscales (0.86±0.97, 0.53±0.61, 0.79±0.84, and 0.92±1.24, respectively) were lower than their mean pre-test scores from the same subscales (1.79±1.76, 1.49±1.39, 1.72±1.38, and 1.95±1.82, respectively) and that the difference was statistically significant (p<0.05). There was no statistical difference between pre-test and post-test MENQOL vasomotor, psychosocial, physical, and sexual subscale scores in the control group (p>0.05).

The inter-group comparison of MENQOL subscale scores showed that there were differences between the mandala coloring and control groups in terms of their mean pre-test and post-test vasomotor, psychosocial, physical, and sexual subscale scores (p<0.05). It was observed that the mean pre-test

Table 2. Intra- and inter-group comparisons of the mean state-trait anxiety inventory and menopause-specific quality of life questionnaire scores obtained by the participants in the mandala coloring and control groups.

	Mandala coloring group (n=32)	Control group (n=35)	t*	p_2
STAI-S				
Pre-test	42.90±4.29	41.22±1.47	t=2.174	p=0.33
Post-test	29.71±5.22	41.02±1.20	t=-12.458	p=0.000
t**	t=10.533	t=1.022		
p_1	p=0.000	p=0.314		
MENQOL-vasomotor domain				
Pre-test	1.79±1.76	2.85±0.77	t=-3.242	p=0.002
Post-test	0.86±0.97	2.92±0.74	t=-9.755	p=0.000
t**	t=3.154	t=-1.022		
p_1	p=0.004	p=0.314		
MENQOL-psychosocial domain				
Pre-test	1.49±1.39	2.28±0.54	t=-3.121	p=0.003
Post-test	0.53±0.61	2.32±0.57	t=-12.306	p=0.000
t**	t=3.727	t=-1.435		
p_1	p=0.001	p=0.160		
MENQOL-physical domain				
Pre-test	1.72±1.38	2.47±0.50	t=-2.993	p=0.004
Post-test	0.79±0.84	2.48±0.50	t=-10.068	p=0.000
t**	t=3.797	t=-1.160		
p_1	p=0.001	p=0.254		
MENQOL-sexual domain				
Pre-test	1.95±1.82	3.30±0.83	t=-3.933	p=0.000
Post-test	0.92±1.24	3.26±0.79	t=-9.036	p=0.000
t**	t=3.456	t=1.675		
p_1	p=0.002	p=0.103		

t*: independent-samples t-test; t**: dependent-samples t-test. p_1: intra-group comparisons; p_2: inter-group comparisons; STAI-S, STAI-T: state-trait anxiety inventory; MENQOL: the menopause-specific quality of life questionnaire.

and post-test MENQOL subscale scores of the control group were higher than those of the mandala coloring group (Table 2).

DISCUSSION

The groups in this study were almost similar in terms of sociodemographic and descriptive characteristics (age, BMI, education level, menopausal period, frequency of menopausal symptoms, etc.), which supported the reliability of the study. The results of the study were similar to those of other national and international studies[6,10,20,21].

In this study, the mean STAI scores of the participants who did mandala coloring decreased significantly compared with those of the control group. In a study conducted with 31 pregnant women, Amelia et al. reported that anxiety scores decreased significantly in women who drew and colored mandalas compared with those of the control group[22]. Yakar et al. conducted a quasi-experimental study with 12 women with breast cancer for 8 weeks and found that the anxiety scores of the participants who received mandala art therapy decreased significantly compared with those before the intervention[23]. In their study with 40 nurses, 37 of whom were female, Maguire et al. reported that mandala coloring for 20 min during breaks reduced their anxiety[24]. Akbulak and Can conducted a quasi-experimental study with 84 participants to examine the effect of mandala coloring on reducing the stress experienced by women with early-stage breast cancer during their first chemotherapy session and found that mandala coloring significantly reduced their anxiety levels[25]. In a quasi-experimental study with 200 patients diagnosed with multiple sclerosis, 55% of whom were female, Barati et al. reported that mandala coloring for 4 weeks significantly reduced anxiety scores[26].

The increase in vasomotor symptoms and sleep disturbances in menopause potentiates depression, sleep difficulty, decreased sleep quality with advancing age, as well as moderate to severe climacteric symptoms, which can affect cognitive function[27]. Different aspects such as a decline in hormonal levels, vaginal dryness, genital atrophy with pain, and fear of meeting their partners' expectations contribute to the progressive decline in sexual activity[28]. Additionally, depression and vasomotor symptoms may affect these women's sexual activities, their spouses' health problems, or marital problems[29]. In this study, the menopause-specific quality of life of participants who did mandala coloring was evaluated, and MENQOL vasomotor, psychosocial, physical, and sexual subscale scores decreased significantly compared with those of the control group. There was no study in the literature on the examination of the effect of mandala coloring on menopause symptoms in women in the climacteric period, and the results of our study were parallel to the results of studies conducted with alternative methods used to improve the quality of life of women in the climacteric period[26,30].

The results of many studies conducted to date have supported the application of mandala coloring, which is among the art therapy methods, in the treatment of various health problems. It has been reported in the literature that the effectiveness and reliability of this method have not been reduced by any complications. Mandala, which is an integrative body–mind education method, created a meditative effect with its repetitive patterns and symmetry, increased psychological well-being by developing awareness, and also demonstrated its anxiety-reducing effect. This, in turn, contributed to a significant decrease in STAI and MENQOL subscale scores. Therefore, mandala coloring can be recommended as an effective method to reduce anxiety and menopause-specific symptoms during the climacteric period and improve quality of life.

The results of this randomized controlled trial apply only to the women in this research and cannot be generalized to others. The lack of high-quality RCTs and quasi-experimental studies testing these hypotheses limits the generalization of our results.

CONCLUSION

Our study data show that mandala coloring reduces mean STAI-S and MENQOL vasomotor, psychosocial, physical, and sexual subscale scores of women in the climacteric period and that this practice is an effective intervention that can be used to alleviate anxiety symptoms in the climacteric period and to improve menopause-specific quality of life. Mandala coloring, which has a therapeutic effect, is recommended to be widely used in such populations.

Mandala painting can be applied in more sessions and its effect can be evaluated. The effect of unstructured mandala coloring and structured mandala coloring can be compared. In addition, by comparing different art therapy methods with each other, the method that is effective in reducing psychological symptoms can be investigated.

ETHICS APPROVAL

Ethics approval was obtained from the Çukurova University Medical Faculty Ethics Committee (approval date: October 7, 2022, and decision number: 126/38).

AUTHORS' CONTRIBUTIONS

AŞK: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **EDa:** Formal Analysis, Investigation, Methodology, Writing – original draft. **EDe:** Formal Analysis, Investigation, Methodology, Writing – original draft. **NA:** Investigation, Formal Analysis, Methodology.

REFERENCES

1. Kırca AS, Oksuz SK, Gul DK, Bulut S. Constipation and its effects on quality of life in menopausal women. Int J Caring Sci. 2021;14(1):269.
2. Kanza Gul D, Solt Kirca A. Effects of acupressure on preoperative acute anxiety in cesarean section under spinal anesthesia: a double-blind randomized controlled study. Holist Nurs Pract. 2020;34(6):356-64. https://doi.org/10.1097/HNP.0000000000000413
3. Şolt Kırca A, Kanza Gül D. The effect of music and skin contact with the newborn on pain and anxiety during episiotomy repair in primiparous women: a double-blind randomized controlled study. Explore (NY). 2022;18(2):210-6. https://doi.org/10.1016/j.explore.2020.11.007
4. Şolt Kırca A, Güdücü N, İkiz B. The effect of virtual glasses application on pain and anxiety during episiotomy repair: randomized controlled trial. Pain Manag Nurs. 2023;24(5):e123-30. https://doi.org/10.1016/j.pmn.2023.06.004
5. Solt Kırca A, Kızılkaya T. Effects of music medicine on premenstrual symptoms levels and quality of life: a randomized controlled trial. Complement Ther Clin Pract. 2022;46:101542. https://doi.org/10.1016/j.ctcp.2022.101542
6. Dağli E, Çelik N. The effect of oxytocin massage and music on breast milk production and anxiety level of the mothers of premature infants who are in the neonatal intensive care unit: a self-controlled trial. Health Care Women Int. 2022;43(5):465-78. https://doi.org/10.1080/07399332.2021.1947286
7. Store JS, Jakobsson N. The effect of mandala coloring on state anxiety: a systematic review and meta-analysis. Art Therapy 2021;39(4):173-81. doi:10.1080/07421656.2021. 2003144
8. Liu C, Chen H, Liu CY, Lin RT, Chiou WK. Cooperative and individual mandala drawing have different effects on mindfulness, spirituality, and subjective well-being. Front Psychol. 2020;11:564430. https://doi.org/10.3389/fpsyg.2020.564430
9. Abbing A, Ponstein A, Hooren S, Sonneville L, Swaab H, Baars E. The effectiveness of art therapy for anxiety in adults: a systematic review of randomised and non-randomised controlled trials. PLoS One. 2018;13(12):e0208716. https://doi.org/10.1371/journal.pone.0208716
10. Flett JAM, Lie C, Riordan BC, Thompson LM, Conner T, Hayne H. Preliminary evidence that adult coloring reduces depressive symptoms and anxiety. Creat Res J. 2017;29(4):409-16. https://doi.org/10.1080/10400419.2017.137650
11. Şolt Kırca A, Dağlı E. The effect of mandala coloring on pregnancy-related anxiety. Eur J Obstet Gynecol Reprod Biol. 2024;292:251-8. https://doi.org/10.1016/j.ejogrb.2023.11.035
12. Lith T. Art making as a mental health recovery tool for change and coping. Art Ther. 2015;32(1):5-12. https://doi.org/10.1080/07421656.2015.992826
13. Abbing A, Ponstein A, Hooren S, Sonneville L, Swaab H, Baars E. The effectiveness of art therapy for anxiety in adults: a systematic review of randomised and non-randomised controlled trials. PLoS One. 2018;13(12):e0208716. https://doi.org/10.1371/journal.pone.0208716
14. Khademi F, Rassouli M, Rafiei F, Moayedi S, Torres M, Marzban N, et al. The effect of mandala colouring on anxiety in hospitalized COVID-19 patients: a randomized controlled clinical trial. Int J Ment Health Nurs. 2021;30(Suppl 1):1437-44. https://doi.org/10.1111/inm.12901
15. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. Trials. 2010;11:32. https://doi.org/10.1186/1745-6215-11-32
16. Spielberger CD. State-trait anxiety inventory. In: Weiner IB, Craighead WE, editros. The corsini encyclopedia of psychology. John Wiley & Sons, Inc; 2010. https://doi.org/10.100 2/9780470479216.corpsy0943
17. Öner N, Compte A. Süreksiz durumluk/sürekli kaygı envanteri El Kitabı. Boğaziçi Üniversitesi Yayınları; 1985.
18. Hilditch JR, Lewis J, Peter A, Maris B, Ross A, Franssen E, et al. A menopause-specific quality of life questionnaire: development and psychometric properties. Maturitas. 1996;24(3):161-75. https://doi.org/10.1016/s0378-5122(96)82006-8
19. Kharbouch SB. Şahin NH. Menopozal dönemlerdeki yaşam kalitesinin belirlenmesi. Florence Nightingale Hemşirelik Dergisi. 2007;15(59):82-90.
20. Choi H, Hahm SC, Jeon YH, Han JW, Kim SY, Woo JM. The effects of mindfulness-based mandala coloring, made in nature, on chronic widespread musculoskeletal pain: randomized trial. Healthcare (Basel). 2021;9(6):642. https://doi.org/10.3390/healthcare9060642
21. Czamanski-Cohen J, Wiley JF, Sela N, Caspi O, Weihs K. The role of emotional processing in art therapy (REPAT) for breast cancer patients. J Psychosoc Oncol. 2019;37(5):586-98. https://doi.org/10.1080/07347332.2019.1590491
22. Amelia G, Satiadarma MP, Wati, L. The effects of drawing and coloring mandala on anxiety among pregnant women in their third trimester. In: Proceeding of the The 2nd Tarumanagara International Conference on the Applications of Social Sciences and Humanities (TICASH 2020). Atlantis Press; 2020;997-1002.
23. Yakar HK, Yilmaz B, Ozkol O, Gevher F, Celik E. Effects of art-based mandala intervention on distress and anxiety in cancer patients. Complement Ther Clin Pract. 2021;43:101331. https://doi.org/10.1016/j.ctcp.2021.101331
24. Maguire P, Coughlan A, Drayton D, Lacko H, Reich J. The effect of coloring mandalas on the anxiety of medical-surgical nurses and nursing support staff. MEDSURG Nurs. 2020;29(3):192-9
25. Akbulak F, Can G. Effectiveness of mandala coloring in reducing anxiety in women with early-stage breast cancer receiving chemotherapy for the first time. Explore (NY). 2023;19(1):42-7. https://doi.org/10.1016/j.explore.2022.04.007
26. Barati F, Sarchahi Z, Khaniki SH, Sirjani EB, Taghiabadi M. Effect of software-based therapeutic coloring on stress and anxiety in patients with multiple sclerosis: a quasi-experimental study. Rom J Neurol/Rev Romana Neurol. 2020;19(4):262-7. https://doi.org/10.37897/RJN.2020.4.7
27. Zangirolami-Raimundo J, Sorpreso ICE, Rebouças CMP, Bezerra PCL, Costa LMPRD, Baracat EC, et al. Depression in women in climacteric period: a brief review. Rev Assoc Med Bras (1992). 2023;69(7):e20230385. https://doi.org/10.1590/1806-9282.20230385
28. Fonseca AMD, Bagnoli VR, Massabki JOP, Arie WMY, Azevedo RS, Soares JM, et al. Brazilian women's health after 65 years of age. Rev Bras Ginecol Obstet. 2017;39(11):608-13. https://doi.org/10.1055/s-0037-1604200
29. Hachul H, Bittencourt LR, Soares JM, Tufik S, Baracat EC. Sleep in post-menopausal women: differences between early and late post-menopause. Eur J Obstet Gynecol Reprod Biol. 2009;145(1):81-4. https://doi.org/10.1016/j.ejogrb.2009.03.019
30. Hadizadeh-Talasaz Z, Firoozi M, Khadivzadeh T, Azizi H, Vatanchi A. Effect of auricular acupressure on quality of life in postmenopausal women: a randomized clinical trial. J Midwifery Reprod Health. 2023;11(1):3630-7. https://doi.org/10.22038/jmrh.2022.67427.1977

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240138

Relationship between polycystic ovary syndrome and high periostin level

Semra Eroglu[1*], Elcin Kal Cakmakliogullari[2]

SUMMARY

OBJECTIVE: There is growing evidence suggesting a relationship between periostin levels, inflammation, and ovarian dysfunction. In this prospective case-control study, we aimed to investigate serum periostin levels and their relationship with metabolic parameters in patients with polycystic ovary syndrome.

METHODS: We conducted a prospective case-control study involving 45 polycystic ovary syndrome patients and 45 control subjects, matched in a 1:1 ratio. Serum samples collected from both study and control groups were analyzed using enzyme-linked immunosorbent assay.

RESULTS: The demographic characteristics were similar between the polycystic ovary syndrome and control groups (p>0.05). Periostin levels were significantly higher in patients with polycystic ovary syndrome compared with the control group (4.67±2.46 vs. 2.60±1.41 ng/mL, respectively; p=0.000).

CONCLUSION: Our study revealed a significant elevation in periostin levels among polycystic ovary syndrome patients compared with controls. These findings suggest that periostin could serve as a potential marker for assessing disease severity in polycystic ovary syndrome patients.

KEYWORDS: Polycystic ovary syndrome. Periostin. Inflammation. Insulin resistance.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women during the reproductive period. Patients who meet at least two of the criteria of oligoanovulation, polycystic ovary appearance on ultrasound, and clinical or biochemical hyperandrogenism are diagnosed with PCOS[1]. Chronic inflammation is thought to play a role in pathophysiology. These patients suffer from insulin resistance (IR), obesity, dyslipidemia, infertility, and menstrual irregularities in their lives. The prevalence of obesity varies between 12.5 and 100% in publications[2]. In addition, there is an increase in the risk of type 2 diabetes, cardiovascular diseases, and metabolic syndrome (MS) with advancing age[3,4].

Periostin is a matricellular protein that is embryonically expressed. It has been previously detected in bone, heart, teeth, uterus, and breast tissues[5]. The function of periostin is thought to be cell adhesion, migration, proliferation, and differentiation[6]. It is known that it survives longer than normal cells in hypoxic environments[7]. Periostin-secreting cells have been shown to increase paracrine tissue repair[8]. The relationship between PCOS, obesity, and chronic inflammation has been increasing in recent years[9,10]. It has been shown that periostin level increases with inflammation and IR[11]. It has been revealed that periostin may be involved in some metabolic diseases through JNK-mediated suppression of fatty acid oxidation in the liver[12]. Subsequently, periostin levels were shown to be strongly associated with triglycerides (TG) metabolism, chronic inflammation, and IR[13]. In addition, high glucose concentrations may also increase periostin expression. Besides this, studies on periostin in gynecology are increasing. Studies have shown that periostin increases in endometrial tissue in the midproliferative and early secretory phases and decreases in the late proliferative and late secretory phases. It has been suggested that periostin may play a role in implantation in the endometrium[14].

In the literature, a few studies have examined the PCOS and periostin relationship[11,15]. The aim of this study was to determine the serum periostin level in PCOS patients and examine its relationship with metabolic parameters.

METHODS

This is a prospective case-control study. Women between the ages of 18 and 45 years who applied to Karabuk University Training and Research Hospital Gynecology Polyclinic between April 2017 and June 2017 were included in the study. In this

[1]Samsun University, Department of Gynecology and Obstetrics – Samsun, Turkey.
[2]Alanya Alaaddin Keykubat University, Department of Microbiology – Alanya, Turkey.
*Corresponding author: drsemraeroglu@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 29, 2024. Accepted on March 03, 2024.

study, the participants were designated as the PCOS group (n=45) or control group (n=45) on a 1:1 ratio. The study protocol was approved by the ethics committee of the Medical Faculty of Karabuk University.

Polycystic ovary syndrome was diagnosed according to the 2003 Rotterdam European Society for Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) PCOS Consensus Workshop Group diagnostic criteria[1]. According to the NIH recommendation, there are four PCOS phenotypes evaluated: type A (hyperandrogenism, chronic anovulation, and polycystic ovaries); B (hyperandrogenism and chronic anovulation without polycystic ovaries); C (hyperandrogenism and polycystic ovaries); and D (chronic anovulation and polycystic ovaries without hyperandrogenism)[5,16]. In this study, the PCOS group consisted of phenotype-A patients. Pelvic ultrasound and standard gynecological examinations were performed by the same physician. All participants gave written informed consent.

The control group consisted of healthy individuals with regular menstrual periods, normal ovaries on ultrasonographic evaluation, and normal hormonal status who were matched for age and body mass index (BMI). Among the exclusion criteria were pregnancy status, malignancy history, hypothyroidism, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, inflammatory disease history, androgen-secreting tumor, active infection, and receiving treatment for PCOS or acne, including oral contraceptives, antidiabetics, acne or eyes solutions, glucocorticoids, anti-obesity, and ovulation induction agents. Age, height, weight, BMI, and menstruation findings were collected and recorded for all of the subjects. The BMI was calculated by dividing the weight (kg) by the square of the height (kg/m^2).

Sample processing

Blood samples were retrieved from all of the participants on the second and third days of the menstrual cycle after 8–12 h of overnight fasting. For the laboratory parameters, the ADVIA 1800 system (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) was used to evaluate the fasting and non-fasting glucose, TG, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. IR was determined using the homeostatic model assessment of insulin resistance (HOMA-IR) index using the following formula: a [Fasting insulin (μU/mL) × Fasting Glucose (mg/dL)/405]. The serum periostin levels were stored at -70°C and determined by enzyme-linked immunosorbent assay (ELISA) (Fine test, Wuhan, Hubei, China).

Statistical analysis

The SPSS 22 program was used for data analysis (IBM, NY, USA). The minimum sample size was calculated as 45 per group based on α error of 0.05, power of 0.90, and effect size of 0.7[15].

The Kolmogorov-Smirnov test was used to determine normal distribution of the variables. As the data followed a normal distribution, the Student's t-test was applied to compare the clinical and laboratory parameters. The Pearson correlation analysis was performed to investigate the relationship between the variables. $p<0.05$ was considered statistically significant.

RESULTS

Demographic and laboratory parameters of the PCOS and control groups are shown in Table 1. The mean age and BMI were similar in the PCOS and control groups (p=0.52 and p=0.864). Serum glucose levels and LDL cholesterol levels were observed to be significantly higher in the PCOS group compared with the control group (p=0.042 and p=0.03, respectively).

The levels of periostin in the patients with PCOS and control groups were found as 4.67±2.46 and 2.60±1.41 ng/mL, respectively. The increased level of periostin in PCOS was also statistically significant (p=0.000).

There were no statistically significant correlations between periostin and metabolic variables according to correlation tests (Table 2).

DISCUSSION

In this study, serum periostin levels were found to be significantly higher in PCOS patients. Hyperandrogenism and IR, which are involved in the pathophysiology of PCOS, are thought to be accompanied by chronic low-grade inflammation and are responsible for the reproductive and metabolic dysfunction in PCOS[9]. While polycystic ovarian morphology occurs intrauterinely, IR is usually the postnatal activating factor. Increased serine phosphorylation in the insulin receptor and disruption of the insulin signaling pathway to the ovary were seen as the primary cause. IR causes hyperinsulinemia by increasing ovarian androgen secretion or LH secretion[9]. Findings support some degree of IR in many women with PCOS. Maffazioli and colleagues identified the prevalence of MS in women with PCOS as 27.4%, with hypertension present in 10.9%. The prevalence of MS among normal weight, overweight, and obese women with PCOS was found to be 17.6, 22.6, and 33.9%, respectively[3]. The prevalence of MS

Table 1. Comparison between patients with polycystic ovary syndrome and the control group according to laboratory parameters.

Variables	PCOS group (n=45) Mean±standard deviation	Control group (n=45) Mean±standard deviation	p-values
Age (years, mean±SD)	22.40±5.46	23.02±4.30	0.52
Periostin (ng/mL)	4.67±2.46	2.60±1.41	0.000
Body mass index (kg/m^2)	24.10±9.28	23.65±10.2	0.864
Insulin (mmol/L)	19.26±9.48	17.24±8.64	0.074
Serum glucose (mmol/L)	88.92±5.64	84.26±9.26	0.042
HOMA-IR	4.15±3.26	3.85±3.84	0.146
Serum cholesterol (mg/dL)	164.26±31.26	156.84±42.84	0.120
LDL cholesterol (mg/dL)	102.04±21.34	93.40±24.26	0.03
HDL cholesterol (mg/dL)	62.22±24.28	63.44±26.42	0.74
Triglycerides (mg/dL)	110.24±8.94	102.26±11.24	0.06
FSH (IU/L)	7.46±4.42	8.52±3.24	0.89
LH (IU/L)	9.52±5.28	8.62±4.42	0.06
Estradiol (pg/mL)	58.46±34.28	55.24±36.27	0.82
Total testosterone (ng/mL)	49.26±21.44	44.35±28.24	0.76

The parametric variables are shown as mean±SD, median. p-values were obtained using the Student's t-test. p<0.05 was considered statistically significant. HOMA-IR: homeostatic model assessment-insulin resistance; LDL cholesterol: low-density lipoprotein; HDL cholesterol: high-density lipoprotein; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

appears to have increased in normal-weight individuals with PCOS compared with the general population. In this context, it will not be sufficient to address only menstrual irregularities and infertility issues in PCOS patients encountered in clinics. There is a need for new modalities to prevent or delay the formation of MS. Current treatments are aimed at addressing symptoms rather than resolving the cause of PCOS. For example, although ovulation can occur with ovulatory agents in PCOS, the chances of pregnancy can still be low. This is presumed to be due to the different effects of genetic and morphological changes on endometrial receptivity[10]. Therefore, different markers affecting the endometrium may play a role in physiology. The complexity of PCOS still remains a mystery.

Polycystic ovary syndrome has been divided into four phenotypes based on clinical or biochemical hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology[16]. Differences in treatment methods in long-term PCOS follow-up studies may reveal factors affecting the progression to MS. Baracat et al. emphasized that anovulation and hyperandrogenism are necessary for the diagnosis of PCOS and argued that treatments may differ according to PCOS phenotypes. For example, letrozole may not be suitable for Phenotype C, as indicated by OKS Phenotype D[10]. In a study on 310 PCOS patients followed for 6 years, Soarez et al. used metformin for those with IR, and combined oral contraceptive (COC) and antiandrogen drugs for those without MS or reproductive desire, for menstrual regulation and hirsutism treatment, comparing them with the control group. Significant increases in the visceral adiposity index (VAI), indicating type 2 diabetes mellitus (DM) and cardiovascular risk, were observed only in Phenotype A. Despite treatment, there was no decrease in the

Table 2. Correlation between periostin level and laboratory parameters in polycystic ovary syndrome patients.

	Periostin
Body mass index (kg/m^2)	r=0.046 p=0.820
HOMA-IR	r=0.124 p=0.08
Serum cholesterol (mg/dL)	r=0.042 p=0.644
LDL cholesterol (mg/dL)	r=0.08 p=0.920
HDL cholesterol (mg/dL)	r=-0.62 p=0.54
Triglycerides (mg/dL)	r=0.47 p=0.26
FSH (IU/L)	r=-0.152 p=0.132
LH (IU/L)	r=0.162 p=0.09

p<0.05 is statistically significant.

risk of type 2 DM and MS in Phenotype A. The frequency of MS was found to be unchanged in all types except Phenotype D[4]. Phenotype D is considered the mildest type, and we believe that lifestyle changes and appropriate treatment can prevent future morbidities.

In another study, Iwata et al. divided PCOS patients into three groups in their treatment scheme: COC, Metformin, and COC+Metformin. Ultimately, they found that IR improved only in PCOS patients given Metformin treatment alone. No improvement in IR was observed in those using Metformin+COC treatment. However, using COC alone showed an improvement in acne, Ferriman-Gallwey index, and menstrual cycle index and a decrease in testosterone and androstenedione levels[17]. After that, Medeiros et al. published an article about dysglycemia prediction in PCOS patients. They enrolled 648 PCOS and 330 control subjects. In non-PCOS women, low levels of thyroid-stimulating hormone (TSH) and high levels of testosterone predict estimated average glucose (EAG), whereas in PCOS Phenotype A, low HDL cholesterol (HDL-C) and high estradiol levels predict EAG. In Phenotype D, EAG is predicted with high HDL-C. Finally, anthropometric, hormonal, and lipid parameters did not provide much benefit in predicting dysglycemia. The authors emphasized the need to evaluate outcomes across different phenotypes of PCOS[18].

Similar to our study, Chen et al. demonstrated that periostin levels are elevated in PCOS patients and that there is a positive correlation between periostin and HOMA-IR[11]. Previous research has suggested that insulin may stimulate the theca cells of the ovary to produce excess testosterone, leading to clinical symptoms of hyperandrogenism such as acne, hirsutism, and alopecia[19]. Therefore, it is plausible to speculate that increased periostin levels may contribute to the development of IR in women with PCOS and play a role in the pathogenesis and clinical manifestations of the syndrome. Elevated periostin levels and chronic inflammation may explain hyperandrogenism. In Chen et al's study, glucose and LDL levels were significantly higher in the PCOS group, while HOMA-IR levels were independently associated with periostin. Moreover, periostin was positively correlated with BMI, uric acid, and HOMA-IR[11]. However, in our study, no significant relationship was found between metabolic parameters and periostin levels. Similarly, Gonulalan et al. also found no correlation between periostin and metabolic parameters[15]. Our findings are consistent with the existing literature. Based on these results, we believe that periostin levels may contribute to the pathophysiology of PCOS through chronic inflammation.

Periostin is a protein secreted from fibroblasts, which are a component of the extracellular matrix. It is known to be secreted from the lung, breast, thyroid, placenta, ovary, skin, and periodontal ligaments[5]. Its role in promoting the occurrence of various diseases is to activate different signaling pathways by combining with integrins. Recent studies have shown that periostin may be associated with inflammation, IR, glucose, and lipid metabolism. In addition, increased periostin expression was observed in the liver of obese rodents and humans and was associated with hepatosteatosis and hypertriglycidemia. Periostin overexpression was linked to decreased expression of peroxisome proliferator-activated receptor α (PPARα) protein[13]. Fatty acid oxidation caused by dyslipidemia can promote TG expression in the liver, while excessive activation of JNK signaling leads to the development of fatty liver, obesity, and IR[20]. Lu et al. revealed that periostin may be involved in some metabolic diseases through JNK-mediated suppression of fatty acid oxidation in the liver[12]. Subsequently, periostin levels were shown to be strongly associated with TG metabolism, chronic inflammation, and IR[13]. In addition, high glucose concentrations may also increase periostin expression. Glucose is the main redox substrate of cells in the mononuclear circulation. In this process, the formation of reactive oxygen species (ROS) is induced. This leads to the activation of NF-kB, the transcription factor that is associated with the expression of pro-inflammatory mediators such as TNF or IL-6[21]. Additionally, the hyperglycemic state may promote the secretion of steroidogenic molecules, which may result in hyperandrogenemia[22].

It is believed that periostin plays an active role in the female reproductive system. Studies have shown that periostin is involved in various reproductive processes[14,23]. For instance, Hiroi et al. demonstrated in mice that periostin is crucial for implantation. Additionally, periostin mRNA levels in human endometrium fluctuate throughout the menstrual cycle, with significant increases during the mid-proliferative and early secretory phases and decreases during the late proliferative, mid-secretory, and late secretory phases. This suggests that periostin expression is regulated by ovarian steroid hormones in both rat uterus and human endometrium[23]. Furthermore, periostin mRNA levels have been monitored in the endometrium of pregnant sheep at 12–14 days, indicating a potential role for periostin in promoting trophoectoderm cell retention and migration, particularly in sheep treated with progesterone[24]. Subsequent studies have demonstrated that periostin enhances migration, adhesion, and invasion of endometrial tissues in the

context of endometriosis[25]. In addition, periostin levels have been found to vary in cases of spontaneous abortion compared with voluntary abortions, suggesting a potential role for periostin in early pregnancy maintenance[6]. In our previous study, while serum periostin levels were lower in the spontaneous abortion group compared with the voluntary abortion group, tissue values were found to be similar[14]. Collectively, these findings indicate that periostin may act as a regulatory molecule in the female reproductive system, potentially influencing menstrual cycles, ovulation, and early pregnancy processes.

This study represents one of the initial investigations on periostin levels among patients with PCOS, thereby enriching our understanding of the interplay between PCOS pathophysiology, inflammation, and IR. Nevertheless, it is important to note that variations across different PCOS phenotypes were not explored in this study. In future studies, to comprehend whether elevated periostin leads to PCOS or if periostin levels elevate due to IR in PCOS, structural alterations that may occur can be monitored through electron microscopy or ovarian biopsy.

In conclusion, the levels of periostin are elevated in patients with PCOS. Periostin can serve as a marker for assessing the severity of the disease in PCOS patients. Understanding the role of periostin in the pathogenesis of the disease may pave the way for identifying therapeutic targets for future treatment of PCOS.

ACKNOWLEDGMENTS

This work was supported by Karabuk University under Grant number 17-KP-348.

AUTHORS' CONTRIBUTIONS

SE: Investigation, Software, Supervision, Validation, Visualization, Writing – review & editing. **EKC:** Formal Analysis, Methodology, Software, Validation.

REFERENCES

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25. https://doi.org/10.1016/j.fertnstert.2003.10.004
2. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The androgen excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91(2):456-88. https://doi.org/10.1016/j.fertnstert.2008.06.035
3. Maffazioli GDN, Lopes CP, Heinrich-Oliveira V, Lobo RA, Hayashida SAY, Soares JM, et al. Prevalence of metabolic disturbances among women with polycystic ovary syndrome in different regions of Brazil. Int J Gynaecol Obstet. 2020;151(3):383-91. https://doi.org/10.1002/ijgo.13374
4. Soares-Jr JM, Hayashida SAY, Marcondes JAM, Maciel GAR, Barcellos CRG, Maffazioli GN, et al. Influence of phenotypes on the metabolic syndrome of women with polycystic ovary syndrome over a six-year follow-up in Brazil. Biomedicines. 2023;11(12):3262. https://doi.org/10.3390/biomedicines11123262
5. Idolazzi L, Ridolo E, Fassio A, Gatti D, Montagni M, Caminati M, et al. Periostin: the bone and beyond. Eur J Intern Med. 2017;38:12-16. https://doi.org/10.1016/j.ejim.2016.11.015
6. Morelli M, Misaggi R, Cello A, Zuccalà V, Costanzo F, Zullo F, et al. Tissue expression and serum levels of periostin during pregnancy: a new biomarker of embryo-endometrial cross talk at implantation. Eur J Obstet Gynecol Reprod Biol. 2014;175:140-4. https://doi.org/10.1016/j.ejogrb.2013.12.027
7. Zhang Z, Nie F, Kang C, Chen B, Qin Z, Ma J, et al. Increased periostin expression affects the proliferation, collagen synthesis, migration and invasion of keloid fibroblasts under hypoxic conditions. Int J Mol Med. 2014;34(1):253-61. https://doi.org/10.3892/ijmm.2014.1760
8. Chow T, Rogers IM. Periostin is critical for improving the therapeutic properties of adipocyte-derived stem cells. Stem Cell Res Ther. 2015;6:214. https://doi.org/10.1186/s13287-015-0215-x
9. Lakkakula BV, Thangavelu M, Godla UR. Genetic variants associated with insulin signaling and glucose homeostasis in the pathogenesis of insulin resistance in polycystic ovary syndrome: a systematic review. J Assist Reprod Genet. 2013;30(7):883-95. https://doi.org/10.1007/s10815-013-0030-1
10. Baracat EC, Baracat MCP, José MSJ. Are there new insights for the definition of PCOS?. Gynecol Endocrinol. 2022;38(9):703-4. https://doi.org/10.1080/09513590.2022.2121387
11. Chen X, Huo L, Ren L, Li Y, Sun Y, Li Y, et al. Polycystic ovary syndrome is associated with elevated periostin levels. Exp Clin Endocrinol Diabetes. 2019;127(9):571-7. https://doi.org/10.1055/a-0752-0061
12. Lu Y, Liu X, Jiao Y, Xiong X, Wang E, Wang X, et al. Periostin promotes liver steatosis and hypertriglyceridemia through downregulation of PPARα. J Clin Invest. 2014;124(8):3501-13. https://doi.org/10.1172/JCI74438
13. Luo Y, Qu H, Wang H, Wei H, Wu J, Duan Y, et al. Plasma periostin levels are increased in chinese subjects with obesity and type 2 diabetes and are positively correlated with glucose and lipid parameters. Mediators Inflamm. 2016;2016:6423637. https://doi.org/10.1155/2016/6423637
14. Eroglu S, Colak E, Erinanc OH, Ozdemir D, Ceran MU, Tasdemir U, et al. Serum and placental periostin levels in women with early pregnancy loss. J Reprod Immunol. 2020;140:103138. https://doi.org/10.1016/j.jri.2020.103138
15. Gonulalan G, Guney I, Sackan F, Acar S. The relationship between polycystic ovary syndrome and serum periostin level. J Coll Physicians Surg Pak. 2021;31(11):1291-5. https://doi.org/10.29271/jcpsp.2021.11.1291

16. National Institutes of Health. Evidence-based methodology workshop on polycystic ovary syndrome. 3–5 December 2012. Executive Summary. 2022. [cited on 2022 Dec 08]. Available from: https://prevention.nih.gov/docs/programs/pcos/finalreport.pdf

17. Iwata MC, Porquere L, Sorpreso IC, Baracat EC, Soares Júnior JM. Association of oral contraceptive and metformin did not improve insulin resistance in women with polycystic ovary syndrome. Rev Assoc Med Bras (1992). 2015;61(3):215-9. https://doi.org/10.1590/1806-9282.61.03.215

18. Medeiros SF, Medeiros ALWY, Medeiros MAS, Silva Carvalho AB, Yamamoto MW, Soares JM, et al. Anthropometric, metabolic, and endocrine parameters as predictors of estimated average glucose and other biomarkers of dysglycemia in women with different phenotypes of polycystic ovary syndrome. Horm Metab Res. 2024;1:10. https://doi.org/10.1055/a-2207-0739

19. Traub ML. Assessing and treating insulin resistance in women with polycystic ovarian syndrome. World J Diabetes. 2011;2(3):33-40. https://doi.org/10.4239/wjd.v2.i3.33

20. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. Nature. 2002;420(6913):333-6. https://doi.org/10.1038/nature01137

21. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2002;26(7):883-96. https://doi.org/10.1038/sj.ijo.0801994

22. Duleba AJ, Dokras A. Is PCOS an inflammatory process? Fertil Steril. 2012;97(1):7-12. https://doi.org/10.1016/j.fertnstert.2011.11.023

23. Hiroi H, Momoeda M, Nakazawa F, Koizumi M, Tsutsumi R, Hosokawa Y, et al. Expression and regulation of periostin/OSF-2 gene in rat uterus and human endometrium. Endocr J. 2008;55(1):183-9. https://doi.org/10.1507/endocrj.k07-073

24. Ahn HW, Farmer JL, Bazer FW, Spencer TE. Progesterone and interferon tau-regulated genes in the ovine uterine endometrium: identification of periostin as a potential mediator of conceptus elongation. Reproduction. 2009;138(5):813-25. https://doi.org/10.1530/REP-09-0208

25. Zheng QM, Lu JJ, Zhao J, Wei X, Wang L, Liu PS. Periostin facilitates the epithelial-mesenchymal transition of endometrial epithelial cells through ILK-Akt signaling pathway. Biomed Res Int. 2016;2016:9842619. https://doi.org/10.1155/2016/9842619

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240141

The impact of herbal treatments on cervicovaginal human papillomavirus infection: a systematic review and meta-analysis

Nuran Gençtürk[1], Aysu Yildiz Karaahmet[2*], Dilan Cömert[3]

SUMMARY

OBJECTIVE: This systematic review and meta-analysis aimed to investigate the effects of herbal treatments on cervicovaginal human papillomavirus infection.

METHODS: A comprehensive literature search was conducted in PubMed, Scopus, Science Direct, and the Cochrane Library until December 2023, following Cochrane guidelines. Data were analyzed using the Review Manager computer program (Version 5.4.1).

RESULTS: Five randomized controlled trials involving a total sample size of 662 women were included in the study. The pooled odds ratio for individuals testing negative for human papillomavirus after herbal intervention among human papillomavirus-positive patients was 1.86 (95% confidence interval (CI) 0.64–5.43), according to the fixed-effects model. Three out of the five studies indicated a significant relationship. The relationship between positive human papillomavirus infection and herbal treatments, measured by the fixed-effects model, resulted in a pooled odds ratio of 0.41 (95%CI 0.17–1.01), reporting a significant association (p=0.05). Subgroup analysis revealed a significant reduction in the relationship between herbal treatment and atypical squamous cells of undetermined significance (OR 0.16, 95%CI 0.03–0.88, p=0.04) but no significant impact on the relationship between herbal treatment and low-grade squamous intraepithelial lesion (OR 0.33, 95%CI 0.01–8.77, p=0.51).

CONCLUSION: The meta-analysis suggests that herbal treatments reduce human papillomavirus infections. While herbal treatments show a significant reduction in atypical squamous cells of undetermined significance, they do not significantly impact the regression of low-grade squamous intraepithelial lesions.

KEYWORDS: HPV infection. Cervicovaginal lesions. Herbal. Treatment. Randomized controlled trial.

INTRODUCTION

According to the 2020 cancer statistics from the Global Cancer Observatory (GLOBOCAN) by the International Agency for Research on Cancer (IARC), cervical cancer is the fourth most common cancer globally[1]. Human papillomavirus (HPV) is the most prevalent sexually transmitted infection and forms the foundation for the development of cervical cancer neoplasia. HPV is detected in 99.7% of cervical cancer cases[2]. A large portion of HPV cases resolves spontaneously without any symptoms[2,3]. The World Health Organization (WHO) estimates the global prevalence of HPV to be between 9 and 13% of the population[3,4]. While prophylactic vaccines effectively prevent new infections, they do not eliminate existing infections[5]. HPV infections can be treated with methods such as laser, conization, and surgery. Invasive treatments are generally effective in high-grade intraepithelial lesions (HSIL) cases. However, these invasive treatments may have various side effects, such as early delivery, late miscarriages, cervical stenosis, and the possibility of recurrence[6]. Therefore, there is a need for non-invasive treatments that can eliminate lesions and HPV infections[7]. As one of these non-invasive methods, herbal treatments are reported to be effective in clearing HPV and reducing abnormal cytology associated with HPV[8,9].

This systematic review and meta-analysis aimed to examine the impact of herbal treatments on cervicovaginal HPV infection. This study represents the first meta-analysis solely evaluating the effectiveness of herbal treatments. The meta-analysis of randomized controlled trials (RCTs) on herbal treatments for patients with cervicovaginal HPV infection was conducted to obtain more concrete results.

METHODS

A systematic review and meta-analysis were conducted to evaluate the impact of herbal treatments on cervicovaginal

[1]Istanbul University-Cerrahpaşa, Faculty of Health Sciences, Department of Midwifery – İstanbul, Turkey.
[2]Halic University, Faculty of Health Sciences, Department of Midwifery – İstanbul, Turkey.
[3]Istanbul University-Cerrahpaşa, Graduate Education Institute, Department of Midwifery, Doctoral Program – İstanbul, Turkey.
*Corresponding author: aysuyildiz@halic.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on March 05, 2024. Accepted on March 17, 2024.

HPV infection. The study aimed to address the following questions:

1. What is the effect of herbal treatments on the treatment of HPV infection?
2. What is the impact of herbal treatments on cytological regression?

Protocol registration

The PRISMA (preferred reporting items for systematic reviews and meta-analysis statement) guidelines was followed in preparing this systematic review and meta-analysis. Throughout the study, there were no instances requiring deviation from the protocol, and the study was concluded following the protocol registered in the PROSPERO database (CRD42023491610).

Eligibility criteria

The selection of studies adhered to the following criteria (PICOS): Participant (P): Women with cervicovaginal HPV infection. Intervention (I): Herbal interventions, including (1) oral polyphenon E; (2) curcumin vaginal capsule; (3) vaginal spray containing olive oil; (4) myrtle vaginal suppository; (5) oral epigallocatechin gallate (EGCG); and (6) their combinations. Comparison (C): Comparison with a placebo or a group not receiving any treatment. Outcomes (O): (1) Results related to the effectiveness of herbal treatments on the treatment of HPV infection and (2) results related to the effectiveness of herbal treatments on cytological regression. Study Design (S): Experimental randomized controlled studies published in English and Turkish until December 2023 were included.

Exclusion criteria encompassed individuals under 18 years old, pregnant and breastfeeding women, those with known or suspected cervical cancer, HIV-positive patients, individuals with known allergies to herbal methods, animal studies, studies with unavailable full texts, and all studies not related to herbal treatments and incorporated theoretical studies, editorial comments, non-experimental studies such as only protocol studies and review papers, and articles using measurement tools with questionable validity. The inclusion of studies was limited to those with results or outcome tables available in English or Turkish.

Search strategy

For this systematic review, the literature search was conducted until December 20, 2023, using databases such as PubMed, Scopus, Science Direct, and the Cochrane Library.

The keywords included "HPV infection" OR "human papillomavirus" OR "cervicovaginal lesions" AND "curcumin" OR "polyphenon E" OR "myrtle" OR "epigallocatechin gallate" OR "herbal treatment." Additionally, systematic reference lists of articles and previous systematic reviews were searched.

Selection of studies and data extraction

Data were extracted by one reviewer (DC) using a data extraction form and checked by a second reviewer (AYK). Discrepancies between the two were resolved by a third researcher (NG). General characteristics of the studies (e.g., author, country, publication year, and study design), average age of participants, sample size of groups, type of intervention, duration of follow-up, intervention method, and primary outcome variables were included for each study (Table 1).

Quality assessment

Bias risk was assessed for seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. Other bias sources of this study included fundamental group imbalances and potential confounding factors. The risk of bias for each study was assessed as low, high, or unclear. The bias assessment was independently conducted by two researchers (AYK and DC), and in case of disagreement, the researchers reviewed the full text together to reach a consensus.

Data analysis

The relationships between herbal treatments and HPV parameters were estimated using pooled odds ratios (OR) and 95% confidence intervals (CI) with the Mantel-Haenszel method. Heterogeneity between studies was assessed using Cochran's Q test and Higgins' I^2, where I^2 greater than 50% indicated significant heterogeneity. Random-effects results were considered when I^2 was greater than 50%, and fixed-effects results were considered when I^2 was less than or equal to 50%. Various sensitivity analyses were conducted to assess the robustness of our results, excluding some articles considered of low quality. All analyses were performed using Review Manager 5.4.1 (The Nordic Cochrane Center, Copenhagen, Denmark). All tests were two-tailed, and a p-value less than 0.05 was considered statistically significant.

RESULTS

Study selection

Figure 1 presents the PRISMA flowchart summarizing the literature search and study selection process. The remaining

Table 1. Features of the included studies.

References, country	Study design	Study period	Age	Sample size	Intervention type	Intervention time and method	Control group	Outcomes
Aragona et al.[1], Italy	RCT	June 2022 to August 2022	Intervention: 37.35±2.60 Control: 37.65±2.48	Intervention: 20 Control: 20	Pervistop® [200 mg epigallocatechin gallate (EGCG), 400 µg folic acid (FA), 1 mg vitamin B12, and 50 mg hyaluronic acid (HA)]	Time: 3 months Method: Oral Pervistop® (200 mg epigallocatechin gallate) once daily	Control	HPV clearance: Intervention group: 17/20; Control group: 5/20 Cytological regression Intervention group: 3/20; Control group: 15/20
Nikakhtar et al.[7], Iran	RCT	November 2016 to December 2017	Intervention: 31.12±7.92 Placebo: 33.58±6.58	Intervention: 27 Placebo: 25	Myrtle	Time: 3 months Method: 20 vaginal suppositories for each month, 60 vaginal suppositories in total	Placebo	HPV clearance: Intervention group: 25/27; Placebo group: 17/25
Baleka Mutombo et al.[6], Congo	RCT	July 2015 to July 2017	Intervention: 41.6±10.6 Placebo: 42.4±11.1	Intervention: 168 Placebo: 159	Antiviral AV2®	Time: 6 months Method: spraying spray on cervix	Placebo	HPV clearance: Intervention group: 73/168; Placebo group: 61/159 Cytological regression Intervention group: 5/168; Placebo group: 13/159
Garcia et al.[17], United States of America	RCT	N/A	Intervention: 28.48±8.78 Placebo: 28.27±8.05	Intervention: 41 Placebo: 41	Polyphenon E	Time: 4 months Method: oral once daily, Polifenon E 800 mg	Placebo	HPV clearance: Intervention group: 10/41; Placebo group: 12/41
Basu et al.[18], India	RCT	N/A	Intervention: 37.5 (35.8–39.2) Placebo: 38.3 (35.7–38.9)	Intervention: 79 Placebo: 82	Curcumin	Time: 4 months Method: Curcumin vaginal capsule (500 mg per application)	Placebo	HPV temizleme: Intervention group: 75/79; Placebo group: 81/82

RCT: randomized control trials; N/A: data not reported.

21 full-text articles were assessed for eligibility, and five articles that met the criteria for RCTs were included in the analysis (Figure 1).

Study characteristics

This systematic review and meta-analysis encompass five studies involving a total of 662 women, aiming to examine the impact of herbal treatments on cervicovaginal HPV infection. The studies were conducted in India[10], the United States[11], Iran[7], Congo[12], and Italy[13]. The study design for all included studies was RCTs. Table 1 provides a summary of the characteristics of the studies. In all studies within the scope of this review, herbal treatments were applied to women in the intervention group. The efficacy of herbal treatments on cervicovaginal HPV infection was assessed in all studies. In the control group, Aragona et al.[1] did not administer any intervention, while the other four studies[7,10-12] implemented a placebo intervention.

Quality of bias assessment

Except for one study[13], all studies established a sufficient method for randomly assigning participants to cognitive-behavioral intervention groups. Therefore, we assessed the risk of bias as low in this domain for these studies. Except for Aragona et al.,[1] all studies reported sufficient allocation concealment for randomization and were evaluated as having a low risk of bias, except for one study[11] where dropouts were balanced between intervention and control groups or were considered to have a low risk of bias due to the small number of dropouts that would not significantly impact the study.

In three studies included in the meta-analysis[10-12], we considered a high risk of bias because they discussed significant results, including adverse outcomes, and matched the reports in their records. For each included study, we explained significant concerns about other potential sources of bias not addressed in the above categories. Specifically, we sought a conflict of interest statement and a funding source. None of the studies reported any other bias risk.

Meta-analysis

The meta-analysis results depicting the relationship between herbal interventions and HPV infection in women were presented as a forest plot. All included studies reported an association between herbal interventions and testing negative for HPV after the application to individuals initially testing positive. The measurements of the relationship between HPV and herbal treatments in the selected studies varied between 0.23 (95%CI 0.03–2.12) and 17.00 (95%CI 3.46–83.44). These studies showed a significant degree of heterogeneity (I2:76, p=0.002). According to the fixed-effects model, the pooled OR was 1.86 (95%CI 0.64, 5.43). Among the five studies, three demonstrated a significant relationship between herbal treatment and HPV infection (Figure 2a). All included studies reported an association between herbal interventions and a positive assessment for HPV infections. Measurements of the relationship between HPV and herbal treatments in the selected studies varied between 0.06 (95%CI 0.01, 0.29) and 1.28 (95%CI 0.48, 3.42). These studies exhibited a significant degree of heterogeneity (I2:68, p=0.01). According to the fixed-effects model, the pooled OR was 0.49 (95%CI 0.16–1.45). Among the five studies, three demonstrated a significant relationship between herbal treatment and HPV infection (Figure 2b). Two included studies reported an association between herbal interventions and atypical squamous cells (ASCUS). Measurements of the relationship between herbal treatments and ASCUS in the selected studies varied between 0.06 (95%CI 0.01–0.29) and 0.34 (95%CI 0.12–0.99). These studies showed a significant degree of heterogeneity (I2:70, p=0.07). According to the fixed-effects model, the pooled OR was 0.16 (95%CI 0.03, 0.88). Two studies demonstrated a significant relationship between herbal treatment and ASCUS (p=0.04) (Figure 2c). One included study reported an association between herbal interventions and low-grade squamous intraepithelial lesions (LSIL). Measurements of the relationship between herbal treatments and LSIL in the selected studies varied between

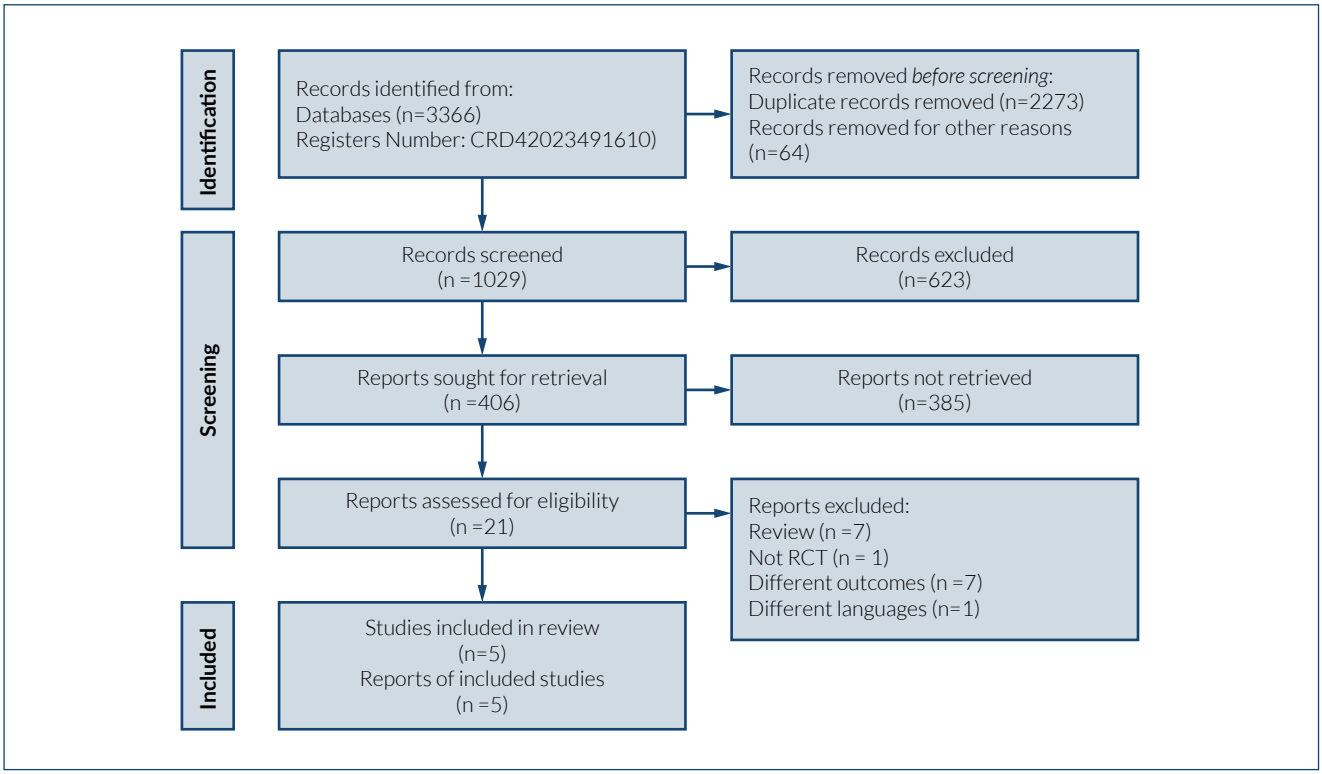

Figure 1. Preferred reporting items for systematic reviews and meta-analysis 2020 flow diagram for new systematic reviews which included searches of databases and registers only.

0.06 (95%CI 0.01–0.29) and 1.68 (95%CI 0.48–5.87). These studies exhibited a significant degree of heterogeneity (I2:91, p=0.001). According to the fixed-effects model, the pooled OR was 0.33 (95%CI 0.01, 8.77). It indicated no significant difference between herbal treatments and LSIL (p=0.51) (Figure 2d).

DISCUSSION

This systematic review and meta-analysis delved into the efficacy of herbal treatments on cervicovaginal HPV infection. According to the meta-analysis results, herbal treatments were found to be effective in treating HPV infection. Furthermore, secondary outcomes indicated the effectiveness

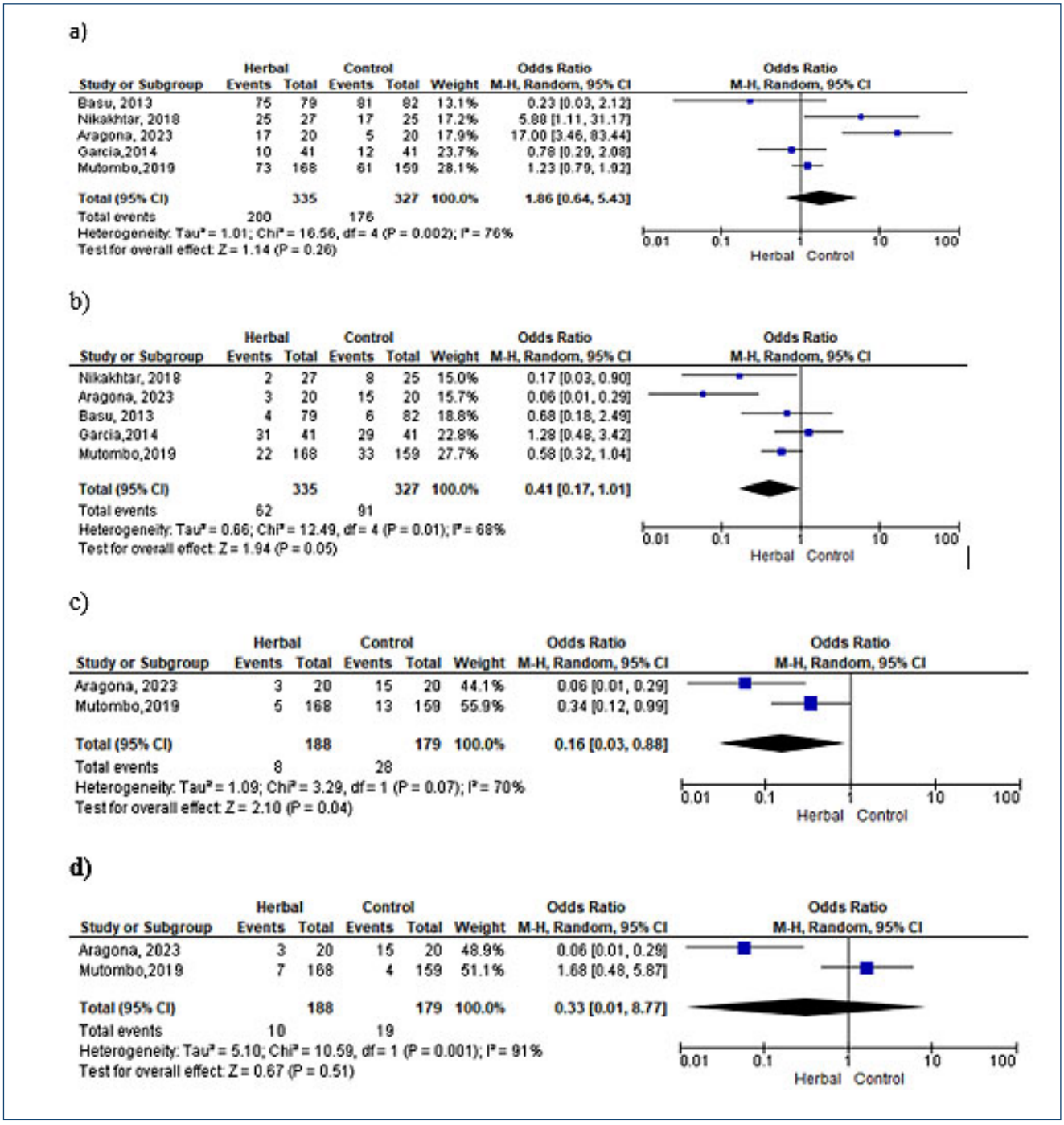

Figure 2. Forest plots showing the association between herbal treatment and (a) negative human papillomavirus, (b) positive human papillomavirus, (c) Atypical squamous cells rate, and (d) Low grade squamous intraepithelial lesions rate.

of herbal treatments in terms of ASCUS. However, they did not show a significant effect in regressing lesions concerning LSIL.

While HPV infection can be treated with invasive methods[14], there is a substantial need for acceptable, safe, cost-effective, and non-surgical treatments to prevent cervical cancer. Herbal treatments emerge as one of these non-invasive methods[15,16]. In this study, herbal treatments were determined to be effective in clearing HPV infection (p=0.05). Although there is no meta-analysis specifically evaluating the effectiveness of herbal treatments in HPV treatment in the literature, a meta-analysis found a significant impact of biological and herbal studies on HPV clearance in a subgroup analysis similar to this study (p=0.01)[11].

A literature search identified meta-analysis studies evaluating the efficacy of non-surgical treatments on HPV. The results, similar to this study, indicated a significant effect on clearing HPV: Xiong et al.[10] (p<0.00001), Huang et al.[5] (p <0.0001), and Zhuang and Yang[11] (p<0.01). While these studies are up-to-date, providing a comprehensive assessment of non-surgical treatment methods, this study is crucial for focusing exclusively on the impact of herbal treatments and obtaining more specific data.

This study evaluated the regression of ASCUS and LSIL with herbal treatment methods as a secondary outcome. A regression in lesions concerning ASCUS was determined with herbal treatments (p=0.04). However, a significant effect concerning LSIL (p=0.51) could not be determined. In their subgroup analysis of a meta-analysis, Xiong et al.[5] found a significant impact of biological and herbal studies on cytological regression similar to this study (p=0.02). The same study found the overall effectiveness of non-surgical treatments on cytological regression to be significant (p=0.001). In a meta-analysis[15], it was determined that non-surgical treatment methods had a significantly higher level of regression in mild abnormal cytology compared with the control group (p<0.00001). The inability to obtain significant results regarding LSIL in this study may be due to the limited number of studies considered for evaluation.

Strengths and limitations

One of the robust aspects of this study is that this is the first meta-analysis examining the efficacy of herbal treatments on HPV infection. The study aimed to provide less heterogeneous data by solely analyzing herbal treatments, contributing valuable insights to the field and literature. Another strength lies in the diverse participant background, with individuals from studies representing various income levels and different advantage-disadvantage groups, enhancing the generalizability of the findings.

The study's meticulous approach to scanning multiple databases and involving multiple researchers in the data extraction process ensures low bias and error. In addition, the methodological quality of the included studies was collaboratively assessed independently by each researcher, reaching a consensus after individual evaluations. However, one notable limitation is the inclusion of only English or Turkish publications, potentially excluding relevant studies published in other languages.

CONCLUSION

According to the meta-analysis results, herbal treatments demonstrated a reduction in HPV infections. While herbal treatments were effective in decreasing atypical squamous cells of undetermined significance, no significant impact was observed in the regression of low-grade squamous intraepithelial lesions. Nevertheless, further meta-analyses considering the effectiveness of herbal treatments require more RCTs to draw a more conclusive result.

AUTHORS' CONTRIBUTIONS

NG: Data curation, Formal Analysis, Investigation, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **AYK:** Conceptualization, Data curation, Formal Analysis, Investigation, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **DC:** Data curation, Formal Analysis, Investigation, Resources, Validation, Visualization, Writing – review & editing.

REFERENCES

1. Aragona C, Bezerra Espinola MS, Bilotta G, Porcaro G, Calcagno M. Evaluating the efficacy of Pervistop®, a new combination based on EGCG, folic acid, vitamin B12 and hyaluronic acid on patients with human papilloma virus (HPV) persistent infections and cervical lesions: a pilot study. J Clin Med. 2023;12(6):2171. https://doi.org/10.3390/jcm12062171
2. Arbyn M, Xu L. Efficacy and safety of prophylactic HPV vaccines. A Cochrane review of randomized trials. Expert Rev Vaccines. 2018;17(12):1085-91. https://doi.org/10.1080/14760584.2018.1548282
3. Eisenberg MC, Campredon LP, Brouwer AF, Walline HM, Marinelli BM, Lau YK, et al. Dynamics and determinants of HPV infection: the Michigan HPV and oropharyngeal cancer (M-HOC) study. BMJ Open. 2018;8(10):e021618. https://doi.org/10.1136/bmjopen-2018-021618

4. Bruno MT, Ferrara M, Fava V, Barrasso G, Panella MM. A prospective study of women with ASCUS or LSIL pap smears at baseline and HPV E6/E7 mRNA positive: a 3-year follow-up. Epidemiol Infect. 2018;146(5):612-8. https://doi.org/10.1017/S0950268818000250
5. Huang S, Qi Y, Chen S, He B, Chen X, Xu J. Effect of heat-clearing and dampness-eliminating Chinese medicine for high-risk cervical cancer papillomavirus infection: a systematic review and meta-analysis of randomized controlled trials. Front Med (Lausanne). 2023;10:1022030. https://doi.org/10.3389/fmed.2023.1022030
6. Baleka Mutombo A, Tozin R, Kanyiki H, Geertruyden JP, Jacquemyn Y. Impact of antiviral AV2 in the topical treatment of HPV-associated lesions of the cervix: results of a phase III randomized placebo-controlled trial. Contemp Clin Trials Commun. 2019;15:100377. https://doi.org/10.1016/j.conctc.2019.100377
7. Nikakhtar Z, Hasanzadeh M, Hamedi SS, Najafi MN, Tavassoli AP, Feyzabadi Z, et al. The efficacy of vaginal suppository based on myrtle in patients with cervicovaginal human papillomavirus infection: a randomized, double-blind, placebo trial. Phytother Res. 2018;32(10):2002-8. https://doi.org/10.1002/ptr.6131
8. Przybylski M, Pruski D, Millert-Kalinska S, Zmaczynski A, Baran R, Horbaczewska A, et al. Remission of HPV infection after LEEP-conization - a retrospective study. Ginekol Pol. 2022. https://doi.org/10.5603/GP.a2021.0164
9. Quinlan JD. Human papillomavirus: screening, testing, and prevention. Am Fam Physician. 2021;104(2):152-9. PMID: 34383440
10. Xiong Y, Cui L, Bian C, Zhao X, Wang X. Clearance of human papillomavirus infection in patients with cervical intraepithelial neoplasia: a systemic review and meta-analysis. Medicine (Baltimore). 2020;99(46):e23155. https://doi.org/10.1097/MD.0000000000023155
11. Zhuang Y, Yang H. The significance of nonsurgical therapies for cervical infection of high-risk human papilloma virus: a systematic review and meta-analysis. J Obstet Gynaecol Res. 2023;49(9):2213-31. https://doi.org/10.1111/jog.15726
12. Sundaram N, Voo TC, Tam CC. Adolescent HPV vaccination: empowerment, equity and ethics. Hum Vaccin Immunother. 2020;16(8):1835-40. https://doi.org/10.1080/21645515.2019.1697596
13. Phumaphi J, Gautam KC, Mason E. Increased production and comprehensive guidelines needed for HPV vaccine. Lancet. 2020;395(10221):319-21. https://doi.org/10.1016/S0140-6736(19)32976-9
14. McClung NM, Gargano JW, Park IU, Whitney E, Abdullah N, Ehlers S, et al. Estimated number of cases of high-grade cervical lesions diagnosed among women - United States, 2008 and 2016. MMWR Morb Mortal Wkly Rep. 2019;68(15):337-43. https://doi.org/10.15585/mmwr.mm6815a1
15. Rodriguez AM, Zeybek B, Vaughn M, Westra J, Kaul S, Montealegre JR, et al. Comparison of the long-term impact and clinical outcomes of fewer doses and standard doses of human papillomavirus vaccine in the United States: a database study. Cancer. 2020;126(8):1656-67. https://doi.org/10.1002/cncr.32700
16. Basu P, Dutta S, Begum R, Mittal S, Dutta PD, Bharti AC. et al. Clearance of cervical human papillomavirus infection by topical application of curcumin and curcumin containing polyherbal cream: a phase II randomized controlled study. Asian Pacific J Cancer Prevent. 2013;14(10):5753-9. https://doi.org/10.7314/apjcp.2013.14.10.5753
17. Garcia FA, Cornelison T, Nuño T, Greenspan DL, Byron JW, Hsu CH, et al. Results of a phase II randomized, double-blind, placebo-controlled trial of Polyphenon E in women with persistent high-risk HPV infection and low-grade cervical intraepithelial neoplasia. Gynecol Oncol. 2014;132(2):377-82. https://doi.org/10.1016/j.ygyno.2013.12.034
18. Shanmugasundaram S, You J. Targeting persistent human papillomavirus infection. Viruses. 2017;9(8):229. https://doi.org/10.3390/v9080229

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240226

Efficacy of probiotics, paraprobiotics, and postbiotics in colorectal cancer cell line and their role in immune response

Gülçin Alp Avci[1*], Ülkü İrem Yilmaz[1], Emre Avci[1]

SUMMARY

OBJECTIVE: The aim of this study was to reveal certain features (anti-tumor/microbial activities) of postbiotics and heat-inactivated paraprobiotics obtained from two different bacteria with determined probiotic properties, which are thought to contribute to human health.

METHODS: In the study, Lactobacillus reuteri ENA31 and L. rhamnosus GAA6 strains were used. Supernatants of postbiotically active cultures were used. Paraprobiotics were obtained by exposing probiotic bacteria to high temperatures. The cytotoxic effects of probiotics, paraprobiotics, and postbiotics were evaluated by the MTT method. IL-1/-10/-12/-13, TNF-α, IFN-γ, and neopterin parameters were determined via the ELISA method in immunity studies.

RESULTS: It was detected that biotics had a cytotoxic effect on cancer cells with rising concentrations (paraprobiotic<probiotic<postbiotics, respectively). Intercalarily, with these biotic applications, a decline in the values of IL-1, IFN-γ, TNF-α, and neopterin and a rise in the values of IL-10/-12/-13 were observed in cancer cells.

CONCLUSION: Our study shows that biotics, which are widely used and beneficial to health, are also available for use in immunocompromised individuals. The resulting paraprobiotics and postbiotics will both increase the conscious use of probiotics and provide the opportunity for use in immunocompromised individuals.

KEYWORDS: Probiotics. Cytotoxicity. Antimicrobial. Immunity.

INTRODUCTION

In the last few years, the use of structures known as paraprobiotics, which are non-living microorganisms, and postbiotics, which are metabolic by-products from bacteria to the external environment or resulting from the breakdown of bacteria, have become widespread as alternatives to probiotics. The crucial role of probiotics in maintaining intestinal homeostasis and in some gastrointestinal system disorders has been known for a long time, but there are still some pathways in the underlying mechanism that remain unclear[1]. Additionally, viability checks limit their use in the food and pharmaceutical industries. For this reason, the focus of studies is increasingly shifting from live probiotic microorganisms to non-living paraprobiotics, and biomolecules derived from probiotics, that is, postbiotics[1,2]. Postbiotics are a complex product of metabolic products with structural properties, such as enzymes, proteins, short-chain fatty acids, vitamins, peptides, and organic acids, secreted by probiotics in cell-free supernatants[3,4]. Paraprobiotics are inactivated microbial cells containing probiotics, which are intact or lysed structures or crude cell extracts containing cell components such as peptidoglycans, teichoic acids, and surface proteins[4].

Like probiotics, paraprobiotics and postbiotics have been reported to exert a range of strain-specific health-promoting activities in individuals, including maintaining intestinal regularity at the physiological level, enhancing immunomodulatory activity, reducing inflammation, and inhibiting tumor development. However, although there are many studies on the efficacy of these para-post biotics, the pathways or mechanisms through which they exert these effects have not been fully explained. For these reasons, in our study, we aimed to obtain heat-inactivated paraprobiotics and postbiotics released from probiotics that can be used instead of probiotics and to contribute to the elucidation of the process by revealing some of their properties (cytotoxic, antimicrobial, and antioxidant activities) that may benefit human health.

[1]University of Health Sciences, Faculty of Gulhane Dentistry, Department of Basic Medical Sciences – Ankara, Turkey.
[2]University of Health Sciences, Gülhane Vocational School of Health, Department of Pathology – Ankara, Turkey.
[3]University of Health Sciences, Faculty of Gulhane Pharmacy, Department of Biochemistry – Ankara, Turkey.
*Corresponding author: gulcin.alpavci@sbu.edu.tr

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this study was supported by the University of Health Sciences, Scientific Research Projects (2022/077).
Received on March 05, 2024. Accepted on March 17, 2024.

METHODS

Selection, isolation, and identification of possible probiotic properties

In this study, a total of 15 lactobacilli were isolated from 5 breastfed infants' fecal samples. Isolate studies used the method of Hadadji et al.[5]. Bacterial DNA was extracted from gram-positive and catalase-negative strains using a genomic DNA extraction kit (27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-AAGGAGGGTGATCCAGCC-3'). *L. reuteri* ENA31 and *L. rhamnosus* GAA6 strains were selected according to their possible probiotic characteristics in studies.

In the selection of probiotics, the acid resistance, bile tolerance, and EPS production of the strains were determined using the method applied by Alp and Aslım[6]. The disk diffusion method was used to determine the antibiotic susceptibilities of the strains. The study included the six most commonly used antibiotics, including ampicillin, gentamicin, vancomycin, tetracycline, chloramphenicol, and ofloxacin. The inhibition zone diameter was measured with the help of calipers, and the data were evaluated together with the Antimicrobial Drug Susceptibility Testing Conduct Standard.

Preparation of paraprobiotics

Bacteria were grown in MRS medium at 37°C for 18–24 h and then centrifuged at 5,000 g at 4°C for 10 min to obtain pellets. Afterward, it was washed three times with a saline solution and suspended in distilled water. Suspended bacteria were killed by subjecting them to heat at 80°C for 20 min, and a pellet was obtained by centrifuging at 5,000 g at 4°C for 10 min. The resulting pellets were suspended in distilled water and then lyophilized[7].

Preparation of postbiotics (cell-free)

The strains were grown in MRS broth at 37°C for 18–24 h and centrifuged at 2,000 g for 10 min. The supernatant was lyophilized[8].

The chemical compositions of postbiotics were determined by the GC-MS and identified using calculated linear retention indices and mass spectra with those reported in the National Institute of Standards and Technology (NIST) database 2005[7,8].

Determination of exopolysaccharide and bacteriocin as a postbiotic

Exopolysaccharide production of the strains was determined using the method applied by Alp and Aslım[7]. For bacteriocin, cultures were centrifuged to remove the cells, and the pH value of the resulting supernatants was adjusted to 7 with NaOH. Notably, 50% ammonium sulfate was added and mixed at 4°C for 24 h, and after the mixture was centrifuged under the same conditions, the pellets were collected and suspended in 25 mL of 0.05 M potassium phosphate buffer. The bacteriocin was partially purified by adding 15 mL of a methanol/chloroform (1:2 v/v) mixture and incubating at 4°C for 1 h.

In vitro cell culture studies

The MTT method was utilized in cytotoxic studies. The viability of treated cultures with <70% test extract compared to untreated control cultures was considered to have cytotoxic effect according to ISO 10993-5.

Determination of cellular immunity

In our study, IL-1/-10/-12/-13, TNF-α, IFN-IFN-γ, and neopterin parameters were examined using a commercially available enzyme-linked immunosorbent assay (ELISA, Rel Assay, Türkiye) kit.

Statistical analysis

Differences were determined by applying one-way ANOVA and Tukey analysis using the data obtained from the studies using the IBM SPSS 22.0 statistical program, and the results were shown as mean±standard deviation. The statistical significance value was accepted as $p<0.05$. The parameters included in the research studies, total antioxidant capacity and scavenging activities of DPPH free radicals, were evaluated logarithmically on a graph.

RESULTS

Selection of bacteria

In our study, based on the probiotic properties of 15 lactobacilli isolated from the fecal samples of 5 breastfed babies, the 2 strains (L. rhamnosus GAA6 and L. reuteri ENA31) with the highest acid resistance (pH 3.0; 8.54±0.09 and 7.98±0.07, respectively), bile resistance (0.3%; 7.54±0.05 and 7.20±0.02, respectively), and EPS production (101.24 mg/L and 86.45 mg/L, respectively) were selected for use in other studies. Antibiotic sensitivity was also considered in strain selection: ampicillin (15.21±0.12), gentamicin (7.45±0.08), vancomycin (5.70±0.02), tetracycline (17.10±0.20), chloramphenicol (18.10±0.31), and ofloxacin (5.25±0.03).

Exopolysaccharide and bacteriocin production

It was determined that the EPS production of the GAA6 strain (109.68 mg/L) was higher than the EPS production of the ENA31 strain (94.26 mg/L). The antimicrobial activity of the bacteriocin obtained from ENA31 and GAA6 was observed against some pathogenic bacteria. The findings obtained are presented in Table 1.

Cytotoxicity

In our study, the proliferative effect of both GAA6 as a probiotic and postbiotics and paraprobiotics obtained from it was determined in the L929 cell line, which was included as the control group. It has been determined that GAA6 and the postbiotics and paraprobiotics derived from it have a cytotoxic effect on the cancer cell CaCO2 in parallel with the increasing concentration, and this effect is more visible in postbiotics and paraprobiotics (Figure 1).

In our study, the proliferative effect of both ENA31 as a probiotic and postbiotics and paraprobiotics obtained from it was determined in the L929 cell line, which was included as the control group. It has been determined that ENA31 and the postbiotics and paraprobiotics derived from it have a cytotoxic effect on the cancer cell $CaCO_2$ in parallel with the increasing concentration, and this effect is more visible in postbiotics and paraprobiotics (Figure 2).

Determination of cellular immunity

A decrease in the levels of inflammatory cytokines (IL-1, TNF-α, and IFN-γ) and neopterin was determined in the control and the cells treated with probiotics, postbiotics, and paraprobiotics. On the other hand, an increase in the levels of IL-10/-12/-13 was detected. TNF-α level was 3.12±1.7 pg/mL in control-CaCO2 cells; after the addition of paraprobiotics, postbiotics, and probiotics, it was determined to be 2.54±0.4, 2.59±0.1 and 2.81±0.6 pg/mL, respectively. While the IFN-γ value was 1.24±0.6 pg/mL in control-$CaCO_2$ cells, it was observed that it decreased to 1.06±0.2, 1.10±0.1 and 1.10±0.2 pg/mL with the addition of paraprobiotics, postbiotics, and probiotics, respectively. While the IL-1 value was 0.97±0.7 pg/mL in control-$CaCO_2$ cells, it was determined to be 0.92±0.3, 0.86±0.1, and 0.79±0 pg/mL with the addition of

Table 1. Effect of bacteriocin obtained from Lactobacillus reuteri and Lactobacillus rhamnosus against gram-negative and gram-positive bacteria.

Bacteriocin	Zone of inhibition (mm)				
	Gram-positive bacterial strains		Gram-negative bacterial strains		
	S. aureus ATCC 25923	E. faecalis ATCC 29212	P. aeruginosa ATCC 27853	E. coli ATCC 25922	B. subtilis ATCC 6633
L. reuteri	18.4±0.4	14.7±0.3	11.4±0.9	29.1±2.4	12.6±0.8
L. rhamnosus	19.6±1.6	14.9±0.8	11.8±1.1	29.4±2.1	13.8±0.2

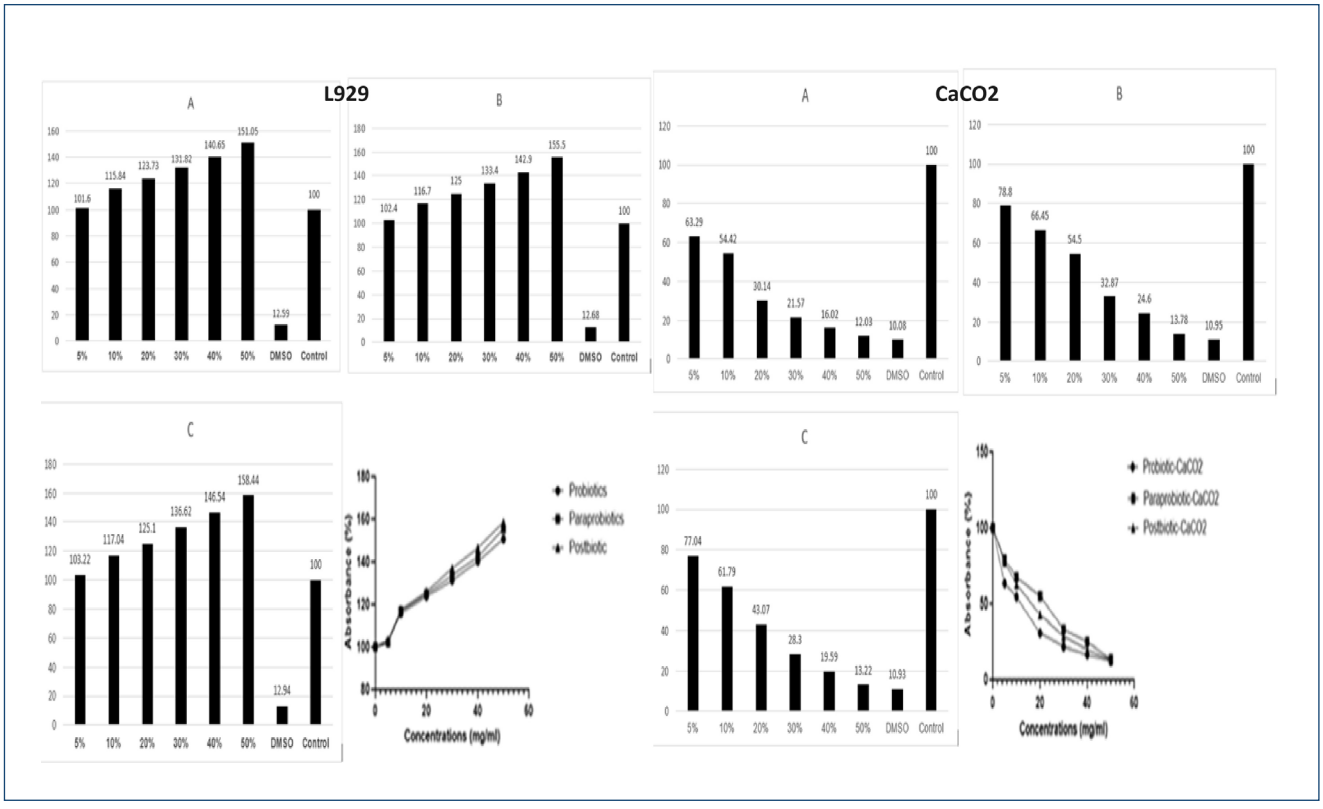

Figure 1. Effect of GAA6 on L929 and $CaCO_2$ cell lines viability of probiotics (A), paraprobiotics (B), and postbiotics (C).

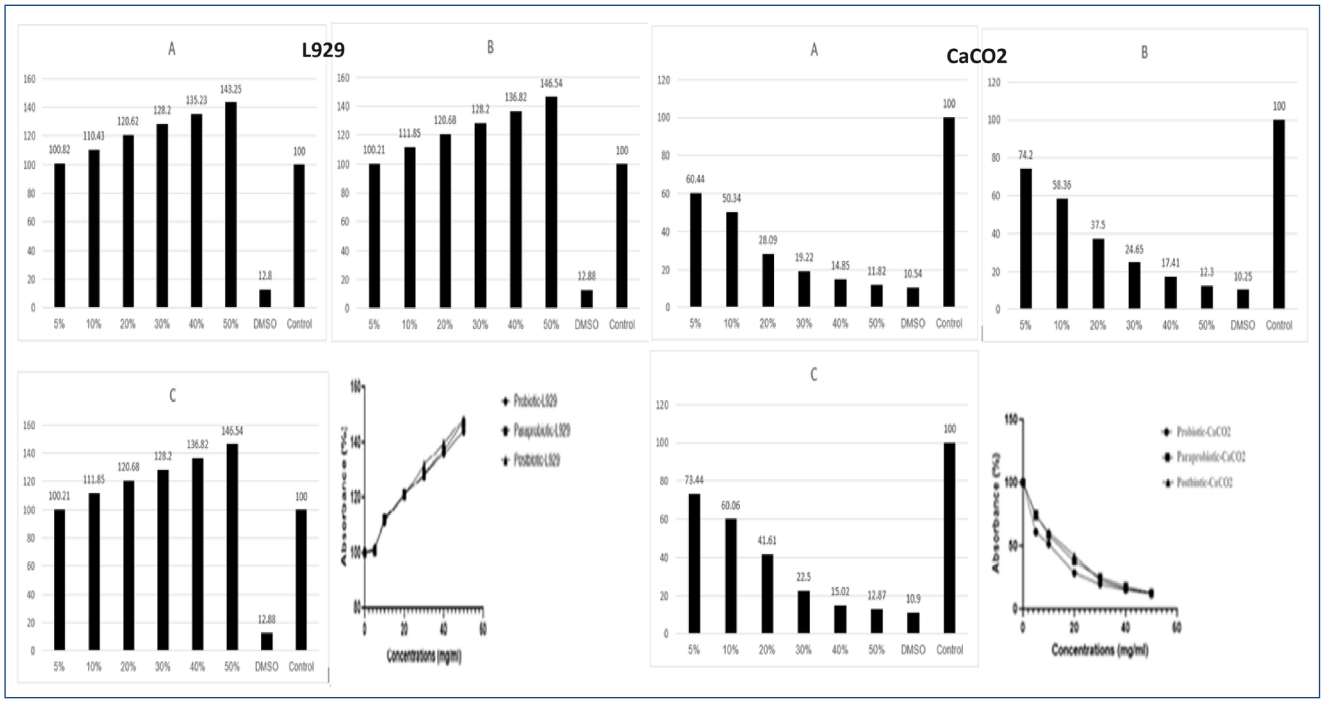

Figure 2. Effect of ENA31 on L929 and $CaCO_2$ cell lines viability of probiotics (A), paraprobiotics (B), and postbiotics (C).

paraprobiotics, postbiotics, and probiotics, respectively. While the IL-10 value was 2.21±1.0 pg/mL in control-$CaCO_2$ cells, it was raised to 2.66±1.0, 2.51±0.8, and 2.34±1.2 pg/mL, respectively, with the addition of paraprobiotics, postbiotics, and probiotics. While the IL-12 value in control-$CaCO_2$ cells was 2.05±0.9 pg/mL, with the addition of paraprobiotics, postbiotics, and probiotics, it was determined to be 2.48±1.7, 2.28±1.1, and 2.21±1.4 pg/mL, respectively. While the IL-13 value was 0.94±0.3 pg/mL in control-$CaCO_2$ cells, it increased to 1.34±1.7, 1.25±1.4, and 1.14±1.6 pg/mL with the addition of paraprobiotics, postbiotics, and probiotics, respectively.

DISCUSSION

In this context, studies reveal that postbiotics and paraprobiotics exhibit bioactivities such as anti-inflammatory, immunomodulatory, antioxidant, and antimicrobial as well as anticarcinogenic benefits[9-11]. Lactic acid bacteria have been reported to be beneficial to health by secreting lactic acid, peptidoglycan, bacteriocin, and metabolites that may be postbiotics. For example, it is reported that lactate and short-chain fatty acids produced by fermentation are postbiotics and affect the anti-inflammatory and anticarcinogenic properties of the intestine[12]. Recently, especially EPS, bacteriocins, and biosurfactants have attracted attention as postbiotics. Studies emphasize the importance of biotics with high EPS production capacity, biosurfactant production, and bacteriocin activity. Biotics with these features will contribute much more to the benefit of human health.

It has been reported in the literature that paraprobiotics inactivated by heat treatment do not interfere with the increase in the production of immune-supporting cytokines in macrophages, and thus the paraprobiotic has a positive effect on immunity[13,14]. One of the health problems for which the effects of paraprobiotics and postbiotics have been investigated is colon cancer. Several paraprobiotic fractions (heat-inactivated cells, cell walls, peptidoglycan, and cytoplasmic structures) derived from *Lactobacillus* spp. that have antiproliferative effects against human cancer cells are reported in the literature[13]. In a study conducted by Cicenia et al. on the effect of postbiotics on the colon, it was stated that smooth muscle cells in the human colon, *L. rhamnosus*, protected postbiotic chemicals that mediate postbiotic reactions from lipopolysaccharides that cause myogenic damage. Studies have concluded that paraprobiotics and/or their cell wall extracts can alleviate inflammation in ways similar to those of live bacteria[14]. In a study by Chuah et al., different postbiotics derived from *L. plantarum* strains exhibited selective cytotoxic activity through antiproliferative effects and induction of apoptosis against cancer cells, showing that they are strain-specific and cancer cell type-specific[9].

The immune system neutralizes cellular and humoral agents through different mechanisms. The literature reveals that the gut microbiome has a vital role, especially in the development of the host's immune system and the regulation of metabolic events[15]. Studies prove that paraprobiotics and postbiotics are effective on the immune system. *Bifidobacterium* spp. act against active ulcerative colitis and exacerbations of this disease. It has been observed that the application of paraprobiotic *Bifidobacteria* in fermented milk triggers the production of IL-10, an anti-inflammatory cytokine, and suppresses the secretion of IL-8, a pro-inflammatory cytokine, in epithelial cells[15]. Riaz et al. studied the cell-free supernatant of the liquid culture of three *L. rhamnosus* strains isolated from human breast milk and showed the antioxidant activities against radicals[16]. Song et al. studied the use of both live and heat-inactivated samples conducted with *L. brevis* B13-2 and showed that both forms showed antioxidant activity, while the paraprobiotic form exhibited both stability and immunomodulatory activity. It has been shown that they can be used as functional components[17]. Balzaretti et al. reported that an exopolysaccharide derived from *L. paracasei* DG as a postbiotic inhibited proinflammatory cytokines in the human monocytic cell line[18]. Qi et al. found that different postbiotic compounds, derived from *L. rhamnosus* GG, including surface layer protein, genomic DNA, and unmethylated cytosine-phosphate-guanine containing oligodeoxynucleotides, were activated by mitogen-activated protein kinases in lipopolysaccharide-stimulated mouse macrophage cells[19].

CONCLUSION

Hundreds of probiotic products are sold and used commercially around the world. However, although these products are not products of our country, they are brought from abroad and are widely used because they are beneficial to health, without their scientific data being researched in detail with academic studies. With the data obtained within the scope of our study, both the acquisition of new probiotics and the diversification of existing products—extending the shelf life—can be achieved. Considering all these study results, postbiotics and paraprobiotics show beneficial activity in the health process in the regulation of the immune system in the host. It is an important finding that this situation will be a safer alternative for premature infants, elderly individuals, and transplant patients, especially for immunocompromised and affected individuals, and some of the disadvantages that probiotics may cause in these individuals will be eliminated.

AUTHORS' CONTRIBUTIONS

GAA: Conceptualization, Data curation, Formal Analysis, Writing – original draft. ÜİY: Data curation, Formal Analysis. **EA:** Conceptualization, Data curation, Formal Analysis, Writing – original draft.

REFERENCES

1. You S, Ma Y, Yan B, Pei W, Wu Q, Ding C, et al. The promotion mechanism of prebiotics for probiotics: a review. Front Nutr. 2022;9:1000517. https://doi.org/10.3389/fnut.2022.1000517
2. Kerry RG, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: a review. J Food Drug Anal. 2018;26(3):927-39. https://doi.org/10.1016/j.jfda.2018.01.002
3. Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Fact. 2014;13(1):S3. https://doi.org/10.1186/1475-2859-13-S1-S3
4. Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microb Cell Fact. 2020;19(1):168. https://doi.org/10.1186/s12934-020-01426-w
5. Hadadji M, Benama R, Saidi N, Henni DJ, Kihal M. Identification of cultivable Bifidobacterium species isolated from breast-fed infants feces in West-Algeria. Afr J Biotechnol. 2005;4(5):422-30.
6. Bibiloni R, Pérez PF, Antoni GL. An enzymatic-colorimetric assay for the quantification of Bifidobacterium. J Food Prot. 2000;63(3):322-6. https://doi.org/10.4315/0362-028x-63.3.322
7. Alp G, Aslim B. Relationship between the resistance to bile salts and low pH with exopolysaccharide (EPS) production of *Bifidobacterium* spp. isolated from infants feces and breast milk. Anaerobe. 2010;16(2):101-5. https://doi.org/10.1016/j.anaerobe.2009.06.006
8. Fujiki T, Hirose Y, Yamamoto Y, Murosaki S. Enhanced immunomodulatory activity and stability in simulated digestive juices of *Lactobacillus plantarum* L-137 by heat treatment. Biosci Biotechnol Biochem. 2012;76(5):918-22. https://doi.org/10.1271/bbb.110919
9. Chuah LO, Foo HL, Loh TC, Mohammed Alitheen NB, Yeap SK, Abdul Mutalib NE, et al. Postbiotic metabolites produced by *Lactobacillus plantarum* strains exert selective cytotoxicity effects on cancer cells. BMC Complement Altern Med. 2019;19(1):114. https://doi.org/10.1186/s12906-019-2528-2
10. Maryam A, Hassan Z, Imdakim M, Sharifah NRSA. Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). Afr J Microbiol Res. 2012;6(34):6358-64. https://doi.org/10.5897/AJMR12.702
11. Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE. Postbiotics and paraprobiotics: from concepts to applications. Food Res Int. 2020;136:109502. https://doi.org/10.1016/j.foodres.2020.109502

12. Wu X, Wu Y, He L, Wu L, Wang X, Liu Z. Effects of the intestinal microbial metabolite butyrate on the development of colorectal cancer. J Cancer. 2018;9(14):2510-7. https://doi.org/10.7150/jca.25324

13. Malashree L, Vishwanath A, Shivalkar Y, Prabha R. "Postbiotics" - one step ahead of probiotics. Int J Curr Microbiol Appl Sci. 2019;8(1):2319-7706. https://doi.org/10.20546/ijcmas.2019.801.214

14. Cicenia A, Santangelo F, Gambardella L, Pallotta L, Iebba V, Scirocco A, et al. Protective role of postbiotic mediators secreted by *Lactobacillus rhamnosus* GG versus lipopolysaccharide-induced damage in human colonic smooth muscle cells. J Clin Gastroenterol. 2016;50(Suppl 2), Proceedings from the 8th probiotics, prebiotics & new foods for microbiota and human health meeting held in Rome, Italy on September 13-15, 2015:S140-4. https://doi.org/10.1097/MCG.0000000000000681

15. Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou E, Lamprianidou EE, et al. *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. PLoS One. 2016;11(2):e0147960. https://doi.org/10.1371/journal.pone.0147960

16. Riaz Rajoka MS, Zhao H, Mehwish HM, Li N, Lu Y, Lian Z, et al. Anti-tumor potential of cell free culture supernatant of *Lactobacillus rhamnosus* strains isolated from human breast milk. Food Res Int. 2019;123:286-97. https://doi.org/10.1016/j.foodres.2019.05.002

17. Song MW, Chung Y, Kim KT, Hong WS, Chang HJ, Paik HD. Probiotic characteristics of *Lactobacillus brevis* B13-2 isolated from kimchi and investigation of antioxidant and immune-modulating abilities of its heat-killed cells. LWT. 2020;128(3):109452. https://doi.org/10.1016/j.lwt.2020.109452

18. Balzaretti S, Taverniti V, Guglielmetti S, Fiore W, Minuzzo M, Ngo HN, et al. A novel rhamnose-rich hetero-exopolysaccharide isolated from *Lactobacillus paracasei* DG activates THP-1 human monocytic cells. Appl Environ Microbiol. 2017;83(3):e02702-16. https://doi.org/10.1128/AEM.02702-16

19. Qi SR, Cui YJ, Liu JX, Luo X, Wang HF. *Lactobacillus rhamnosus* GG components, SLP, gDNA and CpG, exert protective effects on mouse macrophages upon lipopolysaccharide challenge. Lett Appl Microbiol. 2020;70(2):118-27. https://doi.org/10.1111/lam.13255

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240400

The first study appraising colonic diverticulosis and *Helicobacter pylori* diagnosed by histopathology

Ersin Kuloglu[1], Kubilay Issever[1], Ali Muhtaroglu[2], Gokhan Aydın[3], Sefer Aslan[4], Aykut Ozturan[1], Demet Sengul[5], Esma Cinar[5], Ahmet Cumhur Dulger[1,6], Ilker Sengul[2,7]*

SUMMARY

OBJECTIVE: Colonic diverticulosis might be caused by low-fiber dietary habits, gastrointestinal motility disorders, and colonic wall resistance changes, which might also affect the upper gastrointestinal system mucosa. Therefore, the present study aims to answer whether the gastric histopathological findings of the cases with diverge from those without.

METHODS: This retrospective cross-sectional study included 184 cases who underwent both upper and lower gastrointestinal endoscopy procedures between January 2020 and December 2022. Notably, 84 cases were colonic diverticulosis, while the rest of the study group was control. Their demographic, laboratory, and histopathological findings were compared meticulously.

RESULTS: The median ages for the colonic diverticulosis and control were 67.07±8.14 and 66.29±15.83 years, respectively, and no statistical difference concerning the age and gender distribution between them was recognized. The median levels of white blood cells, neutrophils, glucose, creatinine, and aspartate aminotransferase in colonic diverticulosis were significantly increased compared to control. As for pathological comparison, colonic diverticulosis had a higher prevalence of *Helicobacter pylori* (45.2 vs. 38%), while atrophy and intestinal metaplasia prevalence were nearly the same in the groups, without significance regarding *Helicobacter pylori*.

CONCLUSION: Consequently, colonic diverticulosis should not be overlooked, particularly when the abovementioned laboratory parameters are augmented in a dyspeptic patient. A correlation might be raised between *Helicobacter pylori* and colonic diverticulosis. Eradication therapy might help attenuate the risk of colonic diverticulosis when *Helicobacter pylori* has emerged in a patient.

KEYWORDS: Diverticulum. *Helicobacter pylori*. Gastric mucosa. Metaplasia. Pathology.

INTRODUCTION

Colonic diverticulosis (CoDiv) is characterized by the herniation of the mucosa through weaknesses in the muscle layer due to insufficiency. Diverticulosis commonly occurs when blood vessels penetrate the muscle layer, weakening those areas for diverticula formation, which are frequently found in the distal colon, with approximately 90% of patients exhibiting diverticula in the sigmoid colon in the continents of Europe and USA[1]. The prevalence of diverticulosis increases with age and is observed in about 50% of individuals aged 60 years in Western societies[2]. The prevalence of CoDiv varies from under 10% in individuals aged under 40 years to 50–66% in the geriatric population aged over 80 years[3]. Of note, CoDiv can be asymptomatic. However, 5–20% of the cases experience recurrent abdominal pain, gastrointestinal (GI) bleeding, and diverticulitis[4]. It is highly prevalent, with increased incidence and prevalence alongside rising socioeconomic standards in developed countries[5], and its development in Western societies has been linked to a diet low in fiber and roughage[6]. Along with a low-fiber diet, changes in colon wall resistance and colonic motility disorders are the most widely accepted etiological factors[6]. In addition, constipation, lack of physical activity, smoking, use of nonsteroidal anti-inflammatory drugs, and inflammation are thought to play roles in the occurrence of this phenomenon[7]. Moreover, male gender, alcohol consumption, prediabetic conditions, increased serum triglyceride levels, gut microbiota alterations, and obesity are asserted with diverticula formation[8,9]. Although genetic factors and ethnic background

[1]Giresun University, Faculty of Medicine, Department of Internal Medicine – Giresun, Turkey.
[2]Giresun University, Faculty of Medicine, Department of General Surgery – Giresun, Turkey.
[3]Hatay Iskenderun State Hospital, Department of Gastroenterology – Hatay, Turkey.
[4]Giresun Education and Research Hospital, Department of Internal Medicine – Giresun, Turkey.
[5]Giresun University, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
[6]Giresun University, Faculty of Medicine, Division of Gastroenterology – Giresun, Turkey.
[7]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
*Corresponding author: ilker.sengul.52@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on March 17, 2024. Accepted on March 18, 2024.

play a role in the etiology of CoDiv, its impact is considered less significant than lifestyle and dietary habits[10].

The development of CoDiv and HP infection is related to socioeconomic status, and their prevalences increase with age. Furthermore, *Helicobacter pylori* (*H. pylori*) might cause mucosal changes in the upper GI system, such as atrophic gastritis and intestinal metaplasia[11], which leads us to think that patients with *H. pylori* infection and CoDiv might have some typical characterizations that facere them more prone to mucosal changes in the GI system. However, to the best of our knowledge, a study regarding the relationship between *H. pylori* and CoDiv has been recognized in the English-language literature[5]. Therefore, our study aimed to evaluate the gastric histopathological findings of the patients with CoDiv, establish possible associations with *H. pylori* infection, and compare them with patients without the diverticula.

METHODS

Study design

The present study was conducted according to the Declaration of Helsinki. This retrospective cross-sectional study was conducted at the Giresun Education and Research Hospital, Giresun, Turkey, from January 2020 to December 2022. The study incorporated a total of 184 cases who had undergone upper and lower GI endoscopy procedures. Of these, 84 cases had been diagnosed with pancolonic diverticulosis, while a control of 100 showed similar demographic characteristics without possessing diverticulosis. CoDiv diagnoses were provided through endoscopic examination characterized by the detection of pouches extending from the colonic wall, and the details, such as the location, size, and appearance of the diverticula, were recorded. The control comprised cases with similar symptoms without colonic diverticulum in the endoscopy. All the procedures were performed by a unique gastroenterologist. The demographic data, such as age, sex, and co-morbidities of the cases, were obtained from the digital medical records of the hospital.

Laboratory parameters

All the cases had been examined with white blood cell count (WBC), neutrophils, hemoglobin (Hgb), mean corpuscular volume (MCV), platelet count (Plt), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (Ca), lymphocytes (Lymp), creatinine (Cre), sodium (Na), and potassium (K), which were recorded meticulously.

Endoscopic procedures

The endoscopic procedures with the administration of 0.1 mg/kg Dormicum and 0.5 mg/kg Propofol for sedation purposes were performed by using the Fujinon VP-4450 HD^{Ò} device in the endoscopic unit (Figures 1A, B).

Histopathological evaluation

The endoscopic biopsy materials were immediately fixed in 10% formalin and prepared for histopathological analysis. The evaluation of *H. pylori* presence, atrophy, and intestinal metaplasia in the gastric tissue was performed under a light microscope via hematoxylin and eosin (H&E) and Warthin-Stary (WE) stains, respectively, and documented by experienced pathologists blinded to the clinical data (Figures 1C, D).

Statistical analysis

The data were analyzed utilizing the Statistical Package for the Social Sciences (SPSS) (IBM for Windows, v.26). The study used the Skewness-Kurtosis tests for continuous measurements in order to detect the normal distribution of the variables, for which the reference range was between ±2. Furthermore, the chi-square (Fisher's exact) test was used to compare descriptive characteristics and histopathological findings between the cases with/without pancolonic diverticulosis. For the comparison of measurements exhibiting a normal distribution, the independent-samples t-test was utilized. Meanwhile, the Mann-Whitney U analysis method was employed to compare measurements that did not demonstrate a normal distribution. Statistical significance level (a) was taken as 5% (95% confidence interval) in the calculations.

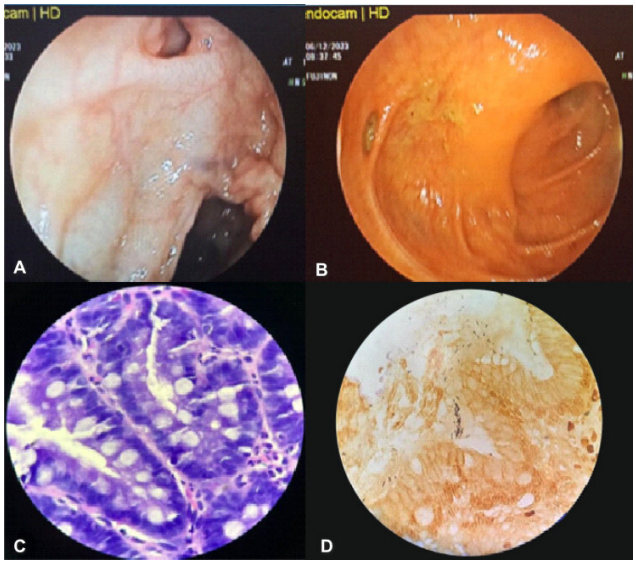

Figure 1. (A) and (B) The endoscopic photographs exhibiting diverticula identified in different colon segments diagnosed with colonic diverticulosis. (C) A microphotograph revealing intestinal metaplasia in colonic diverticulosis, hematoxylin and eosin (H&E) stain, original magnification, 40×. (D) A microphotograph revealing *Helicobacter pylori* positivity in colonic diverticulosis, Warthin-Starry (WS) stain, original magnification, 40×.

RESULTS

In all, 184 patients undergoing endoscopic procedures and the relevant histopathological evaluations were included in the analysis. A total of 45.6% of the cases possessed CoDiv, 47.6% (40) male and 52.4% (44) female, whereas 54.4% did not have CoDiv (control), 45% (45) male and 55% (55) female. A total of 31% of CoDiv were under 65 years old with a mean age of 67.07±8.14 years, while 50% were under 65 years old with a mean age of 66.29±15.83 years in control. These outcomes indicate no significant difference in the gender and age distribution between the cases with and without diverticulosis (p>0.05). In other words, this distribution is homogeneous in both groups (Table 1).

Upon comparing the laboratory parameters, no difference in Lymp, Hgb, MCV, Plt, ALT, Na, K, and Ca values was detected (p>0.05). However, significant differences were observed in WBC, neutrophil, glucose, Cre, and AST values between patients with and without diverticulosis (p<0.05) (Table 2).

In addition, the distribution of *H. pylori*, atrophy, and metaplasia did not differ, whereas the prevalence was higher numerically in CoDiv.

Table 1. Comparison of the laboratory parameters between colonic diverticulosis and control.

Laboratory parameters	CoDiv (n=84)	Control (n=100)	p-value
	Median±SD	Median±SD	
WBC[t]	7510.1±1862.1	6957.6±31892.7	0.048*
Hemoglobin[t]	12.1±1.8	12.6±2.1	0.069
MCV[t]	85.7±6.1	85.2±8	0.885
Platelet[t]	264.72±90.66	262.70±76.78	0.798
Lymphocyte[t]	1843.8±699.8	1990.7±605.4	0.128
Neutrophil	4783.1±1594	3823.8±1040.7	0.001**
Glucose[z]	119±33	105±31.5	0.001**
Creatinine[t]	0.91±0.30	0.80±0.21	0.001**
ALT[z]	18.11±8.62	15.90±8.31	0.061
AST[t]	21.44±8.91	18.90±5.53	0.036*
Na[t]	140.82±3.40	140.46±3	0.174
K[t]	4.41±0.51	4.32±0.4	0.451
Ca[t]	9.54±0.62	9.60±0.40	0.572

*p<0.05, **p<0.01, SD: standart deviation, t: independent-samples t-test, z: Mann-Whitney U test (mean and standard deviation values of the data, as well as median, minimum, and maximum values are given). CoDiv: colonic diverticulosis.

DISCUSSION

The microbial component in the gastrointestinal system is most abundant in the colon, being approximately 10^7 times greater than in the stomach. Some studies suggest that *H. pylori* enhances resistance against human gastrointestinal infections, thereby increasing fecal microbiota diversity. Another theory posits that *H. pylori* disrupts gastric acidity, allowing microorganisms to pass the gastric barrier and reach the colon[12]. A thorough literature review revealed that only one study has examined the relationship between *H. pylori* and CoDiv. Bartels et al. involved 56,001 cases in Denmark in which patients underwent urea breath testing and were followed up for 6 years. The prevalence of *H. pylori* based on the urea breath test was determined to be 20%, and the patients infected with *H. pylori* had a lower CoDiv prevalence (0.87 vs. 1.14%, respectively, OR=0.62, 95%CI: 0.50–0.78). Furthermore, after the eradication, no statistical difference in CoDiv incidence rates was reported[5]. Contrarily, the present study revealed *H. pylori* positivity at a higher rate in cases with CoDiv than those without (45.2 vs. 38%, respectively). However, we anticipate that the difference would be substantial as the present study involves more cases, although no significant difference was revealed between the study groups. In addition, no difference was recognized between them in either intestinal metaplasia or gastric atrophy. Bartels's study identified *H. pylori* non-invasively and noted that they included all the diagnostic codes regarding CoDiv, which might display a heterogenic clinical picture. Nevertheless, our study identified *H. pylori* along with atrophy and intestinal metaplasia status

Table 2. Comparison of histopathological findings between colonic diverticulosis and control.

Histopathological findings		CoDiv (n=84)		Control (n=100)		p-value
		N	%	n	%	
H. pylori	Negative	46	54.8	62	62	0.321
	Positive	38	45.2	38	38	
Atrophy	Negative	75	89.3	90	90	0.874
	Positive	9	10.7	10	10	
Metaplasia	Negative	65	77.4	77	77	0.951
	Positive	19	22.6	23	23	

Chi-square test. CoDiv: colonic diverticulosis.

of gastric mucosa from gastric pathology specimens, the gold standard diagnostic method, and presented CoDiv in elective, planned endoscopic procedures rather than mixed acute and elective cases[13]. Furthermore, we had a much smaller study group without data regarding pre- and post-*H. pylori* eradication comparisons. Finally, the designs of the studies differ as Bartels et al. conducted both a cross-sectional and historical cohort study. At the same time, we performed a retrospective cross-sectional study. Considering these differences between the studies, it should not be surprising to find contradictory results regarding the effect of *H. pylori* on CoDiv progression.

Epidemiological studies have revealed a liaison between *H. pylori* infection and some clinical conditions characterized by persistent and low-grade systemic inflammation and specific diseases such as iron deficiency anemia, idiopathic thrombocytopenic purpura, and vitamin B12 deficiency[14]. Our outcomes, revealing a higher *H. pylori* prevalence and worse laboratory findings in CoDiv, are coherent with this study. However, there are also refutatory articles in the literature. Another Danish study found a lower prevalence of Crohn's and coeliac diseases but not ulcerative colitis in *H. pylori*-positivity[15]. A review investigating the liaison between *H. pylori* infection and inflammatory bowel diseases (IBD) revealed a steady negative association between gastric *H. pylori* infection and IBD[16]. These inconsistent results, along with the result of our study, manifest that the proposed immunomodulatory effects of *H. pylori*, an infectious bacteria that might lead to mucosal inflammation, are still debated and need to be proven by large randomized controlled trials[17].

In the present study, WBS, Neu, Glc, Cre, and AST levels were significantly higher in CoDiv. Literature reveals that low-grade chronic inflammation exists in CoDiv, which is thought to be the underlying mechanism for the risk factors of this disease, such as obesity, smoking, physical inactivity, high red meat consumption, and low-fiber diet. Acute phase reactants increase further in more severe forms of CoDiv, such as symptomatic uncomplicated diverticular disease and acute diverticulitis, in which low-grade chronic inflammation might cause leukocyte and neutrophil elevation in our patient group, although they all incidentally possessed asymptomatic diverticulosis. It was shown that components of metabolic dysregulation, such as hypertension, hyperlipidemia, and hepatosteatosis, are associated with the presence and severity of CoDiv[18-25]. Considering this fact, considering higher Glc, Cre, and AST levels in CoDiv is unsurprising. Consequently, all the laboratory analyses were compatible with the current literature on CoDiv. To the best of our knowledge, this is the first study evaluating the liaison of *H. pylori* with CoDiv via gastric biopsy analysis and the second study to investigate this association in the literature. The observed elevations in WBC, Neu, Glc, Cre, and AST levels may affect future clinical guidelines and patient management strategies. This research might open avenues for further studies investigating the pathophysiological mechanisms behind these associations and their clinical implications.

Limitations

The limitations of the present study are its retrospective nature, with a small study group without data regarding pre- and post-*H. pylori* eradication comparisons, a lack of inquiry into patients' socioeconomic levels, dietary habits, defecation routines, physical activity levels, smoking and alcohol use, lipid profiles, and nonsteroidal anti-inflammatory drug use regarding diverticular disease.

CONCLUSION

The outcomes of the present study reveal significant associations between pancolonic diverticulosis, elevated laboratory parameters, and *H. pylori* infection in dyspeptic patients, indicating an inflammatory and metabolic components in this condition, which challenge its traditional perception as an asymptomatic condition and highlight its potential influence on dyspeptic symptomatology. These results emphasize the need to consider pancolonic diverticulosis in the differential diagnosis of dyspepsia, advocating for a more comprehensive diagnostic approach in gastroenterology, which merits further investigation.

ACKNOWLEDGMENTS

The authors thank all the study participants and the head nurse of the endoscopic unit, Aysun Guler, and her team for their dedicated efforts.

AUTHORS' CONTRIBUTIONS

EK: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. KI: Formal Analysis, Investigation, Methodology, Project administration, Validation, Visualization. AM: Project administration, Validation, Visualization. GA: Investigation, Validation, Visualization. SA: Investigation, Validation, Visualization. AO: Investigation, Validation, Visualization. DS: Investigation, Software, Supervision, Validation, Visualization, Writing – review & editing. EC: Investigation, Software, Validation, Visualization. ACD: Methodology, Project administration, Resources, Validation, Visualization. IS: Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Stollman NH, Raskin JB. Diverticular disease of the colon. J Clin Gastroenterol. 1999;29(3):241-52. https://doi.org/10.1097/00004836-199910000-00004
2. T□nase I, P□un S, Stoica B, Negoi I, Gaspar B, Beuran M. Epidemiology of diverticular disease -- systematic review of the literature. Chirurgia (Bucur). 2015;110(1):9-14. PMID: 25800310
3. Tursi A. Acute diverticulitis of the colon--current medical therapeutic management. Expert Opin Pharmacother. 2004;5(1):55-9. https://doi.org/10.1517/14656566.5.1.55
4. Shahedi K, Fuller G, Bolus R, Cohen E, Vu M, Shah R, et al. Long-term risk of acute diverticulitis among patients with incidental diverticulosis found during colonoscopy. Clin Gastroenterol Hepatol. 2013;11(12):1609-13. https://doi.org/10.1016/j.cgh.2013.06.020
5. Bartels LE, Jepsen P, Tøttrup A, Vilstrup H, Dahlerup JF. *Helicobacter pylori* infection is associated with reduced prevalence of colonic diverticular disease. Helicobacter. 2017;22(4):e12384. https://doi.org/10.1111/hel.12384
6. Crowe FL, Appleby PN, Allen NE, Key TJ. Diet and risk of diverticular disease in Oxford cohort of European Prospective Investigation into Cancer and Nutrition (EPIC): prospective study of British vegetarians and non-vegetarians. BMJ. 2011;343:d4131. https://doi.org/10.1136/bmj.d4131
7. Mario F, Comparato G, Fanigliulo L, Aragona G, Cavallaro LG, Cavestro GM, et al. Use of mesalazine in diverticular disease. J Clin Gastroenterol. 2006;40(Suppl 3):S155-9. https://doi.org/10.1097/01.mcg.0000225509.98041.4b
8. Yamamichi N, Shimamoto T, Takahashi Y, Sakaguchi Y, Kakimoto H, Matsuda R, et al. Trend and risk factors of diverticulosis in Japan: age, gender, and lifestyle/metabolic-related factors may cooperatively affect on the colorectal diverticula formation. PLoS One. 2015;10(4):e0123688. https://doi.org/10.1371/journal.pone.0123688
9. Daniels L, Philipszoon LE, Boermeester MA. A hypothesis: important role for gut microbiota in the etiopathogenesis of diverticular disease. Dis Colon Rectum. 2014;57(4):539-43. https://doi.org/10.1097/DCR.0000000000000078
10. Böhm SK, Kruis W. Lifestyle and other risk factors for diverticulitis. Minerva Gastroenterol Dietol. 2017;63(2):110-8. https://doi.org/10.23736/S1121-421X.17.02371-6
11. Brito BB, Silva FAF, Soares AS, Pereira VA, Santos MLC, Sampaio MM, et al. Pathogenesis and clinical management of *Helicobacter pylori* gastric infection. World J Gastroenterol. 2019;25(37):5578-89. https://doi.org/10.3748/wjg.v25.i37.5578
12. Chen CC, Liou JM, Lee YC, Hong TC, El-Omar EM, Wu MS. The interplay between *Helicobacter pylori* and gastrointestinal microbiota. Gut Microbes. 2021;13(1):1-22. https://doi.org/10.1080/19490976.2021.1909459
13. Huh CW, Kim BW. Diagnosis of *Helicobacter pylori* infection. Korean J Gastroenterol. 2018;72(5):229-36. https://doi.org/10.4166/kjg.2018.72.5.229
14. Franceschi F, Zuccalà G, Roccarina D, Gasbarrini A. Clinical effects of *Helicobacter pylori* outside the stomach. Nat Rev Gastroenterol Hepatol. 2014;11(4):234-42. https://doi.org/10.1038/nrgastro.2013.243
15. Bartels LE, Jepsen P, Christensen LA, Gerdes LU, Vilstrup H, Dahlerup JF. Diagnosis of *Helicobacter pylori* infection is associated with lower prevalence and subsequent incidence of Crohn's disease. J Crohns Colitis. 2016;10(4):443-8. https://doi.org/10.1093/ecco-jcc/jjv229
16. Castaño-Rodríguez N, Kaakoush NO, Lee WS, Mitchell HM. Dual role of *Helicobacter* and *Campylobacter* species in IBD: a systematic review and meta-analysis. Gut. 2017;66(2):235-49. https://doi.org/10.1136/gutjnl-2015-310545
17. Wang YK, Li C, Zhou YM, Zeng L, Li YY, Huang SL, et al. Histopathological features of *Helicobacter pylori* infection in gastric mucosa. J Inflamm Res. 2022;15:6231-43. https://doi.org/10.2147/JIR.S383075
18. Tursi A, Scarpignato C, Strate LL, Lanas A, Kruis W, Lahat A, et al. Colonic diverticular disease. Nat Rev Dis Primers. 2020;6(1):20. https://doi.org/10.1038/s41572-020-0153-5
19. Sengul D, Sengul I. Frequency of *Helicobacter pylori* and association of location, six age groups, and assessment of borderline of 50-year base-age, based on the anatomic pilot region with the degree of *Helicobacter pylori* colonization. Med J Bakirkoy. 2018;14(4):381-8. https://doi.org/10.4274/BTDMJB.20180327085510
20. Ozturk T, Sengul D, Sengul I. *Helicobacter pylori* and association between its positivity and anatomotopographic settlement in the stomach with the host age range. Ann Afr Med. 2021;20(1):1-8. https://doi.org/10.4103/aam.aam_69_19
21. Muhtaroglu A, Sengul I, Sengul D, Kesicioglu T, Seker D, Aydin M, et al. Does enteral nutrition through a percutaneous endoscopic gastrostomy, attenuate *Helicobacter pylori* colonization?: is it worth mentioning? Rev Assoc Med Bras (1992). 2023;69(4):e20221733. https://doi.org/10.1590/1806-9282.20221733
22. Hjern F, Wolk A, Håkansson N. Smoking and the risk of diverticular disease in women. Br J Surg. 2011;98(7):997-1002. https://doi.org/10.1002/bjs.7477
23. Strate LL, Liu YL, Aldoori WH, Giovannucci EL. Physical activity decreases diverticular complications. Am J Gastroenterol. 2009;104(5):1221-30. https://doi.org/10.1038/ajg.2009.121
24. Humes DJ, Simpson J, Smith J, Sutton P, Zaitoun A, Bush D, et al. Visceral hypersensitivity in symptomatic diverticular disease and the role of neuropeptides and low grade inflammation. Neurogastroenterol Motil. 2012;24(4):318-e163. https://doi.org/10.1111/j.1365-2982.2011.01863.x
25. Pantic I, Lugonja S, Rajovic N, Dumic I, Milovanovic T. Colonic diverticulosis and non-alcoholic fatty liver disease: is there a connection? Medicina (Kaunas). 2021;58(1):38. https://doi.org/10.3390/medicina58010038

REVIEW ARTICLE

https://doi.org/10.1590/1806-9282.20231436

Polycystic ovary syndrome: emerging stem cell therapies

Karimat Adeola Busari[1], Pinar Tulay[1,2,3]*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine disorder that affects females aged 15 to 49 years, with a frequency of up to 20%[1]. PCOS is a heterogeneous condition and is characterized by chronic anovulation and hyperandrogenism. In addition to being linked to metabolic abnormalities, PCOS raises the risk of developing type 2 diabetes, cardiovascular disease (CVD), and endometrial cancer. Insulin resistance (IR) is thought to be the cause of the metabolic abnormalities associated with PCOS and the inefficient metabolism of carbohydrates, which raises the risk of CVD. PCOS is a major threat to public health because of its metabolic, reproductive, and psychological features. It has been diagnosed in more than 105 million females aged 15–49 years globally. PCOS can cause a variety of symptoms, which include abnormal menstrual periods, infertility, enormous growth of the hair, and issues with pregnancy. Furthermore, PCOS is linked to psychological disorders such as depression, anxiety, and also low self-esteem. As age increases, the condition advances from reproductive illness to a metabolic disorder[1-3].

Obesity (BMI ≥30 kg/m^2) and overweight (BMI 25–29.9 kg/m^2) are risk factors linked to PCOS that have been identified as significant contributors to global health issues in women. Obesity has also been linked to reproductive health issues such as anovulation. The incidence of anovulation increases substantially with body weight. Gaining abdominal fat indicates a higher risk of IR, which is another factor contributing to difficulties with reproduction. Increased testosterone production and anovulation have been linked to IR in obese women[4]. It has been proposed that bisphenol A is an endocrine disruptor that mimics estrogen. BPA is an exogenous substance that is regarded as a xenoestrogen because it imitates the effects of 17-β estradiol. As a result, BPA can interfere with E2 feedback at the ovarian and hypothalamus-pituitary levels, which inhibits the actions of the HPO axis. BPA can also lead to fertility problems, irregular menstruation cycles, insufficient release of hormones, and problems with the growth and performance of the female reproductive system[5]. Researchers discovered that the effect of progesterone may be hampered by hormonal (elevated testosterone levels) and metabolic (elevated insulin levels) abnormalities. In PCOS, progesterone therapy does not completely resolve the histomorphometric abnormalities of the endometrium, which are associated with insulin and androgen levels[6].

Due to the complex causes of PCOS, treatment is typically customized based on the patient's current signs and symptoms rather than being mono-therapeutic. Complementary therapies have been recommended for PCOS treatment and management. The cornerstone of PCOS management is thought to be modifications to diet and lifestyle. Current treatments typically improve symptoms or consequences related to the conditions, such as menstrual disruption, elevated testosterone levels, fertility issues, metabolic abnormalities, and cancer and CVD prevention, without tackling the underlying cause. While ovulation stimulation works, rates of pregnancies are low, necessitating the use of advanced assisted reproductive procedures[7].

The endometrial morphological and genetic alterations may have a major role in PCOS women's fertility. As PCOS is frequently linked to IR, obesity, and metabolic abnormalities, the ability of melatonin to enhance metabolic health is crucial for those who have the illness. As melatonin lowers oxidative stress and increases insulin sensitivity, it provides an all-encompassing strategy for treating the metabolic factors that cause PCOS. Increased sensitivity to insulin can lessen the effects of hyperinsulinemia and the hazards it carries, including diabetes and cardiovascular problems[8]. In the rat model of PCOS (permanent estrus), melatonin seems to restore the regular process of granulosa cell proliferation, according to a study. Melatonin therapy is a low-toxicity, readily available, reliable option. Based on the research, melatonin is beneficial for treating and preventing reproductive issues in PCOS patients[9].

[1]Near East University, Faculty of Medicine, Department of Medical Genetics – Nicosia, Cyprus.
[2]Near East University, DESAM Research Institute – Nicosia, Cyprus.
[3]Near East University, Center of Excellence, Genetics and Cancer Diagnosis-Research Center – Nicosia, Cyprus.
*Corresponding author: pinar.tulay@neu.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 27, 2024. Accepted on March 05, 2024.

In terms of PCOS-specific measures (LH: FSH ratio, serum testosterone, and serum AMH), women with PCOS benefit from a micronutrient supplementation, administered for a minimum of 3 months, which contains omega-3 fatty acids, catechin, folic acid, vitamin E, glycyrrhizin, selenium, and co-enzyme Q10[10]. Following the induction of PCOS in rats, the treatment of clomiphene citrate enhanced the structure and physiological functions of rat ovaries[11]. Co-enzyme Q10 (CoQ10) and clomiphene citrate therapy together significantly increase ovulation rate in PCOS-affected women in a way that is not possible with clomiphene citrate therapy alone. An adjuvant for clomiphene citrate that may be effective is CoQ10. Before attempting a more challenging treatment like gonadotrophins or laparoscopic ovarian drilling, a combination of CoQ10 and clomiphene citrate has been demonstrated to be an effective, economical, and safer way to increase follicular growth in PCOS patients[12].

REGENERATIVE MEDICINE: STEM CELL THERAPY

The primary goal of treatment, such as pharmaceutical therapies, is to alleviate symptoms. In addition, some of the patient's issues can be treated and their severity can be decreased by surgical procedures such as bariatric surgery and assisted reproductive technologies. In general, utilizing pharmaceuticals over a lengthy period elevates the probability of side effects. In addition, surgical approaches can result in risky outcomes. Here, it is clear that PCOS requires a novel, interdisciplinary approach. Stem cells can be used in regenerative medicine to create an effective alternate strategy for treating PCOS. Animal models can also help us understand PCOS's genetic changes and disturbed metabolic pathways. In preliminary clinical stages, they can also be used to test new treatments[13].

Induced pluripotent stem cells

Present research in cell reprogramming employs iPSC derived from patient somatic cells to generate stem cells specific to the patient for studying the disease etiology and generating specific treatments. iPSCs have been used in disease models to know the causes and also test potential treatments. When developed into appropriate cell types, the majority of iPSCs exhibit detectable characteristics particular to the disease. iPSC lines were created from the somatic cells of PCOS patients exhibiting the common symptoms. RNA microarray was used to examine the transcriptional patterns of these iPSC lines, as well as metabolic capabilities and mitochondrial activity in the iPSCs derived from patients in vitro. Following that, metformin was utilized to show response in the iPSCs model derived from the patients to assess the possible benefit of the iPSCs for drug development and future clinical applications. The derived iPSCs provide a novel biological cell framework for studying PCOS pathophysiology and aiding in the discovery of new medications for clinical applications[14].

Mesenchymal stem cells

Mesenchymal stem cells have a homing ability, where they release a variety of growth factors, cytokines, and extracellular vesicles with antifibrotic, immunosuppressive, antiapoptotic, anti-inflammatory, and angiogenic properties. MSCs are gaining popularity as a promising cell-based therapy because they offer various benefits over other sources, such as more accessibility, less ethical issues, and a great capacity for self-renewal and differentiation. MSCs have been studied therapeutically in female infertility by medical researchers. In particular, several research studies have concentrated on MSCs as a method for recovering ovarian function and infertility treatment. MSCs can be obtained from dental pulp, umbilical cord tissue, bone marrow, amniotic fluid, adipose tissue, placental tissue, Wharton jelly, and pluripotent stem cells, among other places. MSCs have therapeutic significance through the capability to divide into diverse cell lineages and influence immune responses through the modulation of the immune system. MSCs have been found to reactivate endometrial function and enhance the outcomes of pregnancy[15-17].

Mesenchymal stem cells express their influence through modulating several molecular and biological processes. miRNAs, particularly exosomal miRNAs, seem to have a major role in modulating MSC impacts and hence represent potential targets for therapy[18]. Studies have enhanced our knowledge of the methods and treatment opportunities of stem cell therapies for gynecologic conditions that affect the function of the reproductive tissue. These studies have paved the way for developing innovative and efficient MSC-based therapies, which have the possibility of helping women suffering from ovarian insufficiency or infertility through the restoration of their reproductive health and enhancing their life quality. Additional study is needed to know the safety and effectiveness of MSC in the treatment of infertility[19]. PCOS patients have elevated levels of expression of the *CYP17A1* and *CYP11A1* genes, which are implicated in androgen synthesis. The efficiency of human MSCs (hMSCs) was investigated as a potential cell therapy through the injection of hMSCs into the ovaries of a mouse model. It demonstrated a substantial decrease in hyperandrogenemia, and hMSCs release a variety of factors in the media, which is referred to as the secretome. The secretome of hMSCs

comprises factors that can suppress androgen production in theca cells. MSCs release proteins that decrease androgen production through the inhibition of steroidogenesis proteins *CYP11A1* and *CYP17A1* gene expression[20,21].

Umbilical cord mesenchymal stem cells

Umbilical cord mesenchymal stem cells are considered to have an unlimited supply of stem cells, and they represent an intriguing stem cell source for regenerative medicine. They can be extracted in vast quantities from human umbilical cords and grown in cultures. It was discovered that after administering UCMSCs, the endocrine function is restored in the synthesis of estrogen with an increase in the weight of estrogen-dependent organs, such as ovaries, and the exocrine function is also restored resulting in successful pregnancies and delivery of the healthy children. Many other researchers revealed similar findings[22].

New research shows that UCMSC transplantation may be useful in improving PCOS pathological changes. It was discovered that transplantation of UCMSC improved ovarian function in mice with DHEA-induced PCOS[21]. This impact was interceded by a decrease in the synthesis of proinflammatory cytokines, such as interleukin 1 beta and tumor necrosis factor-alpha, and fibrosis-associated genes, such as connective tissue growth factor, while increasing the secretion of anti-inflammatory factors like IL-10 in local ovarian and uterine tissues, implying that UCMSCs may change a proinflammatory condition to an anti-inflammatory condition. The finding of this research was that PCOS was alleviated in the mice by stopping inflammation. While these results imply that UCMSC transplantation may be useful for managing ovarian diseases, additional research is needed to create a reliable USMSC-based therapy method[23,24].

Bone marrow mesenchymal stem cells

The transplantation of BM-MSCs significantly enhances folliculogenesis in PCOS-induced mice. Stem cells produced from bone marrow are widely employed for a variety of therapeutic applications, but clinical use for the treatment of female infertility has remained difficult to find so far[16]. Despite this, a recent study using mouse models of PCOS demonstrated improvements in the function of the endocrine, quality of the oocytes, and folliculogenesis due to PCOS's anti-inflammatory, anti-apoptotic, and anti-oxidative qualities[16]. Another study found that after intra-ovarian delivery of BM-MSCs, older women (who had previously been subjected to chemotherapy) had important recovery of ovarian function. The number of oocytes grew enormously as folliculogenesis developed; however, the quantity of primary and pre-antral follicles decreased substantially.

Chugh et al. found out that BM-hMSC and its secreted components (secretome) demonstrate powerful anti-inflammatory properties. In both in vitro and in vivo models of PCOS, the therapeutic effectiveness of BM-hMSC and its secretome was assessed. The effectiveness of intra-ovarian injection of BM-hMSC in the treatment of phenotypes associated with PCOS such as metabolic and reproductive abnormalities was demonstrated. This method might be a potential treatment for PCOS patients. Our findings demonstrate that BM-hMSC can inhibit inflammation induced by PCOS via IL-10 production. The anti-inflammatory cytokine IL-10 performed an important function in relaying BM-hMSC effects. BM-hMSC might be an effective therapeutic option for PCOS[25,26].

Increased inflammation and the synthesis of androgen from ovarian theca cells are PCOS primary characteristics, and approximately 50% of women with PCOS have elevated production of androgen. Therefore, H295R (androgen-producing human adrenocortical-carcinoma cells) serves as an ideal in vitro PCOS model. The effects of H295R cells exposure to the BM-hMSC secretome and IL-10 were studied. Comparable experiments were conducted on primary cultured theca cells derived from PCOS patients. In both H295R cells and primary cultured theca cells, the BM-hMSC secretome inhibited the production of steroids, inflammation, and androgen synthesis[25,27].

CHALLENGES AND FUTURE PERSPECTIVE

One of the many unanswered questions about PCOS is regarding the common factor that connects obesity, hyperandrogenism, and IR. Proper diagnosis and treatments are required for women who may develop infertility during their years of reproduction in order to enhance their prognosis. Although PCOS is manageable with lifestyle modifications and medications, a majority of women with PCOS do not receive proper treatment due to the low rate of accurate diagnosis of this disorder[28-30].

The transplantation of stem cells or their derivatives into the relevant tissues or organs is one of the most potential treatments for many fatal conditions. However, because of the sensitivity and complex nature of the immune system, obtaining immune-competent cells from any patient is challenging. In this context, iPSCs and technologies for gene editing may be useful in generating functional autologous cells. However, with major technological breakthroughs in reprogramming, iPSCs are not yet suitable for transplantation into patients, except for a few in current clinical research. There have been few publications on the equivalent of the molecular background and functions of human ESCs and iPSCs, and the iPSCs genome and epi-genomic integrity must be thoroughly assessed before they

can be used in treatments[31]. Also, when contemplating stem cell treatment, the first issue for the researchers is to unravel the processes behind the way stem cells act in the damaged microenvironment using animal models and then translate the outcomes of these studies to humans. The second issue is to detect and extract stem cells from the tissue before inducing differentiation into the appropriate cell types. The final issue is to prevent immune rejection following the transplantation of stem cells. Immune rejection is a significant impediment to effective stem cell transplantation[32].

DISCUSSION

There is optimism about developing an effective therapy method based on the regenerative features of stem cells for reproductive issues. However, to achieve major developments for the treatment strategy, an accurate and effective study plan, from the isolation of stem cells to informed voluntary consent and cell delivery model, is crucial for the success of initial clinical trials considering previously published studies to overcome the limitations. There is no remedy or cure because therapies have so far focused on the symptoms rather than the syndrome itself. A comprehensive attempt should be made to completely research the condition so as to enhance the treatment and hinder the devastating long-lasting results of this condition on the well-being of patients. For the early diagnosis and screening of PCOS subtypes, crucial genetic polymorphisms may be effective. Finding efficient preventative tactics and therapy modalities will require additional research on the genetics and pathophysiology of PCOS. Prebiotic, probiotic, and synbiotic administration seems to enhance a number of biochemical outcomes and has positive benefits on women with PCOS, while the underlying mechanisms are yet unclear. The importance of these drugs in PCOS therapy or perhaps prevention requires further study.

CONCLUSION

Stem cell studies have brought about major advancements in the treatments of reproductive issues. Still, further pre-clinical and clinical trials are needed to establish the advantages of different stem cell therapies and discover the best stem cell type or source for subsequent studies. According to numerous studies, PCOS-related obesity and metabolic syndrome can be treated with novel or modified medications. Statins, letrozole, and nutrient supplements have been the subjects of recent research studies for the treatment of PCOS. To demonstrate the efficacy of novel and emerging treatments, such as aromatase inhibitors and IL-22 therapy, as well as others, in effectively managing PCOS, more research is needed.

AUTHORS' CONTRIBUTIONS

KAB: Writing – original draft. **PT:** Supervision, Writing – original draft.

REFERENCES

1. Louwers YV, Laven JSE. Characteristics of polycystic ovary syndrome throughout life. Ther Adv Reprod Health. 2020;14:2633494120911038. https://doi.org/10.1177/2633494120911038
2. Medeiros SF, Winck Yamamoto Medeiros AL, Souto Medeiros MA, Silva Carvalho AB, Yamamoto MW, M Soares J, et al. Anthropometric, metabolic, and endocrine parameters as predictors of estimated average glucose and other biomarkers of dysglycemia in women with different phenotypes of polycystic ovary syndrome. Horm Metab Res. 2024. https://doi.org/10.1055/a-2207-0739
3. Soares-Jr JM, Hayashida SAY, Marcondes JAM, Maciel GAR, Barcellos CRG, Maffazioli GN, et al. Influence of phenotypes on the metabolic syndrome of women with polycystic ovary syndrome over a six-year follow-up in Brazil. Biomedicines. 2023;11(12):3262. https://doi.org/10.3390/biomedicines11123262
4. Rosenberg S. The relationship between PCOS and obesity: which comes first? Sci J Lander Coll Arts Sci. 2019;13(1). Available from: https://touroscholar.touro.edu/sjlcas/vol13/iss1/5
5. Urbanetz LAML, Junior JMS, Maciel GAR, Simões RDS, Baracat MCP, Baracat EC. Does bisphenol A (BPA) participates in the pathogenesis of polycystic ovary syndrome (PCOS)? Clinics (Sao Paulo). 2023;78:100310. https://doi.org/10.1016/j.clinsp.2023.100310
6. Baracat MCP, Baracat EC, Simões RS, Simões MJ, Maciel GAR, Azziz R, et al. Hormonal and metabolic factors influence the action of progesterone on the endometrium of women with polycystic ovary syndrome. Diagnostics (Basel). 2023;13(3):382. https://doi.org/10.3390/diagnostics13030382
7. Baracat EC, Baracat MCP, José MSJ. Are there new insights for the definition of PCOS?. Gynecol Endocrinol. 2022;38(9):703-4. https://doi.org/10.1080/09513590.2022.2121387
8. Patel A, Dewani D, Jaiswal A, Yadav P, Reddy LS. Exploring melatonin's multifaceted role in polycystic ovary syndrome management: a comprehensive review. Cureus. 2023;15(11):e48929. https://doi.org/10.7759/cureus.48929
9. Lombardi LA, Mattos LS, Simões RS, Florencio-Silva R, Sasso GRDS, Carbonel AAF, et al. Melatonin may prevent or reverse polycystic ovary syndrome in rats. Rev Assoc Med Bras (1992). 2019;65(7):1008-14. https://doi.org/10.1590/1806-9282.65.7.1008
10. Hager M, Nouri K, Imhof M, Egarter C, Ott J. The impact of a standardized micronutrient supplementation on PCOS-typical parameters: a randomized controlled trial. Arch Gynecol Obstet. 2019;300(2):455-60. https://doi.org/10.1007/s00404-019-05194-w

11. Lombardi LA, Mattos LS, Simões RS, Florencio-Silva R, Simões MJ, Franco PC, et al. Histomorphometric and immunohistochemical changes in interstitial cells and ovarian follicles of rats with polycystic ovaries treated with clomiphene citrate. Gynecol Endocrinol. 2021;37(6):554-7. https://doi.org/10.1080/09513590.2020.1843622
12. Jamal H, Waheed K, Mazhar R, Sarwar MZ. Comparative study of combined co-enzyme q10 and clomiphene citrate vs clomiphene citrate alone for ovulation induction in patients with polycystic ovarian syndrome. J Pak Med Assoc. 2023;73(7):1502-5. https://doi.org/10.47391/JPMA.7299
13. Arjmand B, Alaei S, Heravani NF, Alavi-Moghadam S, Payab M, Ebrahimpour M, et al. Regenerative medicine perspectives in polycystic ovary syndrome. Adv Exp Med Biol. 2021;1341:125-41. https://doi.org/10.1007/5584_2021_623
14. Min Z, Gao Q, Zhen X, Fan Y, Tan T, Li R, et al. New insights into the genic and metabolic characteristics of induced pluripotent stem cells from polycystic ovary syndrome women. Stem Cell Res Ther. 2018;9(1):210. https://doi.org/10.1186/s13287-018-0950-x
15. Chugh RM, Park HS, Andaloussi A, Elsharoud A, Esfandyari S, Ulin M, et al. Mesenchymal stem cell therapy ameliorates metabolic dysfunction and restores fertility in a PCOS mouse model through interleukin-10. Stem Cell Res Ther. 2021;12(1):388. https://doi.org/10.1186/s13287-021-02472-w
16. Kalhori Z, Azadbakht M, Soleimani Mehranjani M, Shariatzadeh MA. Improvement of the folliculogenesis by transplantation of bone marrow mesenchymal stromal cells in mice with induced polycystic ovary syndrome. Cytotherapy. 2018;20(12):1445-58. https://doi.org/10.1016/j.jcyt.2018.09.005
17. Prayitno GD, Lestari K, Sartika CR, Djuwantono T, Widjaya A, Muharam R, et al. Potential of mesenchymal stem cells and their secretomes in decreasing inflammation markers in polycystic ovary syndrome treatment: a systematic review. Medicines (Basel). 2022;10(1):3. https://doi.org/10.3390/medicines10010003
18. Tamaddon M, Azimzadeh M, Tavangar SM. microRNAs and long non-coding RNAs as biomarkers for polycystic ovary syndrome. J Cell Mol Med. 2022;26(3):654-70. https://doi.org/10.1111/jcmm.17139
19. Esfandyari S, Chugh RM, Park HS, Hobeika E, Ulin M, Al-Hendy A. Mesenchymal stem cells as a bio organ for treatment of female infertility. Cells. 2020;9(10):2253. https://doi.org/10.3390/cells9102253
20. Park HS, Chugh RM, Pergande MR, Cetin E, Siblini H, Esfandyari S, et al. Non-cytokine protein profile of the mesenchymal stem cell secretome that regulates the androgen production pathway. Int J Mol Sci. 2022;23(9):4633. https://doi.org/10.3390/ijms23094633
21. Xie Q, Xiong X, Xiao N, He K, Chen M, Peng J, et al. Mesenchymal stem cells alleviate DHEA-induced polycystic ovary syndrome (PCOS) by inhibiting inflammation in mice. Stem Cells Int. 2019;2019:9782373. https://doi.org/10.1155/2019/9782373
22. Mohamed SA, Shalaby S, Brakta S, Elam L, Elsharoud A, Al-Hendy A. Umbilical cord blood mesenchymal stem cells as an infertility treatment for chemotherapy induced premature ovarian insufficiency. Biomedicines. 2019;7(1):7. https://doi.org/10.3390/biomedicines7010007
23. Witchel SF, Oberfield SE, Peña AS. Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. J Endocr Soc. 2019;3(8):1545-73. https://doi.org/10.1210/js.2019-00078
24. Dennett CC, Simon J. The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. Diabetes Spectr. 2015;28(2):116-20. https://doi.org/10.2337/diaspect.28.2.116
25. Chugh RM, Park HS, Esfandyari S, Elsharoud A, Ulin M, Al-Hendy A. Mesenchymal stem cell-conditioned media regulate steroidogenesis and inhibit androgen secretion in a PCOS cell model via BMP-2. Int J Mol Sci. 2021;22(17):9184. https://doi.org/10.3390/ijms22179184
26. Jafarzadeh H, Nazarian H, Ghaffari Novin M, Shams Mofarahe Z, Eini F, Piryaei A. Improvement of oocyte in vitro maturation from mice with polycystic ovary syndrome by human mesenchymal stromal cell-conditioned media. J Cell Biochem. 2018;119(12):10365-75. https://doi.org/10.1002/jcb.27380
27. Chugh R, Ashour D, Garcia N, Park H, Takala H, Ismail N, et al. Towards cell therapy of polycystic ovary syndrome (PCOS): human mesenchymal stem cells secretome inhibits androgen production by pcos theca cells. Cytotherapy. 2019;21(5):S81. https://doi.org/10.1016/j.jcyt.2019.03.493
28. Saeed Y, Liu X. Mesenchymal stem cells to treat female infertility; future perspective and challenges: a review. Int J Reprod Biomed. 2022;20(9):709-22. https://doi.org/10.18502/ijrm.v20i9.12061
29. Chang S, Dunaif A. Diagnosis of polycystic ovary syndrome: which criteria to use and when? Endocrinol Metab Clin North Am. 2021;50(1):11-23. https://doi.org/10.1016/j.ecl.2020.10.002
30. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016;37(5):467-520. https://doi.org/10.1210/er.2015-1104
31. Wang J, Liu C, Fujino M, Tong G, Zhang Q, Li XK, et al. Stem cells as a resource for treatment of infertility-related diseases. Curr Mol Med. 2019;19(8):539-46. https://doi.org/10.2174/1566524019666190709172636
32. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments. Gynecol Endocrinol. 2021;37(10):869-74. https://doi.org/10.1080/09513590.2021.1958310