Ramb'S 2021
Journal Citation
Reports-Impact Factor:
1,712

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

RAMB

Journal of The Brazilian Medical Association

Volume 69, Number 2
February, 2023

AMB
Associação Médica Brasileira

WMA

RAMB

Journal of The Brazilian Medical Association

Ramb'S 2021 Journal Citation Reports-Impact Factor: 1,712

AMB Associação Médica Brasileira

WMA

Volume 69, Number 2, February, 2023

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
Andrea Pio de Abreu

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: Cesar 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/10.1590/1806-9282.20221160

Healthcare compliance: pioneer experience in a public hospital

Fabio Roberto Cabar[1*], Matheus Abelo de Oliveira[2], Maria Luiza Gorga[3]

INTRODUCTION

The judicialization of health is an irreversible reality not only for professionals, but also for public or private institutions, with an exponential increase in actions against public entities, hospitals, and professionals. Such actions can result in indemnification for alleged malpractice, accountability for various illegal acts, in addition to requiring the right to treatments, hospitalizations, and surgeries. The Brazilian National Council of Justice published a report showing a 130% increase in cases involving health-related aspects between 2008 and 2017, compared to a 50% increase in cases in general[1]. A detailed analysis identified that medications, orthotics, prostheses and auxiliary means, examinations, procedures, and hospitalization beds are the main causes related to the search for judicial guardianship. On the contrary, actions with allegations of medical error represented a higher number than the demands for transplants, for example.

Another very sensitive subject is hospital mortality related to the occurrence of preventable adverse events. In a survey by the Institute for Supplementary Health Studies, it was demonstrated that mortality from this cause could be prevented in almost 40% of cases. In addition, the prevention of serious adverse events could provide fence one million beds for hospitalizations for other causes every year[2]. In addition to the direct and indirect damages caused to patients and families, there is undeniable and enormous financial damage, with the consequent misuse of resources that could be used in the treatment of other patients.

Some of the possible causes of this situation may be the lack of hierarchical definitions, tolerance for individualistic measures and practices, poverty in available information, and fear of punishment. Adjunct to these aspects, conducts known to be unethical or even illegal can be taken in professional practice, which should not be seen as mere individual inappropriate conduct, but as systemic problems in hospital institutions[3].

In this scenario, it is evident that measures need to be adopted to try to prevent or reduce harm, such as the establishment of a non-punitive culture of care security, so that failures are seen as real opportunities for improvement, and the training of health professionals to know and use measures to prevent these failures. It is essential to make professionals aware of the consequences of harmful attitudes, even when they are routine and apparently harmless. As a result, it is urgent to implement a compliance culture in health practices.

COMPLIANCE IN THE HEALTH SECTOR

In the current reality, it is indispensable to apply compliance programs in the health area, even though this is one of the most complex areas to implement the program. This is due to the fact that there is a very high level of specialties, techniques, and procedures to be strictly followed, surrounded by several contradictions and dilemmas, mainly ethical[4]. Compliance in the health sector should be oriented toward the observation of administrative, ethical, and legal rules present in the various resolutions of regulatory agencies, codes of conduct (such as those of the National Accreditation Organization), codes of ethics of professional councils, and, ultimately, the legal system that regulates health practices. Without any doubt, the adequacy of conducts to national and international standards, the adoption of educational methods for the use of the best care protocols, and the improvement of patient safety are attitudes that can be improved with an active compliance program help[5].

"Defensive medicine" is a medical practice that prioritizes the adoption of diagnostic and/or therapeutic conducts with the primary objective of avoiding lawsuits, since the doctor can be triggered in the ethical courts, and most of these processes focus on actions in the judiciary, whether in civil justice or even criminal justice. An UK study showed that 63.8% of physicians adopted defensive practices such as unnecessarily referring patients to other

[1]Universidade de São Paulo, Faculty of Medicine, Department of Obstetrics and Gynecology – São Paulo (SP), Brazil.
[2]Centro Universitário, Faculdade das Américas – São Paulo (SP), Brazil.
[3]Centro Universitário, Faculdade São Camilo – São Paulo (SP), Brazil.
*Corresponding author: fabio.cabar@hc.fm.usp.br
Conflicts of interest: The authors declare there is no conflict of interest. Funding: None.
Received on August 30, 2022. Accepted on September 03, 2022.

doctors, performing unindicated control tests, and requesting unjustified complementary tests[6,7]. In the United States, in the 1990s, 17.6% of medical care investments were related to defensive medicine practices, with amount greater than $10 billion[8,9].

In Brazil, the daily reality of the practice of defensive medicine is not much different. In our country, in addition to the aspects already pointed out, this practice also occurs due to poor medical training, causing the professional to use diagnostic means of difficult public access (and expensive) instead of adequate communication with the patient and a detailed and enlightening clinical examination. Deficient professional training can be explained by the indiscriminate creation of medical schools, many without the minimum operating conditions, added to the fact that most of these trained physicians do not have access to medical residency or specialization courses[10].

The adequacy of the norms does not aim at a definition of a standard of conduct that can interfere with the autonomy of professionals. Its objective is to rule out possible frameworks and punishments, while enabling a rational use of scarce health resources[11]. In fact, what is sought are prevention mechanisms and not only compensation after the occurrence of the act performed in noncompliance with the rules and laws[12].

Two other important aspects that must be faced by health professionals and institutions and that go through the activities of these programs are (1) acts of corruption in hospitals, such as deviations from public resources or bribes to circumvent lines of care or receive priority care and (2) compliance with the recently approved General Data Protection Law[5].

In relation to corruption in the public health system, in addition to the damage caused by diverted financial values, such situations demonstrate that it is easy for professionals to find some ways to enrich themselves using the Public Health money, a fact that directly compromises the image and efficiency of public health. These situations may be a constant source of concern for the administrators of these institutions so that they can curb such practices. These should combat illicit attitudes in a systemic way, acting in the relationship between health providers and public authorities, as well as between these suppliers and various civil servants[13]. The relationships of these professionals with the industry, such as the rich distribution of free samples of medicines, sponsorship for event participation, donation of gifts and gifts, and hiring for lectures and studies, are situations that are in a border zone between morality and legality, which can generate dangerous conflicts of interest.

The General Data Protection Act (LGPD – from Portuguese) was created with the aim of protecting citizens' personal data, maintaining the privacy of these users, and protecting them from the collection and misuse of their personal data. Among the various impacts in the health area, the entry of this law will generate the need for users' consent, expand the concept of sensitive data, and decrease the possibility for other users to access the data.

There is no doubt that the implementation of the LGPD by health institutions, clinics, hospitals, health plan operators, laboratories, pharmacies, and other companies in the sector will be a major challenge since patients are the true owners of their personal data. All these institutions, whether public or private, in the implementation of this legal provision, must be based on what is recommended by the LGPD. Every process of collecting, storing, and transmitting patients' personal data must be carried out in systems, with encryption, and by software approved by institutions, such as the Brazilian Society of Health Informatics. Furthermore, the confidentiality between doctor and patient, a presupposition long provided for in the Code of Medical Ethics, must be guaranteed in digital medicine tools[14].

Disobedience to the LGPD causes high fines to establishments that do not comply with the standards, which makes the dissemination of the culture of proper data processing, from the high-dome manager to the receptionist, fundamental. Thus, the implementation and application of LGPD terms in public health facilities is another challenge for compliance programs.

EXPERIENCE OF A COMPLIANCE PROGRAM IN A BRAZILIAN PUBLIC HOSPITAL

The complex structure of private hospitals in Brazil and competition in the sector caused them to start looking at their management with a strategic vision, with the implementation of a system that integrated the main management processes and generated speed in the quality of services provided for the benefit of patients. Especially after the validity of law 12.846/13 (Anti-corruption Law), there was a need for private hospitals to protect themselves from the danger of illicit acts that could be committed by employees, which would cause economic and image damage to public opinion. Thus, there was a need for these institutions to give more attention to the compliance area. The same did not occur in hospitals and public sector institutions.

The Hospital of Clinics of the Faculty of Medicine of the University of São Paulo (HCFMUSP) is the largest hospital complex in Latin America, which is considered one of the most important Brazilian centers for dissemination of technical and scientific information, serving as a center of excellence and reference in the fields of teaching, research, and care, as well as a pioneer in several medical, technical, scientific, and administrative activities in Brazil and Latin America. In March 2018, HCFMUSP became the first

Brazilian public hospital to establish a compliance program aimed at guiding the actions and professional conduct of approximately 23,000 employees of this hospital complex.

Its board guides the conduct of those who work at HCFMUSP to minimize the risks arising from actions taken in noncompliance with regulations and legislation. This is in line with the concept of Fair Culture, which is defined by the governance model that has as one of its principles that not all errors or violations of conduct are the result of bad intentions, according to which punishment is not effective, since the problem is not found in the individual itself, but in the institution[15,16].

Hospital of Clinics of the Faculty of Medicine of the University of São Paulo (HCFMUSP's) pioneering compliance program focuses on the role of guiding the ethical and legal conduct to be adopted, seeking to change the organizational culture and make clear the expected conducts and those that are restricted to employees, focusing mainly on situations of conflicts of interest – the great doors of access to improper practices. Examples of pillars of the guidelines include sponsorship of education activities, donations, sponsorships, events, and clinical research; the adequacy of prescriptions; patient enrollment; the correct relationship with suppliers; confidentiality obligations; the use of social media; and respect in the handling of privileged information. The seals are clear, grounded, and justified to employees, bearing in mind that the practice of unreasonable and unexplained prohibitions can generate an attitude of weariness and a lack of commitment to the rules.

The dissemination of the guidelines occurs through periodic training for the teams, as well as the dissemination of matters of interest and clarification summaries in official internal publications, maintenance of online portal, and direct communication channel, with the possibility of clarifying doubts, receiving complaints and providing guidance[17]. The HCFMUSP Complex' booklet of the Compliance Board, launched in 2018 and later updated, is distributed to employees (in its printed version) and maintained in the electronic portal of the institution, with ample access to employees and also to all users of the worldwide computer network[18].

Another aspect of the way this compliance board operates is its proximity and synergy with other hospital complex boards, such as the medical and health boards of all institutes, internal communication, information technology, the law center, and, more importantly, the clinical and executive boards of the hospital. Thus, there is no conflict or invasion of competencies, generating a harmonic and aligned discourse, which generates security for all employees and allows greater compliance from all those involved in the policy and principles of compliance.

At present, there is no doubt that this is a successful model. The results speak for themselves: in the 3 years since its installation, there have been hundreds of e-mails and requests for guidance, participation in more than a hundred corporate and board meetings, publication of more than 120 articles, referrals of complaints, and appointments to indictment committees to investigate possible administrative infractions, with clear indicators that show the growth of employees' compliance policies and acceptance of their guidelines year after year.

On the contrary, the support of the various areas of management is also evident, with the expansion of the sector, the ratification of its decisions, and the insertion of its board members in other institutional committees, such as the Bioethics Committee, among others[17].

CONCLUSION

The health sector, in its various segments and institutions, presents great vulnerability and enormous potential for illegal misconduct to occur. It is noted that self-regulation is already consolidated in private institutions, with the establishment of policies and a culture of fraud and damage control, either through guidance, investigation, or even punishment of the various parties involved in its production chain.

On the contrary, this philosophy and these practices are slow in the public sector, especially due to the lack of resources, managerial culture, and the presence of bureaucratic controls that are often archaic and harmful to new regulatory policies.

The experience of the largest hospital complex in Latin America, a public entity, demonstrates the feasibility of implementing tools for the institution of structural and cultural changes, with the use of accessible resources, through the collaboration of administrative bodies and their employees; the focus of this action, based on the culture of education and orientations, without the punitive aspect, undoubtedly contributed to the adhesion of all those involved.

This scenario demonstrates that models of self-control and pipeline restructuring, such as those adopted by HCFMUSP, can and should be replicated in other public hospitals in order to obtain the real confidence of the population and, gradually, and reduce deviations of funds and purposes, resulting in an improvement in healthcare for the entire population, which is one of the constitutional purposes of the Unified Health System.

AUTHORS' CONTRIBUTIONS

FRC: Conceptualization, Formal Analysis, Project administration, Supervision, Validation, Writing – review & editing. **MAO:** Visualization, Writing – original draft. MLG: Conceptualization, Project administration, Supervision, Validation.

REFERENCES

1. Conselho Nacional de Justiça. Judicialização da saúde no Brazil: perfil das demandas, causas e propostas de solução. Brazília (BR): Conselho Nacional de Justiça; 2019. Available from: https://www.researchgate.net/publication/333659130_Judicializacao_da_Saude_no_Brasil_perfil_das_demandas_causas_e_propostas_de_solucao
2. Instituto de Estudos de Saúde Suplementar. II anuário da segurança assistencial hospitalar no Brazil: propondo as prioridades nacionais. Belo Horizonte (BR): UFMG; 2018. Availble from: https://www.iess.org.br/sites/default/files/2021-04/Anuario2018_0.pdf
3. Abramovicius AC. Estudo dos erros de prescrição relacionados aos medicamentos utilizados no hospital das clínicas da faculdade de medicina de Ribeirão Preto [tese]. Ribeirão Preto (BR): Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo; 2007. Available from: https://teses.usp.br/teses/disponiveis/17/17139/tde-06032008-153619/pt-br.php
4. Mânica FB. Compliance no setor de saúde. In: Nohara IP, Pereira FLB, editors. Governança, compliance e cidadania. São Paulo (BR); 2018. p. 461-76. Available from: https://thomsonreuters.jusbrasil.com.br/doutrina/secao/1197015376/compliance-no-setor-de-saude-governanca-compliance-e-cidadania
5. Gorga ML. Compliance médico em um hospital público. In: de Souza LA, editor. Compliance no direito penal. São Paulo (BR): Revista dos Tribunais; 2020. p. 205-26. Available from: https://thomsonreuters.jusbrasil.com.br/doutrina/secao/1188256719/9-compliance-medico-em-um-hospital-publico-compliance-no-direito-penal
6. Minossi JG, da Silva AL. Medicina defensiva: uma prática necessária? Rev Col Bras Cir. 2013;40(6):494-501. https://doi.org/10.1590/S0100-69912013000600013
7. Rodríguez Almada H. De la medicina defensiva y la medicina asertiva. Rev Med Urug. 2006;22(3):167-8. Available from: http://www.scielo.edu.uy/scielo.php?script=sci_arttext&pid=S1688-03902006000300001
8. Reynold S, Rizzo JA, Gonzalez ML. The cost of medical professional liability. JAMA. 1987:257(20):2779-81. PMID: 3573273
9. Tena-Tamayo C, Sanches-Gonzalez JM. Medicina asertiva. Una alternativa para mejorar la calidad. In: Tena-Tamayo C, Hernandez-Orozeo F, editors. La comunicación humana em la relación medico-paciente. México: Prado; 2005;22:167-8. Available from: https://www.academia.edu/31243087/MEDICINA_ASERTIVA_Una_alternativa_para_mejorar_la_calidad
10. Conselho Federal de Medicina. Quase 40% das escolas abertas a partir de 2011 estão em locais sem estrutura. Portal CFM. [cited on August 31, 2022]. Available from: https://portal.cfm.org.br/noticias/quase-40-das-escolas-abertas-a-partir-de-2011-estao-em-locais-sem-estrutura/
11. Nieto Martín A. Problemas fundamentales del cumprimento normativo en el Derecho penal. In: Urbina Gimeno IO, editor. Compliance y teoría del derecho penal. Buenos Aires: Marcial Pons; 2013. p. 21-49.
12. Beck U. Sociedade de risco: rumo a uma outra modernidade. São Paulo (BR): Editora 34; 2011. Available from: https://ferreiramacedo.jusbrasil.com.br/artigos/160037557/sociedade-de-risco-rumo-a-uma-outra-modernidade
13. Reaso, J. Human error: models and management. BMJ. 2000;320(7237):768-70. https://doi.org/10.1136/bmj.320.7237.768
14. Bracarens, CM. LGPD na saúde: seus impactos e desafios. Portal Migalhas. [cited on August 31, 2022]. Available from: https://www.migalhas.com.br/depeso/349454/lgpd-na-saude-seus-impactos-e-desafios
15. Boysen PG. Just culture: a foundation for balanced accountability and patient safety. Ochsen J. 2013;13(3):400-6. PMID: 24052772
16. Leveson NG. The systems approach to medicine: controversy and misconceptions. BMJ Qual Saf. 2015;24(1):7-9. https://doi.org/10.1136/bmjqs-2014-003106
17. Dekker WAS, Hugh TB. A just culture after Mid Staffordshire. BMJ Qual Saf. 2014;23(5):356-8. https://doi.org/10.1136/bmjqs-2013-002483
18. Diretoria Clínica. HCFMUSP. Cartilha de compliance. 2021. [cited on August 31, 2022]. Available from: http://conduta.hc.fm.usp.br

https://doi.org/10.1590/1806-9282.2023D692

Use of programmable valve versus fixed pressure valve in the treatment of idiopathic normal pressure hydrocephalus: a systematic review and meta-analysis

Adriano Anzai[1], Armelim Utino[1], Haroldo Katayama[1], Ighor Alexander Zamuner Spir[1], Marcio A. Lemos[1], Mary Martins Nery[1], Mauricio Anhesini[1], Oswaldo Silvestrini Tiezzi[1], Patricia RN Spir[1], Pericles Otani[1], Clara Lucato dos Santos[2], Luca Schiliró Tristão[2], Wanderley M. Bernardo[2,3*]

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct and assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the clinical condition of each patient.
Authorship: Brazilian Medical Association

INTRODUCTION

Idiopathic normal pressure hydrocephalus (iPNH) manifests itself through the clinical triad of gait disorders, dementia, and urinary incontinence, which is associated with radiological images of ventriculomegaly and normal intracranial pressure. The most commonly performed treatment is the placement of a ventriculoperitoneal valve or ventriculoperitoneal shunt when there is a positive response to TAP test 1. Clinical improvement is significant after this procedure, but overdrainage, subdural hematoma, or other complications may occur, making reinterventions necessary. There are numerous types of valves that can be used: fixed pressure ones (slit, membrane, or ball/spring) and second-generation ones, including anti-siphon, gravitational, and adjustable or programmable. Theoretically, programmable or adjustable valves would have advantages over fixed pressure valves. Our aim was to assess whether programmable or adjustable valves are superior to fixed pressure valves.

METHODOLOGY

This systematic review followed the precepts defined by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[1].

Clinical issue

The systematic review began with the elaboration of the following clinical question: Is the treatment of normal pressure hydrocephalus using programmable valves more effective when compared to fixed pressure ones?

PICO

The clinical question was structured from the acronym PICO being:

- P: patients with iPNH
- I: programmable valve
- C: fixed pressure valve
- O: clinical improvement, prognosis, reinterventions, and complications

Search strategy

Searches were performed in Medline (PubMed), Embase, CENTRAL (Cochrane), and LILACS databases with the following terms: (hydrocephalus) AND (ventriculoperitoneal shunt OR programmable valve OR adjustable valve).

Eligibility criteria

- PICO compliant items
- At least one of the outcomes compatible with those evaluated, such as clinical improvement, prognosis, reinterventions, and complications
- Randomized clinical trials (RCTs) to evaluate efficacy
- RCTs and observational studies to assess adverse events and complications
- No period and language restriction

[1]Unimed Presidente Prudente, Medicina Baseada em Evidências Center – Presidente Prudente (SP), Brazil.
[2]Centro Universitário Lusíada, Faculdade de Ciências Médicas de Santos, Center for Evidence-Based Medicine – Santos (SP), Brazil.
[3]Universidade de São Paulo, Guidelines Program of the Brazilian Medical Association – São Paulo (SP), Brazil.
*Corresponding author: wbernardo@usp.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.

Exclusion criteria

In vitro studies, animal studies, case series or case reports, systematic or narrative reviews, and guidelines.

Data analysis

The following information was extracted: author, year of publication, study design, characteristics and number of patients, intervention, comparison, and outcomes (clinical improvement and complications). Each article was described individually in a qualitative analysis of the evidence. Furthermore, quantitative analysis of the results (meta-analysis) was performed whenever possible. For the meta-analysis, Review Manager (RevMan) Version 5.4[2] was used. Comparisons were demonstrated in risk difference (RD) and 95% confidence interval (95%CI). The inconsistency of effects across interventions was assessed using I^2. The random-effects model was used if I^2>50% and the fixed-effects model was used if I^2≤50%. To access possible publication biases, the *funnel plot* was analyzed for asymmetry. The certainty of the evidence was assessed using the GRADE Pro guideline development tool[3] and rated as high, moderate, low, or very low.

Bias analysis

To assess RCT bias, the following were evaluated: randomization, blinded allocation, double blinding, losses (<20%), intention-to-treat analysis, definition of outcomes, sample size calculation, early discontinuation, and prognostic characteristics. For observational studies, the ROBINS-I platform was used[4].

RESULTS

The search was conducted until December 2022 and retrieved a total of 16,882 articles in the primary databases (Medline: 5,879; Embase: 10,507; LILACS: 338 Lilacs; Cochrane: 158). After removing duplicates, they totaled 8,728 articles. All of them had their titles checked, and 223 abstracts were reviewed for inclusion. The reading of 40 complete texts was carried out to verify compatibility with the defined eligibility criteria. Finally, four comparative studies were included[5-8] (Table 1) (Figure 1). Bias analysis showed that the articles have low-to-moderate risk of bias (Table 2 and Figure 2).

Efficacy

Farahmand et al.[5], in a RCT, reported the clinical evolution of their patients. The measure used was the *total standard deviation score*, which involved the Stroop test, the Grooved Pegboard test, walking duration, and number of steps taken. After 6 months, both groups had a statistically different evolution compared to the preoperative period (I: -0.23±1.10 vs. 0.46±0.27; C: 0.09±0.67 vs. 0.52±0.30; p<0.05). However, between groups, there was no statistical difference in any of the assessments until the end of the study, 6 months after valve placement (p>0.05) (Figure 3). In the evaluation of each parameter separately, a significant difference was also found in relation to the baseline in all tests (p<0.05) but without difference between groups.

Complications: randomized clinical trials and observational studies have evaluated complication rates

Complications analyzed through randomized clinical trials

Sæhle et al.[6], an article derived from the same RCT by Farahmand et al.[5], reported complications after valve placement. Notably, six (17.7%) patients with programmable valves had shunt-related complications, four of which had subdural hematomas.

Table 1. Characteristics – comparative studies.

Author	Year	Study design	Groups		Outcomes	Follow-up
			I	C		
Rinaldo et al.[8]	2019	Cohort	Programmable valve (n=98)	Fixed-setting valve (n=250)	Complications	–
Serarslan et al.[7]	2017	Cohort	Programmable valve (n=30) Mean age: 62 years	Fixed-setting valve (n=80) Mean age: 61 years	Complications	72 months
Farahmand et al.[5]	2016	RCT	Programmable valve (n=34) 20 cm H_20 – 4 cm	Fixed-setting valve (n=34) 12 cm H_20	Stroop test, Grooved Pegboard test, Walk time, Walk steps	6 months
Saehle et al.[6]	2014	RCT	Programmable valve (n=34) 20 cm H_20 – 4 cm	Fixed-setting valve (n=34) 12 cm H_20	Complications	6 months

Furthermore, seven patients had symptoms due to excessive drainage. In patients with a fixed valve, seven (20.6%) patients had complications related to the shunt, with five subdural hematomas. Another four patients had symptoms of excessive drainage. All comparisons had p>0.05. In patients with iPNH who underwent implantation of a programmable valve compared to a fixed pressure valve, there was no difference in complications at the 6-month follow-up (Figure 4). The quality of

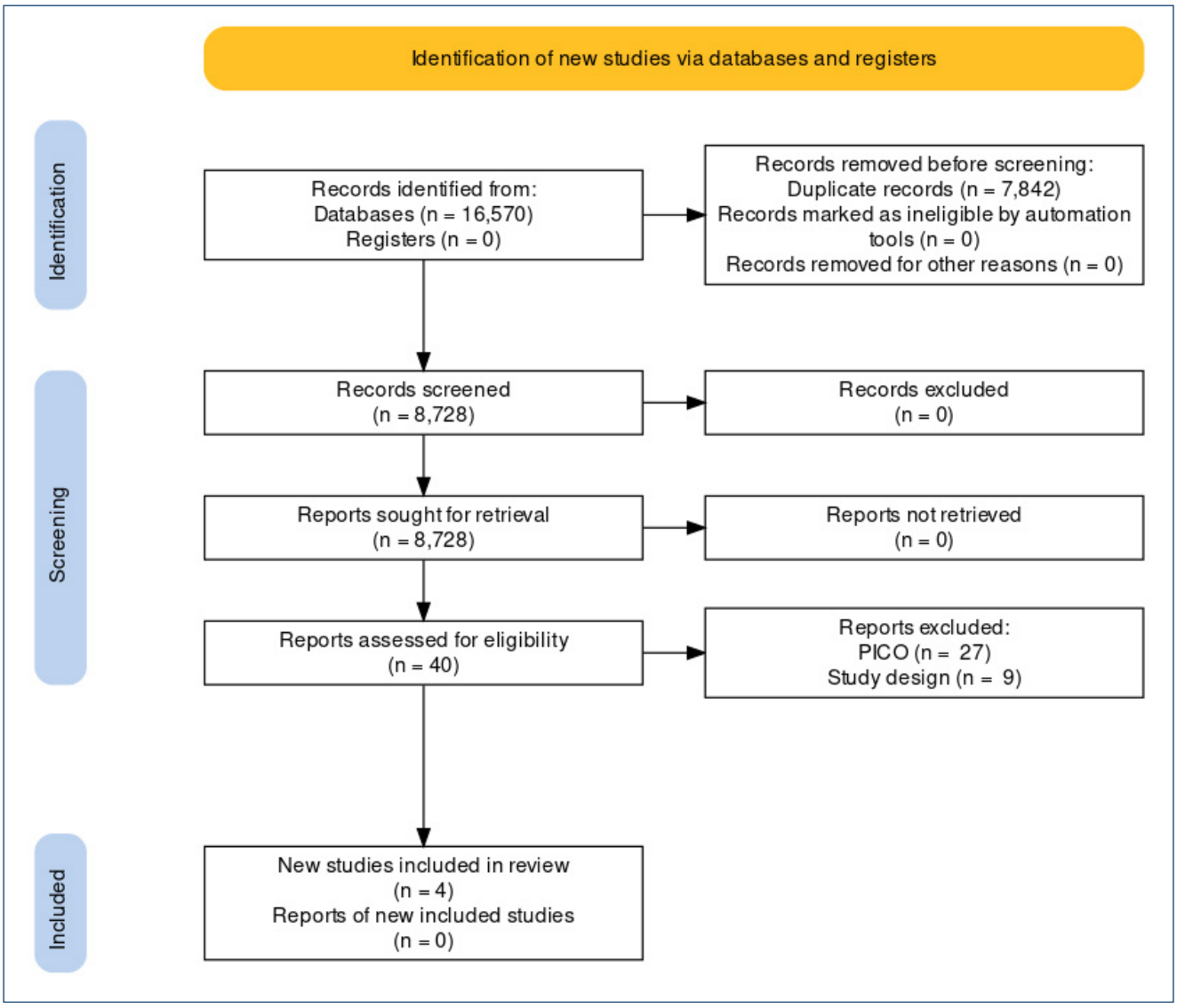

Figure 1. PRISMA flow diagram.

Table 2. Bias – randomized controlled trial.

Studies	Randomization	Allocation	Double blind	Evaluator blind	Losses	Characteristics	Outcomes	ITT analysis	Sample size	Early end
Farahmand et al.[5]										
Saehle et al.[6]										

ABSENCE OF BIAS
ABSENCE OF INFORMATION
PRESENCE OF BIAS

evidence is very low. In patients with iPNH who underwent implantation of a programmable valve compared to a fixed pressure valve, there was no difference in the incidence of overdrainage at the 6-month follow-up (Figure 5). The quality of evidence is very low.

Complications analyzed through cohort studies

Serarslan et al.[7], a cohort, also reported complications in their study. In the group with programmable valves, 26.33% had complications, while in the group with fixed valves, 52.5% had (p=0.02). Subdural effusions occurred in 20% of patients

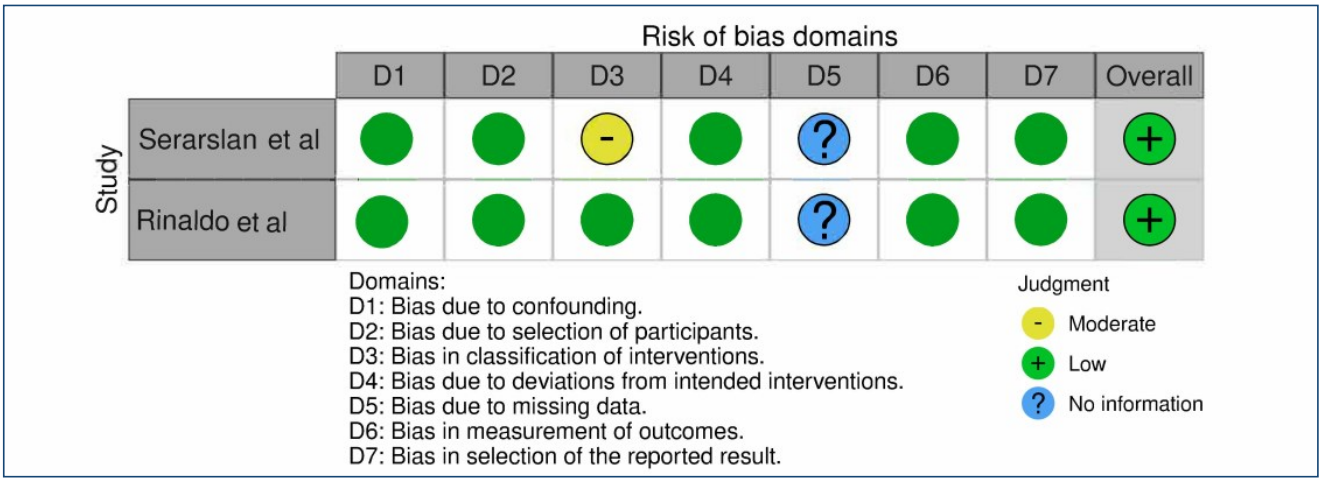

Figure 2. Bias - cohorts (ROBINS-I).

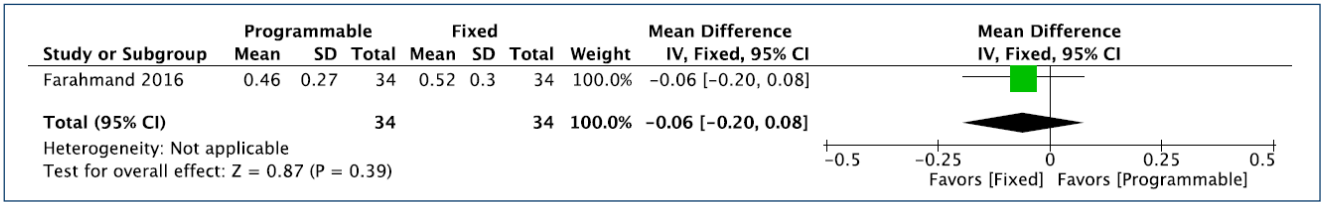

Figure 3. Analysis of clinical evolution (walking test, Stroop test, and Grooved Pegboard test) comparing programmable valve versus valve with fixed pressure.

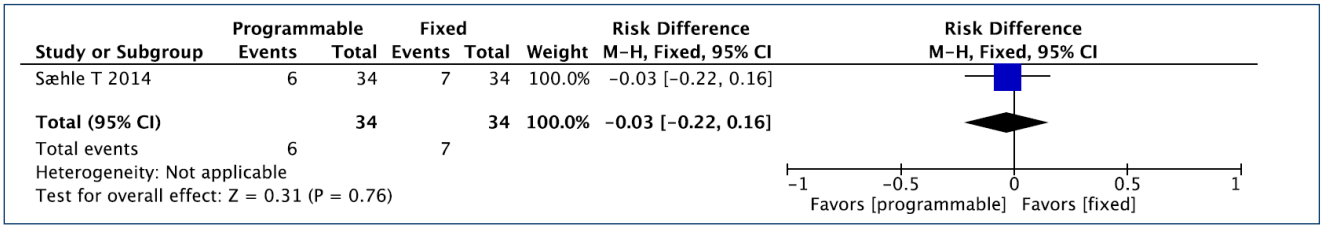

Figure 4. Analysis of complications in comparing programmable valve versus valve with fixed pressure.

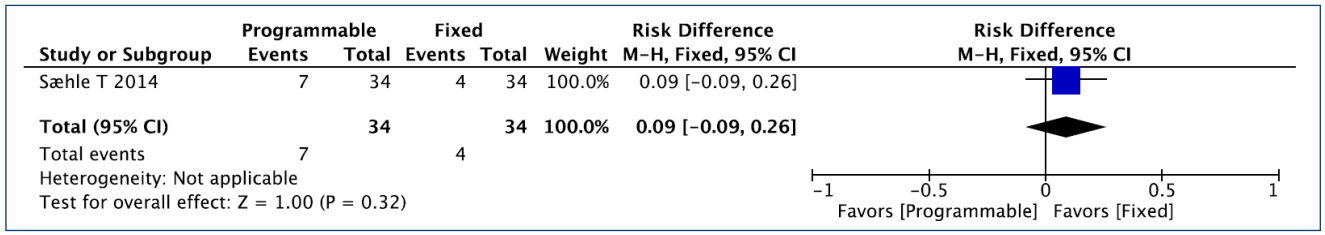

Figure 5. Analysis of the incidence of overdrainage when comparing the programmable valve versus the valve with fixed pressure.

with programmable valves and 22.5% with fixed valves (p=0.78). Nontraumatic subdural hematomas occurred in 11 (13.75%) patients with fixed valves, and of these, 2 died. In the programmable ones, only one patient had this complication (p=0.15).

Rinaldo et al.[8], another cohort, reported that complications occurred in 13.3% of patients with programmable valves and 24.0% of patients with fixed valves (p=0.03). Revision surgery for distal obstruction occurred in 1.0% of those with programmable valves and 6.8% of those with fixed valves (p=0.06), and persistence of symptoms without obstruction in 2.0 and 8.8% (p=0.04), respectively.

Meta-analysis of the complication rate in two observational studies[7,8] revealed that patients with programmable valves had a lower risk of complications than those with fixed valves (RD=-0.16; 95%CI -0.30, -0.02; p=0.03; I^2=51%; random model; certainty of evidence: very low) (Figure 6).

DISCUSSION

Efficacy and complications analyzed through randomized clinical trials

There are no randomized trials directly comparing programmable valves and conventional valves in patients with iPNH. However, comparing these patients with the use of programmable valves with gradual pressure reduction (independent of symptoms) and with fixed pressure, no differences were found in clinical evolution, complications, or overdrainage. The evidence supporting these conclusions is of very low quality.

Complications in observational studies

Several single-arm observational studies have reported complications in patients with programmable valves. Feletti et al.[9], in a cohort of 142 patients, reported 30 cases of symptoms due to poor drainage and 10 due to excessive drainage. In addition, 43 shunt adjustments were performed. Finally, 7 patients had subdural hematoma and 10 had hygroma. Ma et al.[10] reported that the complication rate was 40% (41/102), with the most prevalent being subdural hematoma and hygroma, with 28 cases. They also reported the need for 85 shunt adjustments. Shaw et al.[11] reported 3 subdural hematomas and 3 shunt revisions among 45 patients involved in their study. Oliveira et al.[12] reported 4 subdural hematomas, 1 empyema, 2 malfunctions, and 1 valve exposure in 24 patients involved in their study. Finally, Zemack et al.[13] reported 14 subdural hematomas or hygromas, 2 proximal catheter obstructions, and 138 shunt adjustments in 147 patients involved in their study.

Limitations

This review has some limitations. Only two RCTs that responded to PICO were found. Furthermore, both are part of the same series, only reporting different outcomes in each publication. It is evident that there is a flaw in the literature when comparing fixed and programmable valves in patients with iPNH, limiting the conclusions on the subject. Only these two reported outcomes were related to the effectiveness of the techniques, while the observational ones described only adverse events and complications.

CONCLUSION

In patients with iPNH, no evidence is currently available that allows recommending the use of programmable valves in the treatment of these patients, in comparison, or that leads to the discontinuation of the use of conventional (fixed) valves. The quality of the available evidence is very low.

AUTHORS' CONTRIBUTIONS

AA: Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **HK:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **IAZS:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization,

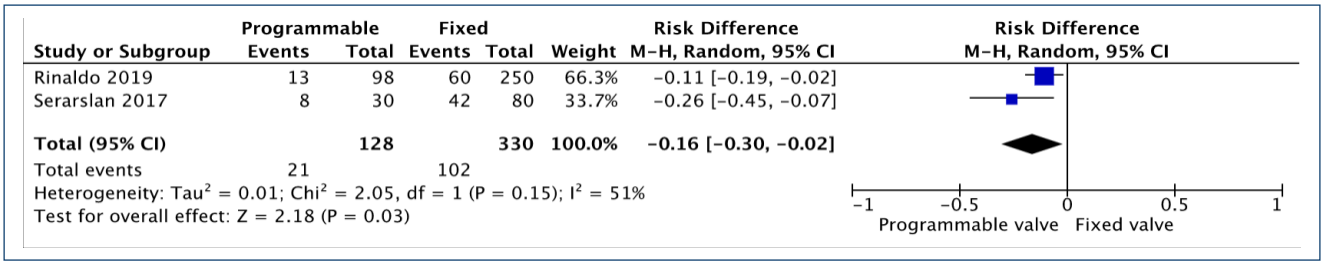

Figure 6. Complications.

Writing – original draft, Writing – review & editing. **MMN:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **MA:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **OST:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **PRNS:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **PO:** Conceptualization, Date curation, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **CLS:** Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **LST:** Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **WMB:** Conceptualization, Date curation, Formal Analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ (Clinical research ed.). 2009;339:b2535. https://doi.org/10.1136/bmj.b2535
2. The Cochrane Collaboration. Review Manager Version 5.4 [Computer program]. 2020. Available from: https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman
3. McMaster University & developed by Evidence Prime, Inc. GRADEpro GDT: GRADEpro Guideline Development Tool. 2020. Available from https://gradepro.org
4. Sterne JA, HernÁn MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;355:i4919. https://doi.org/10.1136/bmj.i4919
5. Farahmand D, Sæhle T, Eide PK, Tisell M, Hellström P, Wikkelsö C. A double-blind randomized trial on the clinical effect of different shunt valve settings in idiopathic normal pressure hydrocephalus. J Neurosurg. 2016;124(2):359-67. https://doi.org/10.3171/2015.1.JNS141301
6. Sæhle T, Farahmand D, Eide PK, Tisell M, Wikkelsö C. A randomized controlled dual-center trial on shunt complications in idiopathic normal-pressure hydrocephalus treated with gradually reduced or "fixed" pressure valve settings. J Neurosurg. 2014;121(5):1257-63. https://doi.org/10.3171/2014.7.JNS14283
7. Serarslan Y, Yilmaz A, Çakır M, Güzel E, Akakin A, Güzel A, et al. Use of programmable versus nonprogrammable shunts in the management of normal pressure hydrocephalus: a multicenter retrospective study with cost-benefit analysis in Turkey. Medicine (Baltimore). 2017;96(39):e8185. https://doi.org/10.1097/MD.0000000000008185
8. Rinaldo L, Bhargav AG, Nesvick CL, Lanzino G, Elder BD. Effect of fixed-setting versus programmable valve on incidence of shunt revision after ventricular shunting for idiopathic normal pressure hydrocephalus. J Neurosurg. 2019;1-9. https://doi.org/10.3171/2019.3.JNS183077
9. Feletti A, d'Avella D, Wikkelsø C, Klinge P, Hellström P, Tans J, et al. Ventriculoperitoneal shunt complications in the european idiopathic normal pressure hydrocephalus multicenter study. Oper Neurosurg (Hagerstown). 2019;17(1):97-102. https://doi.org/10.1093/ons/opy232
10. Ma TS, Sharma N, Grady MS. A simplified pressure adjustment clinical pathway for programmable valves in NPH patients. Clin Neurol Neurosurg. 2017;159:83-6. https://doi.org/10.1016/j.clineuro.2017.05.020
11. Shaw R, Everingham E, Mahant N, Jacobson E, Owler B. Clinical outcomes in the surgical treatment of idiopathic normal pressure hydrocephalus. J Clin Neurosci. 2016;29:81-6. https://doi.org/10.1016/j.jocn.2015.10.044
12. Oliveira MF, Saad F, Reis RC, Rotta JM, Pinto FC. Programmable valve represents an efficient and safe tool in the treatment of idiopathic normal-pressure hydrocephalus patients. Arq Neuropsiquiatr. 2013;71(4):229-36. https://doi.org/10.1590/0004-282x20130007
13. Zemack G, Romner B. Adjustable valves in normal-pressure hydrocephalus: a retrospective study of 218 patients. Neurosurgery. 2008;62(Suppl 2):677-87. https://doi.org/10.1227/01.neu.0000316272.28209.af

AOP https://doi.org/10.1590/1806-9282.20221377

Comment on "Factors affecting the clinical outcomes in pediatric post-cardiotomy patients requiring perioperative peritoneal dialysis"

Wen Peng[1], Yu Pei[2*]

Dear Editor,

In a retrospective article entitled "Factors affecting the clinical outcomes in pediatric post-cardiotomy patients requiring perioperative peritoneal dialysis," the authors investigated factors associated with mortality in pediatric patients undergoing perioperative peritoneal dialysis after cardiotomy[1]. In this study, the authors found that younger preoperative age, longer cardiopulmonary bypass time, prolonged intubation, prolonged inotropic support, and need for extracorporeal membrane oxygenation were associated with a higher risk of mortality. In our opinion, although the findings of this study are of great value, there are some issues that need to be addressed.

First, some continuous variables were not properly expressed. As described in Table 1, the age of the included patients was 11.7±37.6 months. As a result, the standard deviation (37.6) is significantly larger than the mean (11.7), indicating that age is a skewed distribution variable and it should be described as median and interquartile range, not as mean±standard deviation. Similarly, the variables such as weight and preoperative PaO_2 should also be appropriately described as median and interquartile range.

Second, this study did not describe which statistical method was used to screen for risk factors associated with mortality. Providing detailed statistical methods will help improve the reliability and reproducibility of this study. Furthermore, although preoperative lower age is shown to be a risk factor for mortality in this study, the definition of preoperative lower age is unknown. We are curious about this: less than 6 months or 12 months? Evidence from a pediatric cardiac intensive care unit indicated that the age of patient less than 1 month was associated with a higher risk of mortality[2]. Thus, we believe that providing a precise definition of younger preoperative age is helpful for clinicians to give individualized treatment strategies for children undergoing cardiac surgery.

Third, more information after cardiac surgery is unknown. In such case, it is suspected that patients who die may have more red blood cell transfusion and use of vasoactive agents after cardiac surgery. Results from a previous study suggested that red blood cell transfusion was independently associated with a higher risk of mortality in critically ill children[3]. Another study[4] involving 43,441 postoperative pediatric cardiac patients displayed that the use of milrinone alone was associated with a lower risk of in-hospital mortality, while the use of all other vasoactive agents increased the risk of in-hospital mortality at least in one of the subsets. Therefore, it is necessary to provide more information after cardiac surgery (e.g., red blood cell transfusion and the use of vasoactive agents).

AUTHORS' CONTRIBUTIONS

WP: Conceptualization, Investigation, Writing – original draft. **YP:** Project administration, Supervision, Writing – review & editing.

REFERENCES

1. Arslan AH, Aksoy T, Ugur M, Ustunsoy H. Factors affecting the clinical outcomes in pediatric post-cardiotomy patients requiring perioperative peritoneal dialysis. Rev Assoc Med Bras (1992). 2022;68(5):627-31. https://doi.org/10.1590/1806-9282.20211279
2. Morris MC, Ittenbach RF, Godinez RI, Portnoy JD, Tabbutt S, Hanna BD, et al. Risk factors for mortality in 137 pediatric cardiac intensive care unit patients managed with extracorporeal membrane oxygenation. Crit Care Med. 2004;32(4):1061-9. https://doi.org/10.1097/01.ccm.0000119425.04364.cf
3. Kneyber MC, Hersi MI, Twisk JW, Markhorst DG, Plötz FB. Red blood cell transfusion in critically ill children is independently associated with increased mortality. Intensive Care Med. 2007;33(8):1414-22. https://doi.org/10.1007/s00134-007-0741-9
4. Loomba RS, Flores S. Use of vasoactive agents in postoperative pediatric cardiac patients: insights from a national database. Congenit Heart Dis. 2019;14(6):1176-84. https://doi.org/10.1111/chd.12837

[1]Shiyan Maternal and Child Health Hospital Confinement Center – Shiyan, China.
[2]Shiyan Maternal and Child Health Hospital, Department of Maternity – Shiyan, China.
*Corresponding author: py19881105@163.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 13, 2022. Accepted on October 30, 2022.

AOP https://doi.org/10.1590/1806-9282.20221405

Comment on "Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios of overweight children and adolescents"

Hui He[1], Yu Zhong[1*]

Dear Editor,

We read an article entitled "Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios of overweight children and adolescents" by Yazaki et al.[1] In this cross-sectional study, the authors compared the differences in neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) between groups of obesity, overweight, and eutrophic children and adolescents and further verified whether these parameters were related to age, ultrasensitive C-reactive protein (us-CRP), body mass index, and waist-to-height ratio. The results of the study showed that there was no significant statistical difference between the three groups in NLR (p=0.30) and PLR (p=0.68) and that PLR, rather than NLR, was independently associated with overweight. After carefully reading this study, we think that there are some issues that need clarification.

First, as described in Table 2, the purpose of this study[1] was to compare the differences in laboratory variables (e.g., leukocytes, neutrophils, NLR, and PLR) among three groups (i.e., obesity, overweight, and eutrophic groups). However, it is unknown whether there are differences in age and sex among the three groups. It is insufficient to provide the age and gender of the total study population because it cannot reflect the differences in age and gender among the three groups. A possible hypothesis is that there are statistically significant differences in age and gender among the three groups, leading to incomparability between groups. Therefore, it is highly recommended to provide differences in age and gender among the three groups.

Second, the statistical method of this study[1] is not entirely inappropriate. Statistically, the Kruskal-Wallis test[2] should be used to compare variables that are not normally distributed among the three groups. As described in this study[1], the purpose of Table 2 was to compare the differences in laboratory variables among the three groups. Therefore, the Kruskal-Wallis test, rather than the Mann-Whitney test, should be used to analyze the differences in laboratory variables among the three groups.

Third, as described in this study, "*Regarding nutritional status, 106 (62.4%) were eutrophic and 64 (37.6%) were overweight (overweight, obesity, and severe obesity),*" it is known that overweight involves three different groups (i.e., overweight, obesity, and severe obesity). It is well known that severe obesity is a special and vulnerable population[3,4]. From our perspective, it may be more interesting to compare the differences in laboratory variables between this group (severe obesity) and the other three groups (obesity, overweight, and eutrophic groups) in Table 2.

AUTHORS' CONTRIBUTIONS

HH: Conceptualization, Investigation, Project administration, Writing – original draft. **YZ:** Conceptualization, Supervision, Validation, Writing – review & editing.

REFERENCES

1. Yazaki LG, Faria JCP, Souza FIS, Sarni ROS. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios of overweight children and adolescents. Rev Assoc Med Bras (1992). 2022;68(8):1006-10. https://doi.org/10.1590/1806-9282.20211253
2. Yue JK, Yuh EL, Korley FK, Winkler EA, Sun X, Puffer RC, et al. Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study. Lancet Neurol. 2019;18(10):953-61. https://doi.org/10.1016/S1474-4422(19)30282-0
3. Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G. Cardiovascular morbidity, diabetes and cancer risk among children and adolescents with severe obesity. Cardiovasc Diabetol. 2020;19(1):79. https://doi.org/10.1186/s12933-020-01052-1
4. Hoedjes M, Makkes S, Halberstadt J, Noordam H, Renders CM, Bosmans JE, et al. Health-related quality of life in children and adolescents with severe obesity after intensive lifestyle treatment and at 1-year follow-up. Obes Facts. 2018;11(2):116-28. https://doi.org/10.1159/000487328

[1]Shiyan Maternal and Child Health Hospital – Shiyan, China.
*Corresponding author: zy812285475@163.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 18, 2022. Accepted on November 04, 2022.

AOP https://doi.org/10.1590/1806-9282.20221429

Comments on "Comparison of the outcomes of flexible ureteroscopy and mini-percutaneous nephrolithotomy for the treatment of kidney stones: a matched-pair analysis"

André Pontes-Silva[1]*

Dear Editor,

In the article entitled "Comparison of the outcomes of flexible ureteroscopy and mini-percutaneous nephrolithotomy for the treatment of kidney stones: a matched-pair analysis," Rodrigues et al.[1] compared the outcomes of initial mini-percutaneous nephrolithotomy and flexible ureteroscopy. This study has scientific relevance; however, it did not answer an important question: does statistical significance ($p<0.05$) have clinical importance? A comparison of outcomes must consider the clinical relevance of the differences because the p-value only shows statistical significance, in which interpretation translates only a hypothesis test governed by a probability of previously defined error (alpha)[2].

According to Andrade[3], in this context, most persons interpret $p<0.05$ to mean that the probability that chance is responsible for the finding is less than 5% and that the probability that the finding is a true finding is more than 95%. Both these interpretations are incorrect; however, they are widely prevalent because they are an easy way to explain and understand a slightly tricky concept. As this is the first study on this topic, I would like to appreciate as there are suggestions for the authors to be included in future studies.

In the health area, there are several ways to verify the clinical relevance of the comparison of outcomes, e.g., the calculation of the effect size, the minimum detectable change/difference, and the standard error of measurement[4]. I would like to suggest the authors about the calculation of effect size (Cohen's d) for comparison studies (https://www.psychometrica.de/effect_size.html), based on three categories: less than 0.2 (small effect), about 0.5 (moderate effect), and greater than 0.8 (large effect).

ACKNOWLEDGMENTS

The author acknowledges the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade Federal do Tocantins (UFT), Universidade Federal do Maranhão (UFMA), and Universidade Federal de São Carlos (UFSCar).

AUTHORS' CONTRIBUTIONS

APS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Rodrigues JECM, Vicentini FC, Danilovic A, Marchini GS, Torricelli FCM, Batagello CA, et al. Comparison of the outcomes of flexible ureteroscopy and mini-percutaneous nephrolithotomy for the treatment of kidney stones: a matched-pair analysis. Rev Assoc Med Bras. 2022;68(10):1481-5. https://doi.org/10.1590/1806-9282.20221177
2. Pontes-Silva A. Statistical significance does not show clinical relevance: we need to go beyond the p-value. J Clin Exp Hepatol. 2022;12(5):1402. https://doi.org/10.1016/j.jceh.2022.04.017
3. Andrade C. The *P* value and statistical significance: misunderstandings, explanations, challenges, and alternatives. Indian J Psychol Med. 2019;41(3):210-5.https://doi.org/10.4103/IJPSYM.IJPSYM_193_19
4. Armijo-Olivo S. The importance of determining the clinical significance of research results in physical therapy clinical research. Braz J Phys Ther. 2018;22(3):175-6. https://doi.org/10.1016/j.bjpt.2018.02.001

[1]Universidade Federal de São Carlos, Physical Therapy Department, Physical Therapy Post-Graduate Program – São Carlos (SP), Brazil.
*Corresponding author: contato.andrepsilva@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 23, 2022. Accepted on October 26, 2022.

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220293

Comparison of sexual functions in women with and without type 1 diabetes

Selda Celik[1*], Meltem Demirgöz Bal[2], Meral Kelleci[3]

SUMMARY

OBJECTIVE: This study aimed to investigate female sexual function in patients with type 1 diabetes by comparing female sexual function index scores between women with and without type 1 diabetes.

METHODS: A total of 62 women with type 1 diabetes and 69 age-matched women without diabetes but with similar backgrounds were enrolled in the patient and control groups, respectively. All participants were sexually active and had no systemic diseases other than diabetes in the patient group.

RESULTS: The frequency of female sexual dysfunction was significantly higher, and the mean female sexual function index score was significantly lower in women with diabetes compared to the control group (p=0.01). There was a significant relationship between sexual dysfunction and duration of diabetes, glycosylated hemoglobin test, and body mass index (p<0.05).

CONCLUSION: This study demonstrates that female sexual dysfunction is more common among women with type 1 diabetes than among women without type 1 diabetes. Patients with type 1 diabetes should be evaluated in terms of sexual health. Health professionals should give more attention to and provide guidance regarding sexual function in women with type 1 diabetes.

KEYWORDS: Diabetes mellitus, type 1. Sexuality. Sexual dysfunctions, psychological. Sexual dysfunction, physiological.

INTRODUCTION

Diabetes mellitus (DM) is a disease that is rapidly increasing worldwide, and it is one of the most common chronic diseases that is seen in all countries. Diabetes is classified primarily as type 1 diabetes, type 2 diabetes, and gestational diabetes. Type 1 diabetes can affect people at any age, but usually develops in children or young adults[1,2]. Diabetes leads to microvascular (such as retinopathy, nephropathy, and neuropathy), macrovascular (cardiovascular system), and urological (lower urinary system dysfunction, sexual dysfunction, and urinary system infections) complications[3]. Diabetes mellitus is one of the important causes of sexual dysfunction (SD), which is more common and problematic in patients with type 1 diabetes compared to the normal population[4-7]. The pathogenesis of SD in women with diabetes is controversial, and hyperglycemia, infection, vascular, neuronal, and psychosocial disorders have all been implicated[8,9]. Diabetic neuropathy leads to vaginal wall changes, pelvic floor dysfunction, and weakened muscle tone. Neuropathic damage to the autonomic nervous system disrupts the orgasm process and causes delayed stimulation and lower desire. Insufficient vaginal lubrication results in painful sexual intercourse. In hyperglycemic states, dehydration of the mucous membranes and frequent urogenital infections may lead to reduced vaginal lubricity, dyspareunia, burning, itching, tightness, and vaginal dryness or discharge[6,10,11]. Studies on the prevalence of SD in women with type 1 diabetes have yielded varying results. This rate was reported to be 27% by Enzilin et al.[4], 35% by Maiorino Ke et al.[6], and 71% by Doruk et al[5]. These highly discrepant results regarding the prevalence of female SD in patients with type 1 diabetes may be attributed to cultural differences. However, it is now recognized that this issue is not being adequately addressed and remains an important problem.

The evaluation of women's SD is challenging for both patients and healthcare professionals. Personal taboos related to sex, privacy issues, and limited experience with female sexual function are factors that make it difficult to identify sexual problems. Unfortunately, advanced clinical methods for evaluating SD in women are limited. The female sexual function index (FSFI) is an assessment tool developed to standardize the evaluation of female sexual function. The FSFI has gained widespread international acceptance because it comprises subscales

[1]University of Health Sciences, Hamidiye Faculty of Nursing – Istanbul, Turkey.
[2]Marmara University, Health Sciences Faculty, Midwifery Department – Istanbul, Turkey.
[3]Cumhuriyet University, Faculty of Health Sciences, Nursing Department – Sivas, Turkey.
*Corresponding author: seldacelik40@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 21, 2022. Accepted on October 23, 2022.

that facilitate the classification of SD and has been evaluated in validity and reliability studies in different countries[12]. However, women with type 1 diabetes receive less attention in research and clinical practice. In this study, we investigated the prevalence of female SD in type 1 diabetes by comparing the FSFI scores in women with and without type 1 diabetes.

METHODS

Study design

This case-control study compared the sexual function in women with and without type 1 diabetes.

Participants

The study was conducted between October and December 2020. Women who presented to the diabetes outpatient clinic of a training and research hospital for follow-up comprised the case group. This group included 62 women who were diagnosed with type 1 diabetes, were sexually active with a partner, were between the ages of 18 and 45 years, were literate, and consented to participate in the study. Patients who were using any antidepressant, anxiolytic, antiepileptic, or estrogen-based (oral/vaginal) drugs; had a history of vaginal surgery or hysterectomy; were pregnant; had any sexually transmitted disease; or were peri/postmenopausal were excluded from the study. Patients who were directed to the nurse for education and consultancy after the routine diagnosis and treatment are included in the study.

The sample size was calculated based on a power of 80%, confidence interval of 95%, and significance level of $p<0.05$. The nondiabetic control group included 69 volunteers who were sexually active, were between the ages of 18 and 45 years, and had no systemic diseases or depression.

Levels of serum glucose and HbA1c were measured using Cobias Roche diagnostic kits and an auto-analyzer in the biochemistry laboratory of the hospital. Height and weight measurements were obtained with the participants lightly dressed and without shoes. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2).

Data collection tools

The participants (patients with and without type 1 diabetes) filled out forms in a suitable meeting room in the outpatient clinic. Data collection took approximately 20 min.

Information form

This form was developed by the researchers and consisted of two sections. The first section included questions on sociodemographic data including age, education level, employment status, economic status, family type, and duration of marriage. The second part included four questions on the duration of diabetes, BMI, and level of HbA1c.

Female sexual function index

This instrument was developed by Rosen et al.[9] to evaluate female sexual function. The index includes a total of 19 items questioning sexual function/problems within the last week in 6 subdimensions, namely, desire, arousal, lubrication, orgasm, satisfaction, and pain. The first 2 items question the frequency and level of sexual desire (1–5 points); items 3–6 question arousal level, confidence, and satisfaction (0–5 points); items 7–10 question the frequency/difficulty of lubrication and maintaining lubrication (0–5 points); items 11–13 question orgasm frequency, difficulty, and satisfaction (0–5 points); items 14–16 question satisfaction with the amount of closeness with a partner, sexual relationship, and overall sex life (1–5 points); and items 17–19 question the frequency and level of pain during and after penetration (0–5 points). Total FSFI score ranges from a minimum of 2 to a maximum of 36, with scores below 26.55 indicating SD[12]. Aygin and Aslan[13] conducted the reliability and validation study of the FSFI for Turkey in 2005.

Statistical analysis

All data were summarized as mean±standard deviation (minimum–maximum) or as number and percentage. Parametric data were compared using Student's *t*-test and nonparametric data using χ^2 analysis. Multiple regression analysis was used to examine the relationship between the groups. Significance was accepted at $p<0.05$.

Ethical considerations

Written permission was obtained from the Marmara University Non-Interventional Clinical Research Ethics Committee (24.09.2020/53) and the institution where the study was conducted prior to data collection. In addition, all study participants were informed about the nature of the study and that participation was on a voluntary basis. Informed consent was obtained from all participants.

RESULTS

The study sample consisted of 131 participants (62 in the case group, 69 in the control group). The mean ages of the diabetic and control groups were 34.32±7.5 years (range: 20–47

years) and 34.17±7.7 years (range: 20–47 years), respectively. The groups were similar in terms of sociodemographic characteristics (p>0.05) (Table 1).

In the type 1 diabetic group, the mean HbA1c level was 8.16±1.36% (range: 5.6–13.1%), the mean duration of diabetes was 13.2±7.2 (range: 2–31) years, and the mean BMI was 24.9±4.0 (range: 20.7–28.4) kg/m^2. All women in the type 1 diabetic group were receiving intensive insulin therapy (four times a day).

The FSFI total and subscale scores of the women with and without diabetes are shown in Table 2. The diabetic group had significantly lower sexual desire, arousal, satisfaction, as well as total scores compared to the control group (p<0.01).

The frequency of SD was significantly higher in the diabetic group (n=47, 77%) than that in the control group (n=28, 40%) (χ^2=16.01, p=0.01). HbA1c, BMI, and duration of diabetes differed significantly based on the presence of SD (p<0.05) (Table 3).

female sexual function index score and other variables were analyzed by multiple regression analysis. No significant difference was found in the multiple regression analysis to determine the relationship between FSFI scores and HbA1c, BMI, and diabetes durations (F: 1.510, p>0.05) (Table 4).

Table 1. The participants' characteristics.

Features	Women with diabetes (n=62) Mean±SD	Women without diabetes (n=69) Mean±SD	Statistical analysis	
			χ^2	p
Age (years)	34.3±7.5 (range: 18–45)	34.2 ±7.7 (range: 18–45)	35.192	0.276
Educational level (years)				
<8 years	19 (30.6%)	60 (87.0%)	45.087	0.677
>8 years	43 (69.4%)	9 (13.0%)		
Employment status				
Working	20 (32.3%)	11 (15.9%)	4.813	0.588
Not working	42 (67.7%)	58 (84.1%)		
Economic status				
Income < expenses	12 (19.4%)	36 (52.2%)	16.396	0.314
Income ≥ expenses	50 (80.4%)	33 (44.9%)		
Family type				
Nuclear	55 (88.7%)	54 (78.3%)	2.552	0.110
Extended	7 (11.3%)	15 (21.7%)		
Marriage duration (years)	12.7±9.5 (range: 1–28)	14.3±8.7 (range: 1–27)	33.187	0.458

SD: standard deviation.

Table 2. Comparison of semale sexual function index scores in women with and without diabetes.

	Women with diabetes (n=62) Mean±SD	Women without diabetes (n=69) Mean±SD	p
Desire	3.54±1.13	4.31±1.04	<0.01
Arousal	4.00±1.42	4.66±1.03	<0.01
Lubrication	4.91±1.30	5.08±1.21	0.440
Orgasm	4.57±1.17	4.87±1.23	0.153
Satisfaction	3.21±1.05	5.00±1.09	<0.01
Pain	5.00±1.93	5.28±1.14	0.137
Total FSFI score	20.81±4.06	23.27±4.27	<0.01

FSFI: female sexual function index; SD: standard deviation.

Table 3. Comparison of glycosylated hemoglobin test, body mass index, and duration of diabetes in women with type 1 diabetes based on the presence of sexual dysfunction.

	Sexual dysfunction (n=47)	No sexual dysfunction (n=15)	p*
HbA1c (%)	8.00±1.32	8.64±1.38	<0.05
BMI (kg/m^2)	24.89±4.00	26.53±4.23	<0.05
Duration of diabetes (years)	13.09±7.18	13.53±7.42	<0.05

BMI: body mass index. *Student's *t*-test.

Table 4. Multiple regression analysis of female sexual function index scores and variables.

		B	Beta	t	p	95%CI for B	
						Lower bound	Upper bound
FSFI	(Constant)	46.963		2.564	0.013	10.295	83.631
	HbA1c (%)	2.641	0.267	0.026	0.470	0.032	5.250
	BMI (kg/m^2)	0.001	0.002	0.017	0.986	-0.058	0.059
	Duration of diabetes (years)	0.053	0.028	0.223	0.824	-0.422	0.527
	R=0.269, R^2=0.072, Adjusted R^2=0.024, F=1.510, p=0.221						

FSFI: female sexual function index; BMI: body mass index.

DISCUSSION

This study aimed to investigate female sexual function in patients with type 1 diabetes by comparing FSFI scores between women with and without type 1 diabetes. In this study, the mean FSFI score was 20.8±4.1 for patients with type 1 diabetes and 23.3±4.3 for the control group. Similar to our study, Pontiroli et al.[14] determined in their meta-analysis of 3,168 women with diabetes and 2,823 controls that female SD was relatively common and that women with diabetes had lower FSFI scores than controls, indicating greater SD. In a recent study, Bak et al.[15] reported that type 1 diabetes was associated with sexual disorders in a third of affected women. Ahmed et al.[16] found that the mean total FSFI score was significantly lower in type 1 diabetes mellitus (21.1±3.9) than in type 2 diabetes mellitus (26.4±4.2), and both were significantly lower than in the control group (31.5±5.8). Similarly, Zamponi et al.[17] found that female SD (total FSFI score≤19) was significantly more prevalent in patients with type 1 diabetes compared to controls (12/33, 36.4% versus 2/39, 5.2%, respectively; p=0.010).

Several previous studies have shown that women with type 1 diabetes experience sexual problems at rates varying between 18 and 71%[4-7,18-20]. In the study conducted by Doruk et al.[5] in Turkey, SD was reported at a rate of 71% among women with type 1 diabetes and 37% in the control group. Similar to that study, we determined SD rates of 76 and 40% in the type 1 diabetes and control groups, respectively, which are higher than the rates reported in other studies. The high rate of SD in women with diabetes may be due to neurogenic, psychogenic, or vascular factors. Duration of diabetes, age, microvascular complications, cultural factors, and psychological factors are other risk factors. However, there may also be sociocultural reasons related to Turkish society, such as women's perception of sexuality, behaviors such as tending to conceal their problems and feeling ashamed, and reluctance to discuss sexuality-related problems[6]. The wide variation in the prevalence of female SD observed in various studies may be due to differences in the characteristics of the sample groups other than diabetes, the different sociocultural environments in which the studies were conducted, or the use of different measurement tools to evaluate sexual function.

The symptoms of diabetes-related SD are complicated but generally include conditions such as reduced or absent interest, sexual desire, and arousal, a decrease in lubrication and consequent dyspareunia, and difficulty or inability to achieve orgasm[21]. Although various rates have been reported in the literature, diabetes can impact all stages of female sexual function to varying degrees. In the Turkish study conducted by Doruk et al.[5], women with diabetes showed significant decreases in sexual desire, arousal, and lubrication compared to the control group. The most frequently affected domains in women with type 1 diabetes were desire (85%), arousal (76%), orgasm (66%), pain (61%), satisfaction (61%), and lubrication (57%). Enzlin et al.[4] reported SD in the area of desire in 17%, lubrication in 14%, orgasm in 14%, and pain in 12% of women with

type 1 diabetes. In a study by Basson et al.[21], problems with lubrication were reported by 40.2% of women with type 1 diabetes and 34.0% of controls, while dyspareunia was reported by 31.5 and 26.12%, respectively. In contrast, another study of women with type 1 diabetes showed that diabetes affected arousal, lubrication, satisfaction, orgasm, and pain, but not sexual desire[22]. Mazzilli et al.[20] found that women with type 1 diabetes more frequently reported problems with arousal, lubrication, dyspareunia, and orgasm compared to controls, whereas desire was significantly lower in both type 1 and 2 diabetes compared to the control group. Nowosielski et al.[23] determined that women without diabetes experienced more sexual desire, arousal, and orgasm than women with type 1 diabetes. In our study, mean scores for sexual desire, arousal, and satisfaction were significantly lower in women with type 1 diabetes compared to women without diabetes, while there were no significant differences between the two groups in the areas of orgasm and pain. The discrepancies in these results may be related to many biological, physiological, psychological, cultural, and personal reasons, as well as the impact of women's perception of sexuality.

The frequency of SD was significantly associated with the duration of diabetes, HbA1c level, and BMI in this study ($p<0.05$). Maiorino[3] reported a significant association between HbA1c, BMI and diabetes duration, consistent with our study. Interestingly, Maiorino's study was conducted in a much younger population than our study. Similarly, Abu Ali et al.[24] compared women with and without diabetes and determined that the incidence of SD increased with higher BMI and diabetes duration in women with diabetes. Contrary to our findings, in the current systematic review and meta-analysis of Murgel et al., it was determined that there was no relationship between BMI, hirsutism, and SD in women with polycystic ovary syndrome[25]. Contradictions between the results of the study may be due to the fact that sexuality is complex by nature and is affected by individual characteristics. For example, in the study of Lerner et al., women with hypoactive sexual desire were found to have predominant depressive and anxious moods. These women avoided perceiving themselves as sexual and tended to negatively evaluate their capacity to have sexual intercourse with their partners[26]. As a result, since sexuality is multidimensional, it may not be correct to explain the event with a limited number of variables. SD in women with diabetes may be affected by both physiological and psychological factors. In addition, as the time spent with diabetes increases, additional problems caused by diabetes may increase SD.

Study limitations

The results of this study cannot be generalized because it was conducted with individuals who presented to a single hospital within a specific time frame and agreed to participate in the study. In addition, important variables that may affect sexuality such as women's mental states, depression and anxiety, childhood traumas, and erectile dysfunction of women's spouses were not discussed in this study.

CONCLUSION

This study underlined that SD is higher in women affected by type 1 diabetes than in healthy controls. This could be due to the duration of diabetes, HbA1c, and body mass index. However, it may not be correct to explain sexual function only with these variables. Many variables should be considered when evaluating patients with type 1 diabetes in terms of sexual health.

AUTHORS' CONTRIBUTIONS

SC: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **MDB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MK:** Writing – original draft, Writing – review & editing.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes. Classification and diagnosis of diabetes. Diabetes Care. 2022;45(Suppl. 1):17-38. https://doi.org/10.2337/dc22-S002
2. International Diabetes Federation. Diabetes atlas. 10th ed; 2021 [cited on 2022 August, 18]. Available from: https://diabetesatlas.org/atlas/tenth-edition/
3. Arrellano-Valdez F, Urrutia-Osorio M, Arroyo C, Soto-Vega E. A comprehensive review of urologic complications in patients with diabetes. Springerplus. 2014;3:549. https://doi.org/10.1186/2193-1801-3-549
4. Enzlin P, Mathieu C, Van den Bruel A, Bosteels, J, Vanderschueren D, Demyttenaere K. Sexual dysfunction in women with type 1 diabetes: a controlled study. Diabetes Care. 2002;25(4):672-77. https://doi.org/10.2337/diacare.25.4.672
5. Doruk H, Akbay E, Cayan S, Bozlu M, Acar D. Effect of diabetes mellitus on female sexual function and risk factors. Arch Androl. 2005;1(1):1-6. https://doi.org/10.1080/014850190512798
6. Maiorino MI, Bellastella G, Esposito K. Diabetes and sexual dysfunction: current perspectives. Diab Metab Syndr Obes. 2014;6(7):95-105. https://doi.org/10.2147/DMSO.S36455
7. Flotynska J, Uruska A, Michalska A, Araszkiewicz A, Zozulinska-Ziolkiewicz D. Sexual dysfunction is a more common problem in young women with type 1 diabetes than in healthy women. J Sex Marital Ther. 2019;45(7):643-51. https://doi.org/10.1080/0092623X.2019.1610121

8. Corona G, Isidori AM, Aversa A, Bonomi M, Ferlin A, Foresta C, et al. Male and female sexual dysfunction in diabetic subjects: focus on new antihyperglycemic drugs. Rev Endocr Metab Disord. 2020;21(1):57-65. https://doi.org/10.1007/s11154-019-09535-7
9. Kizilay F, Gali HE, Serefoglu EC. Diabetes and sexuality. Sex Med Rev. 2017;5(1):45-51. https://doi.org/10.1016/j.sxmr.2016.07.002
10. Rutherford D, Collier A. Sexual dysfunction in women with diabetes mellitus. Gynecol Endocrinol. 2005;21(4):189-92. https://doi.org/10.1080/09513590400021110
11. Meeking D, Fosbury JA, Cummings MH. Sexual dysfunction and sexual health concerns in women with diabetes. Pract Diabetes. 2013;30(8):327-31. https://doi.org/10.1002/pdi.1805
12. Rosen R, Brown C, Heiman J. The female sexual function index (FSFI): a multidimensional selfreport instrument for the assessment of female sexual function. J Sex Marital Ther. 2000;26(2):191-208. https://doi.org/10.1080/009262300278597
13. Aygin D, Aslan FE. The Turkish adaptation of the female sexual function index. Turkiye Klinikleri J Med Sci. 2005;25(3):393-99. Available from: https://www.researchgate.net/publication/283149104_The_Turkish_adaptation_of_the_Female_Sexual_Function_Index#:~:text=The%2019%2Dquestion%20Female%20Sexual,pain%20%5B12%2C%2013%5D%20
14. Pontiroli AE, Cortelazzi D, Morabito A. Female sexual dysfunction and diabetes: a systematic review and meta-analysis. J Sex Med. 2013;10(4):1044-51. https://doi.org/10.1111/jsm.12065
15. Bak E, Marcisz C, Krzeminska S, Dobrzyn-Matusiak D, Foltyn A, Drosdzol-Cop A. Diabetes modify sexuality and mood of women and men?. Int J Environ Res Public Health. 2018;15(5):958. https://doi.org/10.3390/ijerph15050958)
16. Ahmed MR, Shaaban MM, Sedik WF, Mohamed TY. Prevalence and differences between type 1 and type 2 diabetes mellitus regarding female sexual dysfunction: a cross-sectional Egyptian study. BMC Women's Health. 2020;20:73:176-81. https://doi.org/10.1080/0167482X.2017.1318123
17. Zamponi V, Mazzilli RZ, Bitterman O, Olana S, Loria C, Festa C, et al. Association between type 1 diabetes and female sexual dysfunction. BMC Women's Health. 2020;20(1):73. https://doi.org/10.1186/s12905-020-00939-1
18. Enzlin P, Rosen R, Wiegel M, Brown J, Wessells H, Gatcomb P, et al. Sexual dysfunction in women with type 1 diabetes. Long-term findings from the DCCT/EDIC study cohort. Diabetes Care. 2009;32(5):780-85. https://doi.org/10.2337/dc08-1164
19. Celik S, Golbası Z, Kelleci M, Satman I. Sexual dysfunction and sexual quality of life in women with diabetes: the study based on a diabetic center. Sex Disabil. 2015;33:233-41. https://doi.org/10.1007/s11195-014-9383-3
20. Mazzilli R, Imbrogno N, Elia J, Delfino M, Bitterman O, et al. Sexual dysfunction in diabetic women: prevalence and differences in type 1 and type 2 diabetes mellitus. Diabetes Metab Syndr Obes. 2015;8:97-101. https://doi.org/10.2147/DMSO.S71376
21. Basson RJ, Rucker BM, Laird PG, Conry R. Sexuality of women with diabetes. J Sex Reprod Med. 2001;1(1):11-20. Available from: https://www.pulsus.com/scholarly-articles/sexuality-of-women-with-diabetes.pdf
22. Salonia A, Lanzi R, Scavini M, Pontillo M, Gatti E, Petrella G, et al. Sexual function and endocrine profile in fertile women with type 1 diabetes. Diabetes Care. 2006;29(2):312-16. https://doi.org/10.2337/diacare.29.02.06.dc05-1067
23. Nowosielski K, Drosdzol A, Sipiński A, Kowalczyk R, Skrzypulec V. Diabetes mellitus and sexuality-does it really matter?. J Sex Med. 2010;7(2 Pt 1):723-35. https://doi.org/10.1111/j.1743-6109.2009.01561.x
24. Abu Ali RM, Hajerı RMA, Khader YS, Shegem NS, Ajlouni KM. Sexual dysfunction in Jordanian diabetic women. Diabetes Care. 2008;31(8):1580-81. https://doi.org/10.2337/dc08-0081
25. Murgel ACF, Simões RS, Maciel GAR, Soares JM, Baracat EC. Sexual dysfunction in women with polycystic ovary syndrome: systematic review and meta-analysis. J Sex Med. 2019;16(4):542-50. https://doi.org/10.1016/j.jsxm.2019.01.313
26. Lerner T, Bagnoli VR, Pereyra EAG, Fonteles LP, Sorpreso ICE, Soares JM, et al. Cognitive-behavioral group therapy for women with hypoactive sexual desire: a pilot randomized study. Clinics (Sao Paulo). 2022;77:100054. https://doi.org/10.1016/j.clinsp.2022.100054. eCollection 2022

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220339

Preoperative evaluation of sarcopenia in patients with colorectal cancer: a prospective study

Giovana Domingues Nunes[1], Letícia Zumpano Cardenas[1], Thais Manfrinato Miola[1], Juliana de Oliveira Souza[1], Letícia Nascimento Carniatto[1], Almir Galvão Vieira Bitencourt[1*]

SUMMARY

OBJECTIVE: Colorectal cancer is the third most diagnosed malignant neoplasm in the world and the fourth leading cause of cancer mortality. The loss of muscle mass in oncological patients is the main aspect of cancer-related malnutrition. Associations between sarcopenia and poor outcomes, such as high postoperative mortality, chemotherapy toxicity, and reduced survival, have been recently described. The aim of this study was to prospectively assess the prevalence of preoperative sarcopenia in patients with colorectal cancer using validated methods to evaluate muscle strength, muscle mass, and physical performance.

METHODOLOGY: This study included patients with colorectal cancer undergoing oncological staging at a Cancer Center in Brazil from May 2019 to March 2020 who had images from abdominal computed tomography available for analysis of body composition. The muscle strength test, physical performance, referred fatigue, and clinical and nutritional data were evaluated.

RESULTS: A total of 31 patients were included, and most were diagnosed with colon cancer (77.4%) and clinical stage II in 41.9% of cases. The prevalence of probable sarcopenia was 22.6%; of these patients, sarcopenia was confirmed in 19.4%, and ultimately, 9.7% of the sample was classified as severe sarcopenia. We did not find a significant association between the presence of sarcopenia in our sample and age, sex, tumor staging, nutritional characteristics, referred patient fatigue, or postoperative complications.

CONCLUSION: Considering the criteria established by the EWGSOP, the prevalence of preoperative sarcopenia in colorectal cancer patients was 19.4%.

KEYWORDS: Sarcopenia. Colorectal neoplasms. Preoperative care. Computed tomography.

INTRODUCTION

Colorectal cancer is the third most diagnosed malignant neoplasm in the world and the fourth leading cause of cancer mortality. In cancer patients, cachexia and malnutrition are extremely important complications of clinical practice because of a variety of inherent tumor mechanisms, host response to the tumor, and oncological therapies[1].

Sarcopenia is directly responsible for functional impairment, increased risk of falls, loss of autonomy, reduced respiratory capacity, and reduced immunity[2]. The diagnostic criteria for sarcopenia include reduced muscle strength (criterion 1), low muscle quantity or quality (criterion 2), and poor physical performance (criterion 3). In addition, a classification into different stages is indicated: probable sarcopenia (criterion 1), sarcopenia (criteria 1 and 2), and severe sarcopenia (all criteria)[3].

The gold standard method to quantify muscle mass is a computed tomography (CT) scan to measure the skeletal muscle area at the L3 level and calculate the skeletal muscle index (SMI). Since abdominal CT is routinely performed in colorectal cancer patients for diagnosis, staging, and follow-up, it is suitable to use this method to measure muscle mass in this population[4,5].

Associations between sarcopenia and worse prognosis, such as high postoperative mortality, chemotherapy toxicity, reduced survival, higher infection rates, increased hospital length of stay, and increased mortality, have been pointed out recently[6-13]. Surgical resection in a patient with nonmetastatic colorectal cancer is an important aspect of cancer management, and including the evaluation of sarcopenia as a predictor of perioperative or postoperative morbidity risk can provide prognostic information for surgeons and patients. Thus, ideally, patients with colorectal cancer should be screened for sarcopenia from the beginning of their oncological treatment and be informed of its potential negative effects, highlighting the importance of sarcopenia prevention and treatment strategies[14].

The actual prevalence and impact of sarcopenia in this population are unknown, as most published studies classify sarcopenia solely by the presence of low muscle mass on CT, which may overestimate its prevalence. The aim of this study was to

[1]A.C. Camargo Cancer Center – São Paulo (SP), Brazil.
*Corresponding author: almir.bitencourt@accamargo.org.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 28, 2022. Accepted on October 30, 2022.

prospectively assess the prevalence of preoperative sarcopenia in patients with colorectal cancer using validated methods to evaluate muscle strength, muscle mass, and physical performance.

METHODS

This is a prospective, cross-sectional, single-center, Institutional Review Board-approved study evaluating patients with a diagnosis of colorectal cancer undergoing oncological staging at a cancer center from May 2019 to March 2020. All included patients were candidates for the institution's early recovery protocol after surgery and underwent a CT scan of the abdomen before the surgery. Patients with distant metastases (stage IV), those with previous cancer treatment or neoadjuvant therapy, who did not agree to participate in the study, who were unable to perform the muscle strength and/or physical performance test, those with physical deformities and reduced mobility, or who were bedridden were excluded.

The assessments of muscle strength, physical performance and fatigue, and nutritional status were carried out by the same physical therapist and a nutritionist, respectively, during the preoperative hospital stay. The Brief Fatigue Inventory (BFI) was used to classify the level of fatigue, which was considered mild from 1 to 3, moderate from 4 to 6, and severe from 7 to 10[15,16]. For the muscle strength test, the handgrip test was performed, obtained through manual dynamometry in kilograms (kg), using a manual hydraulic dynamometer brand Saehan®. The measure collected was from the dominant side. The reference values for identifying muscle weakness were below 16 and 27 kg for women and men, respectively[3,17].

The *short physical performance battery (SPPB)* was performed, and a better score represents a better physical performance of the patient. The highest score is 12 points, and values below 8 points are considered low physical performance[3].

The assessment of nutritional status was performed using the body mass index (BMI=weight/height²). The reference values that were used were the World Health Organization[18] for adults and the Pan American Health Organization (OPAS) for the elderly[19].

Muscle mass assessment was performed by the same radiologist. The analysis of CT images to obtain body composition data was performed using the OsiriX® software[4,20]. Axial CT images of the abdomen at the level of L3 were evaluated. To measure the skeletal muscle mass areas (skeletal muscles, including psoas, paravertebral, and abdominal wall muscles), a semiautomatic method with manual correction was used. To identify the skeletal musculature, a density of -29 to +150 Hounsfield units (HUs) was considered, and the SMI was calculated (skeletal muscle area [cm^2]/height [m]²), which is classified as low when the index is less than 55.4 cm^2/m^2 for men and 38.9 cm^2/m^2 for women[21].

The analysis of the presence of sarcopenia was carried out in accordance with the recommendation of the European Working Group on Sarcopenia (EWGSOP)[3]. Postoperative complications were assessed within 30 days after the procedure.

The information collected from the images and assessments was exported to a spreadsheet database using REDCap® Software. For data processing, the Statistical Packcage for Social Science (SPSS) software version 20 was used. Descriptive statistics parameters were used, adopting the usual measures of central tendency (average, median, and mode) and simple and relative frequency calculations. Statistical tests were used to correlate the variables, as indicated: for the correlation between the variables, the chi-square and Fisher's exact tests were used for frequencies of categorical variables; Student's *t*-test was used for continuous variables with normal distribution; and the Mann-Whitney test was used for continuous variables without normal distribution. The significance level adopted was 5%.

RESULTS

During the study period, 181 patients were eligible for the study, but 135 were excluded due to neoadjuvant treatment (n=46), previous treatment (n=30), and impossibility of performing evaluations before surgery (n=59). The remaining 46 individuals underwent preoperative evaluation; however, only 31 had CT images available for analysis and were included in the study.

The characteristics of the included patients are described in Table 1. Most of the patients were male (54.8%), with a mean age of 58 years. Most patients had cancer of the colon (77.4%), and clinical stage II was observed in 41.9% of the cases. Table 1 also shows the nutritional profile of the patients by BMI, muscle strength, physical performance, fatigue, and muscle mass. In the classification of BMI, most cases (41.9%) were classified as eutrophic, and no cases were classified as malnourished. Seven patients had low muscle strength (22.6%), and nine (29.0%) had low physical performance. Most patients had mild fatigue (41.9%), the mean skeletal muscle area was 137.5±33.3 cm^2 (75.2–231.0), and the SMI was 49.3±7.8 (35.3–68.0). Among the cases, 15 (48.4%) patients had a low SMI, and 16 (51.6%) had a normal SMI.

In Figure 1, the flowchart shows the classification of sarcopenia. The prevalence of probable sarcopenia was 22.6%, sarcopenia was confirmed in 19.4%, and 9.7% were classified as severe sarcopenia, while isolated low SMI on CT was observed in 48.4%.

Male patients had a higher rate of low SMI than female patients (p=0.018). There were no statistically significant differences between the presence of sarcopenia and low SMI with patient age, clinical staging, BMI, fatigue, or postoperative complications (Table 2). Postoperative complications were observed in 13 patients (41.9%); however, we did not observe a correlation between the presence of complications and the presence of sarcopenia or low SMI.

Table 1. Characteristics of the included patients (n=31).

Variable	Category	N (%)
BMI (kg/m²)	Min–Max	22.0–40.9
	Median	27.4
BMI (<60 years old)	Malnutrition Eutrophy Overweight Obesity	0 (0.0%) 10 (52.6%) 2 (10.5%) 7 (36.8%)
BMI (≥60 years old)	Malnutrition Eutrophy Overweight Obesity	2 (16.7%) 7 (58.3%) 3 (25.0%) 0 (0.0%)
Handgrip strength (kg)	Min–Max	14–52
	Average Average for women Average for men	28.4±9.6 20.9±4.2 34.7±8.3
SPPB	Min–Max Average	5–11 8.4±1.4
BFI	No fatigue Fatigue mild Fatigue moderate Fatigue severe	9 (29%) 13 (41.9%) 8 (25.8%) 1 (3.2%)
Muscle mass (cm²)	Min–Max Average	75.2–231.0 137.5±33.3
MMI (cm²/m²)	Min–Max Average	35.3–68.0 49.3±7.8

Min.: minimum; Max.: maximum; BMI: body mass index; SPPB: short physical performance battery; BFI: Brief Fatigue Inventory; MMI: muscle mass index.

DISCUSSION

Sarcopenia can be present in colorectal cancer patients at diagnosis, regardless of the presence of traditional nutrition risk factors[14]. The prevalence of sarcopenia in patients with colorectal cancer ranges from 11.9 to 60% in the literature. Miyamoto et al.[22] reported 25% sarcopenia in patients with stage I to stage III colorectal cancer; however, it is noteworthy that the authors used only low skeletal muscle mass as the definition of sarcopenia. Few studies present discussions about the need for an assessment of all aspects, as the consensus suggests[3]. The prevalence of sarcopenia in this study was 19.4% using the proper criteria, against 48.4% of patients with low SMI on CT.

Huang et al.[23] evaluated the impact of sarcopenia on postoperative outcomes in 142 recently operated colorectal cancer patients. They described that 17 patients (12%) were diagnosed with sarcopenia and concluded that including a functional aspect in addition to evaluating only skeletal muscle mass could result in a better prediction of postoperative complications. Nakanishi et al.[24] found a significant association between the higher prevalence of male colorectal cancer patients and the presence of sarcopenia, which was also observed in our results. Likewise, older age is associated with a greater chance of developing sarcopenia[25].

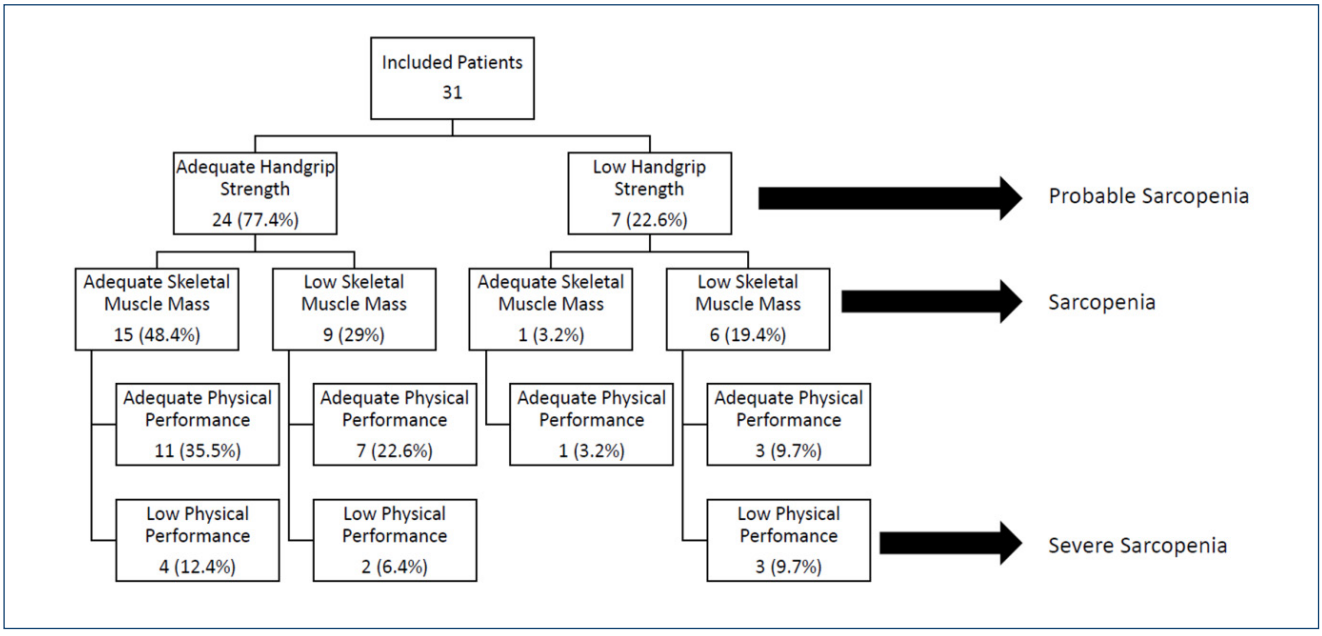

Figure 1. Flowchart of stages of sarcopenia for all patients included in this study.

Table 2. Correlation between the presence of sarcopenia/low muscle mass and evaluated variables.

Variables		Without sarcopenia	With sarcopenia	p	Adequate skeletal muscle mass	Low skeletal muscle mass	p
Gender	Female	12 (85.7%)	2 (14.3%)	0.664	11 (78.6%)	3 (21.4%)	0.018
	Male	13 (76.5%)	4 (23.5%)		5 (29.4%)	12 (70.6%)	
	All patients	25 (80.6%)	6 (19.4%)		16 (51.6%)	15 (48.4%)	
Stage	Stage I	9 (90.0%)	1 (10.0%)	0.439	7 (70.0%)	3 (30.0%)	0.388
	Stage II Stage III	11 (84.6%) 5 (62.5%)	2 (15.4%) 3 (37.5%)		5 (38.5%) 4 (50.0%)	8 (61.5%) 4 (50.0%)	
	All patients	25 (80.6%)	6 (19.4%)		16 (51.6%)	15 (48.4%)	
BMI	Malnutrition	2 (100.0%)	0 (0.0%)	0.157	1 (50.0%)	1 (50.0%)	0.209
	Eutrophy Overweight	11 (64.7%) 5 (100.0%)	6 (35.3%) 0 (0.0%)		6 (35.3%) 4 (80.0%)	11 (64.7%) 1 (20.0%)	
	Obesity	7 (100.0%)	0 (0.0%)		5 (71.4%)	2 (28.6%)	
	All patients	25 (80.6%)	6 (19.4%)		16 (51.6%)	15 (48.4%)	
Fatigue	Mild	11 (84.6%)	2 (15.4%)	0.888	7 (53.8%)	6 (46.2%)	0.849
	Moderate Severe	6 (75.0%) 1 (100.0%)	2 (25.0%) 0 (0.0%)		3 (37.5%) 1 (100.0%)	5 (62.5%) 0 (0.0%)	
	Without	7 (77.8%)	2 (22.2%)		5 (55.6%)	4 (44.4%)	
	Total	25 (80.6%)	6 (19.4%)		16 (51.6%)	15 (48.4%)	
Postoperative complications	Yes	12 (92.3%)	1 (7.7%)	0.359	6 (46.2%)	7 (53.8%)	0.879
	No	13 (72.2%)	5 (27.8%)		10 (55.6%)	8 (44.4%)	
	All patients	25 (80.6%)	6 (19.4%)		16 (51.6%)	15 (48.4%)	

Complications after surgical procedures in nonmetastatic colorectal cancer have been related to the presence of low muscle mass and sarcopenia in some studies. In our sample, 53.8% of patients with low muscle mass had postsurgical complications, compared to 44.4% of patients with normal muscle mass; however, this difference was not statistically significant. In other studies, postsurgical complications were observed in 32.8 to 60% of patients[26,27] with low skeletal muscle mass and were pointed out as an independent predictor of worse overall survival.

Souza et al.[28] evaluated sarcopenia in patients with colorectal cancer and observed a rate of 15% of patients with sarcopenia; among them, most patients were overweight and obese. In this study, most patients were eutrophic at the time of preoperative assessment, including patients with sarcopenia (35.5%) and patients with low muscle mass (64.7%).

Wang et al.[29] analyzed cancer-related fatigue reported in 187 stage III and stage IV patients with cancers at various sites. By using the BFI instrument, this study showed fatigue in 33.7% of the sarcopenic group, and the average score found on the BFI scale was 2.9. It was concluded that fatigue may result in changes in skeletal muscle, resulting in a feeling of tiredness, general weakness, and lack of energy.

On the contrary, in our results, we observed that moderate fatigue was more prevalent both in the population with diagnosed sarcopenia (25.0%) and in patients with isolated low muscle mass (62.5%). Few studies[30] performed a physical performance test in the population with cancer and advanced age, which makes it difficult to identify complications and compare them with frailty. The average score found in our population not testing physical performance (SPPB) was 8.4±1.4, taking into account that below 8 points on the scale, there is low physical performance. Nevertheless, we observed that severe sarcopenia, which in its classification adds poor physical performance, had a prevalence of 9.7% of the collected sample.

Some limitations of this study can be pointed out, such as the limited sample size, which may have impaired the statistical analysis performed. The limited sample was due to different reasons, including the logistical difficulty of performing all the necessary preoperative assessments, without compromising the therapeutic schedule, the nonavailability of CT images in some cases, and, finally, the limitations related to the COVID-19 pandemic during the final period of data collection. In this study, we only evaluated muscle quantity through the measurement of the muscle mass area by CT; however, we did not assess muscle

quality. Schneider et al.[31] recently showed that assessment of muscle quality through the measurement of muscle attenuation on CT can be a better predictor of adverse outcomes than muscle quantity, and it should be considered in future studies.

CONCLUSION

Our study showed that the analysis of muscle mass alone on CT may overestimate the prevalence of sarcopenia in preoperative colorectal patients. Considering the criteria established by the EWGSOP, the prevalence of preoperative sarcopenia in our study was 19.4%, against 48.4% if we consider only low muscle mass on CT. We did not find a significant association between the presence of sarcopenia and age, gender, tumor staging, nutritional characteristics, patient self-reported fatigue, or postoperative complications; however, the small sample size may have limited these analyses. As recommendations for future investigations, we emphasize the importance of reiterating the need to follow the updated sarcopenia consensus for sarcopenia assessment to determine the real impact of the three assessment pillars (muscle strength, muscle mass, and physical performance) in oncological patients.

AUTHORS' CONTRIBUTIONS

GDN: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **LZC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TMM:** Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AGVB:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **JOS:** Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **LNC:** Writing – original draft.

REFERENCES

1. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol. 2011;12(5):489-95. https://doi.org/10.1016/S1470-2045(10)70218-7
2. Dias APTP, Gollner AM, Teixeira MTB. Coloretal cancer-screening, prevention and control. HU Rev. 2007;33(4):125-31.
3. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Aging. 2019;48(1):16-31. https://doi.org/10.1093/aging/afy169
4. Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab. 2008;33(5):997-1006. https://doi.org/10.1139/H08-075
5. Baracos V, Kazemi-Bajestani SMR.Clinical outcomes related to muscle mass in humans with cancer and catabolic illnesses. Int J Biochem Cell Bio. 2013;45(10):2302-8. https://doi.org/10.1016/j.biocel.2013.06.016
6. Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol. 2013;31(12):1539-47. https://doi.org/10.1200/JCO.2012.45.2722
7. Vergara-Fernandez O, Trejo-Avila M, Salgado-Nesme N. Sarcopenia in patients with colorectal cancer: a comprehensive review. World J Clin Cases. 2020;8(7):1188-202. https://doi.org/10.12998/wjcc.v8.i7.1188
8. Golder AM, Sin LKE, Alani F, Alasadi A, Dolan R, Mansouri D, et al. The relationship between the mode of presentation, CT-derived body composition, systemic inflammatory grade and survival in colon cancer. J Cachexia Sarcopenia Muscle. 2022;13(6): 2863-74. https://doi.org/10.1002/jcsm.13097
9. Sato R, Oikawa M, Kakita T, Okada T, Abe T, Yazawa T, et al. Impact of sarcopenia on postoperative complications in obstructive colorectal cancer patients who received stenting as a bridge to curative surgery. J Anus Rectum Colon. 2022;6(1):40-51. https://doi.org/10.23922/jarc.2021-057
10. Fleming CA, O'Connell EP, Kavanagh RG, O'Leary DP, Twomey M, Corrigan MA, et al. Body composition, inflammation, and 5-year outcomes in colon cancer. JAMA Netw Open. 2021;4(8):e2115274. https://doi.org/10.1001/jamanetworkopen.2021.15274
11. Drami I, Pring ET, Gould L, Malietzis G, Naghibi M, Athanasiou T, et al. Body composition and dose-limiting toxicity in colorectal cancer chemotherapy treatment; a systematic review of the literature. Could muscle mass be the new body surface area in chemotherapy dosing? Clin Oncol (R Coll Radiol). 2021;33(12):e540-52. https://doi.org/10.1016/j.clon.2021.05.011
12. Chai VW, Chia M, Cocco A, Bhamidipaty M, D'Souza B. Sarcopenia is a strong predictive factor of clinical and oncological outcomes following curative colorectal cancer resection. ANZ J Surg. 2021;91(5):E292-7. https://doi.org/10.1111/ans.16706
13. Olmez T, Karakose E, Bozkurt H, Pence HH, Gulmez S, Aray E, et al. Sarcopenia is associated with increased severe postoperative complications after colon cancer surgery. Arch Med Sci. 2019;17(2):361-7. https://doi.org/10.5114/aoms.2019.88621
14. Klassen P, Baracos V, Gramlich L, Nelson G, Mazurak V, Martin L. Computed-tomography body composition analysis complements pre-operative nutrition screening in colorectal cancer patients on an enhanced recovery after surgery pathway. Nutrients. 2020;12(12):3745. https://doi.org/10.3390/nu12123745
15. Mendoza TR, Wang XS, Cleeland CS, Morrissey M, Johnson BA, Wendt JK, et al. The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. Cancer. 1999;85(5):1186-96. https://doi.org/10.1002/(sici)1097-0142(19990301)85:5<1186::aid-cncr24>3.0.co;2-n

16. Nunes AF, Bezerra CO, Custódio JDS, Friedrich CF, de Oliveira IS, Lunardi AC. Clinimetric properties of the Brief Fatigue Inventory applied to oncological patients hospitalized for chemotherapy. J Pain Symptom Manage. 2019;57(2):297-303. https://doi.org/10.1016/j.jpainsymman.2018.10.508

17. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: toward a standardized approach. Age Aging. 2011;40(4):423-9. https://doi.org/10.1093/aging/afr051

18. World Health Organization. Obesity: preventing and managing the global epidemic of obesity. Report of the WHO Consultation of Obesity. Geneva: Switzerland; 1997. Available from: https://apps.who.int/iris/handle/10665/63854

19. Organización Panamericana de La Salud. División de Promoción y Protección de La Salud (HPP). Encuesta Multicentricasalud beinestar y envejecimiento (SABE) em América Latina el Caribe: Informe Preliminar [Internet]. In: XXXVI Reunióndel Comité asesor de investigaciones em Salud; 9-11 June 2001; Kingston, Jamaica: OPAS, 2002.

20. Doyle SL, Bennett AM, Donohoe CL, Mongan AM, Howard JM, Lithander FE, et al. Establishing computed tomography-defined visceral fat area thresholds for use in obesity related cancer research. Nutr Res. 2013;33(3):171-9. https://doi.org/10.1016/j.nutres.2012.12.007

21. Prado CMM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumors of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol. 2008;9(7):629-35. https://doi.org/10.1016/S1470-2045(08)70153-0

22. Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J, et al. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Ann Surg Oncol. 2015;22(8):2663-8. https://doi.org/10.1245/s10434-014-4281-6

23. Huang D-D, Wang S-L, Zhuang C-L, Zheng B-S, Lu J-X, Chen F-F, et al. Sarcopenia, as defined by low muscle mass, strength and physical performance, predicts complications after surgery for colorectal cancer. Colorectal Dis. 2015;17(11):O256-64. https://doi.org/10.1111/codi.13067

24. Nakanishi R, Oki E, Sasaki S, Hirose K, Jogo T, Edahiro K, et al. Sarcopenia is an independent predictor of complications after colorectal cancer surgery. Surg Today. 2018;48(2):151-7. https://doi.org/10.1007/s00595-017-1564-0

25. Xiao J, Caan BJ, Cespedes Feliciano EM, Meyerhardt JA, Kroenke CH, Baracos VE, et al. The association of medical and demographic characteristics with sarcopenia and low muscle radiodensity in pa-tients with nonmetastatic colorectal cancer. Am J Clin Nutr. 2019;109(3):615-5. https://doi.org/10.1093/ajcn/nqy328

26. Boer BC, deGraaff F, Brusse-Keizer M, Bouman DE, Slump CH, Slee-Valentijn M, et al. Skeletal muscle mass and quality as risk factors for postoperative outcome after open colon resection for cancer. Int J Colorectal Dis. 2016;31(6):1117-24. https://doi.org/10.1007/s00384-016-2538-1

27. Xiao J, Caan BJ, Cespedes Feliciano EM, Meyerhardt JA, Peng PD, Baracos VE, et al. Association of low muscle mass and low muscle radiodensity with morbidity and mortality for colon cancer surgery. JAMA Surg. 2020;155(10):942-9. https://doi.org/10.1001/jamasurg.2020.2497

28. de Souza BU, Souza NCS, Martucci RB, Rodrigues VD, de Pinho NB, Gonzalez MC, et al. Factors associated with sarcopenia in patients with colorectal cancer. Nutr Cancer. 2018;70(2):176-83. https://doi.org/10.1080/01635581.2018.1412480

29. Wang B, Thapa S, Zhou T, Liu H, Li L, Peng G, et al. Cancer-related fatigue and biochemical parameters among cancer patients with different stages of sarcopenia. Support Care Cancer. 2020;28(2):581-8. https://doi.org/10.1007/s00520-019-04717-0

30. Pamoukdjian F, Paillaud E, Zelek L, Laurent M, Lévy V, Landre T, et al. Measurement of gait speed in older adults to identify complications associated with frailty: a systematic review. J Geriatr Oncol. 2015;6(6):484-96. https://doi.org/10.1016/j.jgo.2015.08.006

31. Schneider M, Hübner M, Becce F, Koerfer J, Collinot J-A, Demartines N, et al. Sarcopenia and major complications in patients undergoing oncologic colon surgery. J Cachexia Sarcopenia Muscle. 2021;12(6):1757-63. https://doi.org/10.1002/jcsm.12771

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220865

Effect of dexmedetomidine on tourniquet-induced skeletal muscle injury

Wenjie Cheng[1], Zhe Wu[1], Jizheng Zhang[1], Wanlu Ren[1]*

SUMMARY

OBJECTIVE: The aim of this study was to investigate whether dexmedetomidine could reduce tourniquet-induced skeletal muscle injury.

METHODS: C57BL6 male mice were randomly assigned to sham, ischemia/reperfusion, and dexmedetomidine groups. Mice in the ischemia/reperfusion and dexmedetomidine groups received normal saline solution and dexmedetomidine intraperitoneally, respectively. The sham group underwent the same procedure as the ischemia/reperfusion group, with the exception of tourniquet application. Subsequently, the ultrastructure of the gastrocnemius muscle was observed, and its contractile force was examined. In addition, Toll-like receptor 4 and nuclear factor-κB expression within muscles was detected by Western blot.

RESULTS: Dexmedetomidine alleviated myocyte damage and increased the contractility of skeletal muscles. Moreover, dexmedetomidine significantly inhibited the expression of Toll-like receptor 4/nuclear factor-κB in the gastrocnemius muscle.

CONCLUSION: Taken together, these results demonstrate that dexmedetomidine administration attenuated tourniquet-induced structural and functional impairment of the skeletal muscle, partly through inactivation of the Toll-like receptor 4/nuclear factor-κB pathway.

KEYWORDS: Dexmedetomidine. Tourniquets. Muscle, skeletal. Surgery.

INTRODUCTION

Tourniquet placement is a universal technique used to create a bloodless operating field[1]. However, tourniquet application leads to significant acute limb ischemia and reperfusion (I/R) injury, which is characterized by inflammation, tissue edema, muscle necrosis, and microvascular perfusion deficits[2,3]. Accordingly, these complications greatly limit tourniquet use[4]. Skeletal muscle injury plays a pivotal role in the tissue response to acute limb I/R[5]. Thus, improving the survival of skeletal myocytes in surgeries requiring tourniquet application would enhance patient recovery.

Toll-like receptors (TLRs), which are ubiquitous in nature, activate the innate immune system in response to pathogens, stressors, and/or cytokines[6]. Binding of a ligand to Toll-like receptor 4 (TLR4), a TLR subtype, leads to activation of nuclear factor (NF)-κB, thereby triggering the transcription of many pro-inflammatory genes[7].

Dexmedetomidine (Dex) is an agent with sedative, anxiolytic, and analgesic effects, which has been applied for surgical patients as an adjuvant anesthetic. Research has demonstrated that Dex elicits a protective effect against I/R injury in multiple organs through antioxidant and anti-inflammatory mechanisms[8,9]. However, to date, no study has evaluated the effects of Dex on tourniquet-induced I/R injury.

Therefore, we investigated whether Dex could attenuate the structural and functional impairment of skeletal muscle in a mouse skeletal I/R injury model and explored the involvement of the TLR4 pathway in the underlying molecular mechanism.

METHODS

Animal experiments were approved by the Animal Care and Use Committee of the Tianjin Medical University and performed in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals. Two sets of C57BL6 male mice (aged 12–13 weeks) were subjected to 3 h of unilateral hind limb tourniquet ischemia, followed by 24 h of reperfusion (I/R), as previously described[1]. For formal experiments, mice were randomly assigned to sham, I/R, and Dex groups (n=16 mice/group) and anesthetized by an intraperitoneal injection of 80 mg/kg pentobarbital sodium. Dex was diluted in 0.9% NaCl. Mice in the I/R and Dex groups received normal saline solution and 9.6 μg/kg Dex intraperitoneally (i.p.), respectively, 30 min before ischemia. Mice in the Dex group were continuously infused with dexmedetomidine (6 μg/kg/h) throughout the 3-h ischemia using an electronic micropump (KD Scientific, Holliston, MA, USA), while mice in the I/R group received an equal amount of 0.9% saline instead. Mice in

[1]Tianjin Hospital, Department of Anesthesiology – Tianjin, China.
*Corresponding author: renwanlu@hotmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 15, 2022. Accepted on October 25, 2022.

the sham group underwent the same procedure as those in the I/R group, except for the application of the orthodontic rubber band. All terminal experiments were performed on mice after 24 h of reperfusion.

Electron microscopy analysis of skeletal muscle

The ultrastructure of gastrocnemius muscles was observed by electron microscopy (EM) analysis. Skeletal muscle tissues were removed from mice in each group, cut into 1–3 mm pieces, and processed in accordance with a previous method described by Yang et al.[10]. Subsequently, sections were stained with uranyl acetate and lead citrate (Sigma-Aldrich, St. Louis, MO, USA) and observed using an H-7500/STEM EM (Hitachi, Tokyo, Japan) at 5,000× magnification.

Detection of gastrocnemius muscle contractile force

The contractile force of gastrocnemius muscles was measured in all experimental groups. Under anesthesia (80 mg/kg pentobarbital sodium, i.p.), the left gastrocnemius muscles were quickly removed and rinsed with ice-cold modified Krebs-Henseleit (K-H) solution containing (in mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4 \bullet 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, and HEPES 10 (pH 7.4±0.05, gassed with 95% O_2 and 5% CO_2). One end of the gastrocnemius muscle was fixed onto the bottom of a small chamber by a micropin. The other end was connected to a force transducer (AD Instruments, Dunedin, NZ, USA). Stimuli were delivered through a bipolar electrode placed in the chamber and connected to the stimulator. The gastrocnemius muscle was continuously perfused with K-H solution at a rate of 15 mL/min at 37°C for at least 1 h before experiments. Individual twitch contractions of the gastrocnemius muscle were induced by stimulation (5 V, 1 Hz, 1-ms pulse). PowerLab Data Acquisition Systems with LabChart 7 (AD Instruments) was used to record and analyze muscle contractions.

Protein expression of toll-like receptor 4 and nuclear factor-κB in gastrocnemius muscles

After 24 h of reperfusion, gastrocnemius muscles from six mice in the sham, I/R, and Dex groups were rapidly collected and stored at -80°C until analysis. Subsequently, tissues were dissected and lysed by RIPA Lysis Buffer (Santa Cruz, Dallas, TX, USA) for homogenization, and the resulting tissue homogenates were centrifuged at 12,000×g for 20 min at 4°C. Total protein concentrations in the centrifuged supernatants were determined with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal volumes of loading buffer were added into the protein samples, which were mixed and boiled for 10 min at 95°C, and then separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein samples were then transferred onto a polyvinylidene difluoride (PVDF) membrane at 200 mA for 3 h. After blocking the membrane with 5% non-fat milk for 1 h, membranes were cut into strips according to their molecular weight and probed overnight at 4°C with a rabbit anti-TLR4 antibody (Abcam, Cambridge, UK) or a rabbit anti-NF-κB antibody (Abcam). After washing membranes with Tris-buffered saline with Tween (TBST), they were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Thermo Fisher Scientific) for 2 h at room temperature. β-Actin (Cell Signaling Technology, Beverly, MA, USA) served as the control. Finally, the PVDF membrane was washed with TBST three times for 15 min each. Specific immunoreactivity was visualized with an enhanced chemiluminescence substrate (Thermo Fisher Scientific), and specific bands were scanned for analysis using ImageJ (http://imagej.nih.gov).

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc, IL, USA). Data were expressed as mean±standard deviation (SD). Continuous variables were compared using the Student's t-test. For multi-group comparisons, one-way analysis of variance (ANOVA) with the Bonferroni post hoc test was used to determine statistical significance. Statistical significance was considered at $p<0.05$.

RESULTS

Subcellular structural changes of gastrocnemius muscle cells

As shown in Figure 1, mice in the sham group displayed normal muscle cell morphology, whereas mice in the I/R group had incomplete membranes with disordered myofilaments and numerous severely swollen mitochondria with disrupted cristae that clustered under myolemma. In addition, swollen sarcoplasmic reticula were observed in gastrocnemius muscle cells of mice in the I/R group. Compared with the I/R group, subcellular characteristics of muscle cells in the Dex group displayed slight disruptions in the intercellular matrix, mild variation in form and size of mitochondria, and mild swelling in mitochondria with several vesicular cristae. Moreover, the number of mitochondria adjacent to the sarcolemma was higher in the Dex group compared with the I/R group.

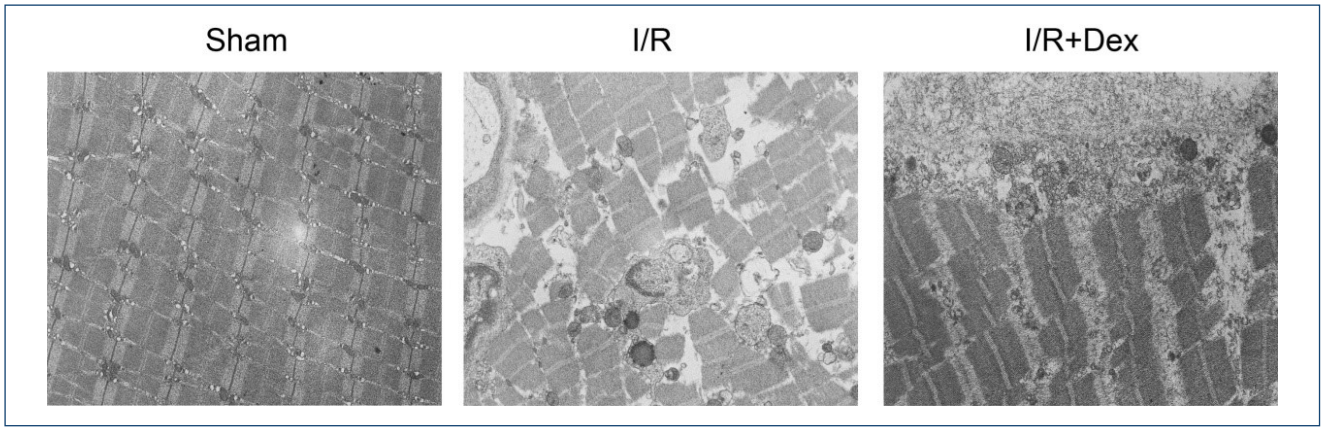

Figure 1. Electron microscopy images of gastrocnemius muscle tissues. Scale bar=20 μm, 5,000× magnification. I/R: ischemia-reperfusion; Dex: dexmedetomidine (n=5 per group).

Contractility of gastrocnemius muscles

As shown in Figure 2, compared with the sham group, gastrocnemius muscle contractility was significantly lower in the I/R group ($p<0.05$). In addition, Dex significantly increased the contractility of muscle tissues compared with the I/R group ($p<0.05$).

Expression of toll-like receptor 4 and nuclear factor-κB in gastrocnemius muscle tissues

As shown in Figure 3, the expression of TLR4 and NF-κB was maintained at a low level in the sham group. However, after 3 h of tourniquet-induced ischemia and 24 h of reperfusion, protein levels of TLR4 and NF-κB were significantly elevated in gastrocnemius muscle tissues ($p<0.05$). Dex pretreatment could observably decrease TLR4 and NF-κB overexpression compared with the I/R group ($p<0.05$).

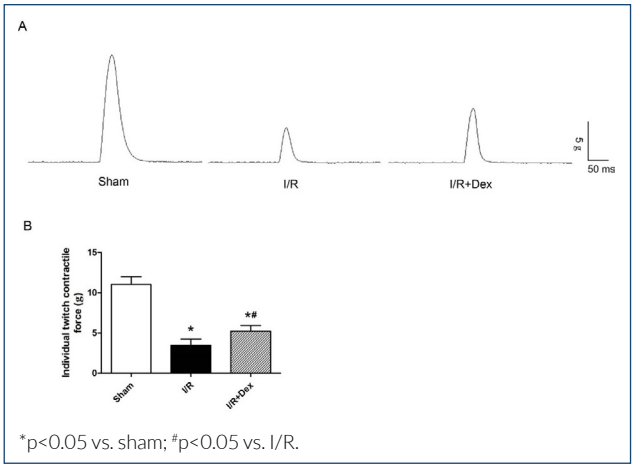

Figure 2. Gastrocnemius muscle contractile forces of experimental groups. **(A)** Individual twitch contraction of the gastrocnemius muscle. **(B)** Quantitative analysis of individual twitch contractile force in each group. I/R: ischemia-reperfusion; Dex: dexmedetomidine. Data are expressed as mean±standard deviation (n=6 per group).

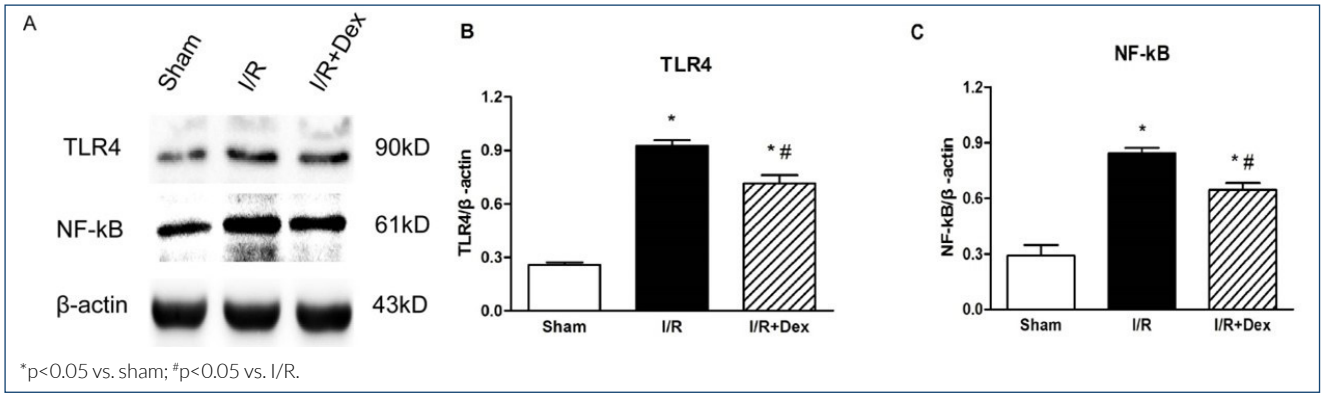

Figure 3. Expression of toll-like receptor 4 and nuclear factor-κB in gastrocnemius muscle tissues detected by western blot. **(A)** Representative band images of toll-like receptor 4 and nuclear factor-κB. **(B,C)** Summary data for expression of toll-like receptor 4 and nuclear factor-κB. I/R: ischemia-reperfusion; Dex: dexmedetomidine. Data are expressed as mean±standard deviation (n=6 per group).

DISCUSSION

Although tourniquets play an important role in vascular and orthopedic surgeries, several local and systemic complications are associated with the use of this device[11]. Prolonged limb tourniquet application and the subsequent restoration of blood flow result in local inflammation in ischemic skeletal muscle, leading to a complex cytokine cascade associated with secondary remote organ damage[12].

Accumulating evidence in animal models demonstrates that Dex has a remarkable protective effect against I/R-induced organ injury[8,9]. Given the prominent role of skeletal muscle injury in tissue responses to limb I/R[5], we hypothesized that Dex could mitigate the severity of skeletal muscle damage. Our EM analysis shows that tourniquet-induced I/R caused morphological damage and leukocyte infiltration into skeletal muscles. However, Dex preconditioning in the setting of limb I/R could reduce both gastrocnemius muscle injury and inflammatory cell infiltration.

Activation of TLRs can trigger the release of a series of inflammatory factors; therefore, blocking TLR activation may be an ideal approach to attenuate tissue damage[7]. TLR4 can activate NF-κB signaling, initiating the transcription of many pro-inflammatory genes[13,14]. Consistent with a previous report, we observed significantly higher expression of TLR4 and NF-κB in the I/R group compared with the sham group[15], indicating an important role for TLR4/NF-κB pathway activation in the pathophysiology of skeletal muscle I/R injury. Moreover, levels of TLR4 and NF-κB in skeletal muscle were significantly lower in mice treated with Dex compared with untreated mice after I/R. Collectively, these results demonstrate that Dex pretreatment inhibited the activation of TLR4 and NF-κB induced by I/R injury. It should be noted that other mechanisms, such as reactive oxygen species (ROS) and miRNA, are involved in skeletal muscle and cerebral I/R injury[16,17]. ROS have crucial roles in both the protective mechanisms and pathogeneses of ischemic preconditioning and postconditioning, whether being come to age or ripening[18]. Indeed, excessive ROS production during the reperfusion phase paves the way for tissue injury. Of the many targets for treatment of I/R injury, attenuation of ROS represents a major mechanism in the early minutes of reperfusion. Future studies are needed to clarify the roles of ROS and miRNA in the protective effect of Dex against I/R injury.

There are some limitations to this study. First, we only investigated a single intraperitoneal dose of Dex. Second, we did not explore the prolonged benefits of Dex, which warrants further investigation.

CONCLUSION

Our findings demonstrate that Dex administration, partly through inactivation of the TLR4/NF-κB pathway, attenuated tourniquet-induced structural and functional impairment of skeletal muscle.

AUTHORS' CONTRIBUTIONS

WC: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **ZW:** Data curation, Formal Analysis, Software. **JZ:** Investigation, Validation. **WR:** Project administration, Supervision, Writing – review & editing.

REFERENCES

1. Corrick RM, Tu H, Zhang D, Barksdale AN, Muelleman RL, Wadman MC, et al. Dexamethasone protects against tourniquet-induced acute ischemia-reperfusion injury in mouse hindlimb. Front Physiol. 2018;9:244. https://doi.org/10.3389/fphys.2018.00244
2. Blaisdell FW. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. Cardiovasc Surg. 2002;10(6):620-30. https://doi.org/10.1016/s0967-2109(02)00070-4
3. Cheng W-J, Liu X, Zhang L, Guo X-Q, Wang F-W, Zhang Y, et al. Chronic intermittent hypobaric hypoxia attenuates skeletal muscle ischemia-reperfusion injury in mice. Life Sci. 2019;231:116533. https://doi.org/10.1016/j.lfs.2019.06.008
4. Kue RC, Temin ES, Weiner SG, Gates J, Coleman MH, Fisher J, et al. Tourniquet use in a civilian emergency medical services setting: a descriptive analysis of the Boston EMS Experience. Prehosp Emerg Care. 2015;19(3):399-404. https://doi.org/10.3109/10903127.2014.995842
5. Wilson H-MP, Welikson RE, Luo J, Kean TJ, Cao B, Dennis JE, et al. Can cytoprotective cobalt protoporphyrin protect skeletal muscle and muscle-derived stem cells from ischemic injury? Clin Orthop Relat Res. 2015;473(9):2908-19. https://doi.org/10.1007/s11999-015-4332-8
6. O'Neill LAJ. How Toll-like receptors signal: what we know and what we don't know. Curr Opin Immunol. 2006;18(1):3-9. https://doi.org/10.1016/j.coi.2005.11.012
7. Cen X, Liu S, Cheng K. The role of Toll-like receptor in inflammation and tumor immunity. Front Pharmacol. 2018;9:878. https://doi.org/10.3389/fphar.2018.00878
8. Ma J, Chen Q, Li J, Zhao H, Mi E, Chen Y, et al. Dexmedetomidine-mediated prevention of renal ischemia-reperfusion injury depends in part on cholinergic anti-inflammatory mechanisms. Anesth Analg. 2020;130(4):1054-62. https://doi.org/10.1213/ANE.0000000000003820

9. Zhang Y, Liu M, Yang Y, Cao J, Mi W. Dexmedetomidine exerts a protective effect on ischemia-reperfusion injury after hepatectomy: a prospective, randomized, controlled study. J Clin Anesth. 2020;61:109631. https://doi.org/10.1016/j.jclinane.2019.109631
10. Hong Y, Zhang B, Yu L, Duan S-S. Cell membrane integrity and revascularization: the possible functional mechanism of ischemic preconditioning for skeletal muscle protection against ischemic-reperfusion injury. Acta Histochem. 2017;119(3):309-14. https://doi.org/10.1016/j.acthis.2017.02.007
11. Mo Y, Chen S, Yang L, Huang L, Jin D, Yu Z, et al. The effect of transcutaneous electrical acupoint stimulation on inflammatory response in patients undergoing limb ischemia-reperfusion. Mediators Inflamm. 2017;2017:8369737. https://doi.org/10.1155/2017/8369737
12. Foster AD, Vicente D, Sexton JJ, Johnston L, Clark N, Leonhardt C, et al. Administration of FTY720 during tourniquet-induced limb ischemia reperfusion injury attenuates systemic inflammation. Mediators Inflamm. 2017;2017:4594035. https://doi.org/10.1155/2017/4594035
13. Zhu L, Ye T, Tang Q, Wang Y, Wu X, Li H, et al. Exercise preconditioning regulates the Toll-like receptor 4/nuclear factor-κB signaling pathway and reduces cerebral ischemia/reperfusion inflammatory injury: a study in rats. J Stroke Cerebrovasc Dis. 2016;25(11):2770-9. https://doi.org/10.1016/j.jstrokecerebrovasdis.2016.07.033
14. Kim E, Kim H-C, Lee S, Ryu H-G, Park Y-H, Kim JH, et al. Dexmedetomidine confers neuroprotection against transient global cerebral ischemia/reperfusion injury in rats by inhibiting inflammation through inactivation of the TLR-4/NF-κB pathway. Neurosci Lett. 2017;649:20-7. https://doi.org/10.1016/j.neulet.2017.04.011
15. Liu Z, Huang W, Chen Y, Du Z, Zhu F, Wang T, et al. Ischemic postconditioning ameliorates acute kidney injury induced by limb ischemia/reperfusion via transforming TLR4 and NF-κB signaling in rats. J Orthop Surg Res. 2021;16(1):416. https://doi.org/10.1186/s13018-021-02565-5
16. Kuroda Y, Togashi H, Uchida T, Haga K, Yamashita A, Sadahiro M. Oxidative stress evaluation of skeletal muscle in ischemia-reperfusion injury using enhanced magnetic resonance imaging. Sci Rep. 2020;10(1):10863. https://doi.org/10.1038/s41598-020-67336-4
17. Neag M-A, Mitre A-O, Burlacu C-C, Inceu A-I, Mihu C, Melincovici C-S, et al. miRNA involvement in cerebral ischemia-reperfusion injury. Front Neurosci. 2022;16:901360. https://doi.org/10.3389/fnins.2022.901360
18. Sengul D, Sengul I. Connection of reactive oxygen species as an essential actor for the mechanism of phenomena; ischemic preconditioning and postconditioning: come to age or ripening? North Clin Istanb. 2021;8(6):644-9. https://doi.org/10.14744/nci.2021.78466

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220929

High diagnostic yield with algorithmic molecular approach on hereditary neuropathies

Gülay Güleç Ceylan[1,2*], Esra Habiloğlu[1], Büşranur Çavdarlı[1], Ebru Tuncez[1], Sule Bilen[3], Özlem Yayıcı Köken[4], C. Nur Semerci Gündüz[1,2]

SUMMARY

OBJECTIVE: Charcot-Marie-Tooth disease covers a group of inherited peripheral neuropathies. The aim of this study was to investigate the effect of targeted next-generation sequencing panels on the molecular diagnosis of Charcot-Marie-Tooth disease and its subtypes in routine clinical practice, and also to show the limitations and importance of next-generation sequencing in the diagnosis of Charcot-Marie-Tooth diseases.

METHODS: This is a retrospective study. Three different molecular methods (multiplex ligation probe amplification, next-generation sequencing, and whole-exome sequencing) were used to detect the mutations related to Charcot-Marie-Tooth disease.

RESULTS: In total, 64 patients (33 males and 31 females) with suspected Charcot-Marie-Tooth disease were analyzed for molecular etiology. In all, 25 (39%) patients were diagnosed by multiplex ligation probe amplification. With an extra 11 patients with normal PMP22 multiplex ligation probe amplification results that were consulted to our laboratory for further genetic analysis, a total of 50 patients underwent next-generation sequencing for targeted gene panels associated with Charcot-Marie-Tooth disease. Notably, 18 (36%) patients had pathogenic/likely pathogenic variants. Whole-exome sequencing was performed on five patients with normal next-generation sequencing results; the diagnostic yield by whole-exome sequencing was 80% and it was higher in the childhood group.

CONCLUSION: The molecular etiology in Charcot-Marie-Tooth disease patients can be determined according to pre-test evaluation, deciding the inheritance type with pedigree analysis, the clinical phenotype, and an algorithm for the genetic analysis. The presence of patients without a molecular diagnosis in all the literature suggests that there are new genes or mechanisms waiting to be discovered in the etiology of Charcot-Marie-Tooth disease.

KEYWORDS: Charcot-Marie-Tooth disease. DNA copy number variations. High-throughput nucleotide sequencing. Exome sequencing.

INTRODUCTION

Charcot-Marie-Tooth disease (CMT) covers a group of inherited peripheral neuropathies. It is also called hereditary motor sensory neuropathy. These neuropathies have heterogeneous clinics in terms of their phenotypic features, inheritance modes, and gene mutations in the etiology[1]. The prevalence is 9.7–82/100.000[2].

The mode of inheritance and genetic cause are important in the classification of CMT[3]. The phenotype of classical CMT contains typically distal weakness (a length-dependent motor sensory neuropathy), a high incidence of foot deformities, and sensory loss. This phenotype can occur in the first/second decade of life in most patients. There is a slow progression of these symptoms and worsening by the time[2]. Nerve conduction studies had a huge help in confirming and classifying CMTs by categorizing patients broadly into demyelinating and axonal or mixt type forms. The key parameters measured by electromyography (EMG) are distal latencies, amplitudes, and velocities of motor and sensory nerves, but the main finding is the median nerve conduction velocity, and 38 m/s is the commonly used cutoff value for differentiating demyelinating from axonal types of CMTs[4].

Genetic heterogeneity of CMT has been revealed by the common use of next-generation sequencing (NGS). Until now, more than 100 genes have been described as having causative mutations for CMT[5]. Especially, four genes are responsible for nearly 80% of genetically inherited CMTs: *PMP22*, *GJB1*, *MFN2*, and *MPZ*[6]. The most common type of CMT is CMT1A, which accounts for nearly 60% of genetically inherited CMT cases. A 1.4 Mb duplication in the short arm of chromosome 17 causes CMT1A, and this region encloses nine genes, including *PMP22* gene[7]. Another inherited neuropathy with pressure palsies has been caused by a deletion in the same gene. This points out the importance of *PMP22* gene and its protein expression level for peripheral nerve

[1]Ankara City Hospital, Department of Medical Genetics – Ankara, Turkey.
[2]Ankara Yıldırım Beyazıt Üniversitesi, Department of Medical Genetics – Ankara, Turkey.
[3]Ankara City Hospital, Neurology Department – Ankara, Turkey.
[4]Akdeniz University, Medical Faculty, Department of Pediatrics, Division of Pediatric Neurology – Antalya, Turkey.
*Corresponding author: gulayceylan23@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on August 30, 2022. Accepted on October 24, 2022.

function. *GJB1*, *MFN2*, and *MPZ* are responsible for CMTX1, CMT2A, and CMT1B, respectively[3]. CMT2A can present in early childhood or infancy period, and it is caused by *MFN2* gene mutations with a more severe phenotype[8].

Recently, NGS has become more cost-effective, suitable, and wide for many genetically inherited diseases, including CMT. Targeted NGS panels include some causative genes related to the diseases[9,10]. This study aimed to describe the effect of targeted NGS panels on the molecular diagnosis of CMT and its subtypes in routine clinical practice, and also to show the limitations and importance of NGS at the diagnosis of CMTs.

METHODS

We reviewed the data of 64 patients who applied for hereditary peripheral neuropathy at the Ankara City Hospital Genetic Diseases Evaluation Center from February 2019 to December 2020. The patients were examined by their pediatric/adult neurologists and were referred to our genetic laboratory for a diagnostic genetic test. Patients who had acquired neuropathy were excluded. Permission for the study was obtained from the Ankara Yıldırım Beyazıt University Ethics Committee (17.02.2021/02). The study followed the guidelines and principles of the Declaration of Helsinki. All patients and formal guardians of the patients under 18 years had signed the written informed consent for the usage of their clinical data and genetic analysis. Genomic DNA was extracted from peripheral blood using QIAcube® automatic DNA isolation system (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's instructions. MRC Holland (Amsterdam, Holland) P033 CMT1 kit was used for multiple ligation-dependent probe amplification (MLPA) method according to the manufacturer's instructions. MLPA using genomic DNA extracted from whole blood was performed to detect the deletion/duplication mutations of *PMP22* gene. Qiagen CMT panel CDHS-17346Z-1897 kit (Qiagen, Hilden, Germany) was used for NGS to detect the single nucleotide variants for the targeted genes (Table 1). The target enrichment process was followed by

Table 1. Charcot-Marie-Tooth disease-related genes (44) included in targeted NGS panel, their corresponding transcript numbers, and heredity types.

Gene name	Transcript ID	Inheritance	Gene name	Transcript ID	Inheritance
AARS	NM_001605.2	AD	KIF5A	NM_004984.2	AD
ARHGEF10	NM_014629.2	AD	LITAF	NM_001136473.1	AD
BSCL2	NM_032667.6	AD,AR	LMNA	NM_170707.3	AD,AR
COX6A1	NM_004373.3	AR	MARS	NM_004990.3	AD
DHTKD1	NM_018706.6	AD	MED25	NM_030973.3	AR
DNMT1	NM_001379.2	AD	MFN2	NM_014874.3	AD,AR
DNM2	NM_001005360.2	AD	MPZ	NM_000530.6	AD
DYNC1H1	NM_001376.4	AD	MTMR2	NM_016156.5	AR
EGR2	NM_000399.3	AD	NDRG1	NM_006096.3	AR
FAM134B	NM_001034850.3	AR	NEFL	NM_006158.4	AD,AR
FIG4	NM_014845.5	AR	PLEKHG5	NM_198681.3	AR
FGD4	NM_139241.2	AR	PMP22	NM_153321.2	AD
GAN	NM_022041.3	AR	PRPS1	NM_002764.3	XLR
GARS1	NM_002047.2	AD	PRX	NM_181882.2	AR,AD
GDAP1	NM_018972.2	AR,AD	RAB7A	NM_004637.5	AD
GJB1	NM_000166.5	XLD	REEP1	NM_022912.2	AD
HSPB1	NM_001540.3	AD	SBF1	NM_002972.2	AR
HSPB8	NM_014365.2	AD	SBF2	NM_030962.3	AR
IGHMBP2	NM_002180.2	AR	SH3TC2	NM_024577.3	AR,AD
IKBKAP	NM_003640.3	AR	TRPV4	NM_021625.4	AD
INF2	NM_022489.3	AD	VCP	NM_007126.3	AD
KIF1B	NM_015074.3	AD	YARS1	NM_003680.4	AD

AD: autosomal dominant; AR: autosomal recessive; XLD: X-linked dominant; XLR: X-linked recessive.

sequencing of the libraries on Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). Whole-exome sequencing (WES) was performed on five patients who had negative duplication/deletion analysis and targeted NGS panel.

Data analysis and variant interpretation

Data analysis was carried out by QIAGEN Clinical Insight (QCITM) software (QIAGEN, Hilden, Germany). Pathogenic, likely pathogenic, and uncertain significant variants were confirmed by Sanger sequencing. The exons of all targeted genes were sequenced at a read depth of 30× or greater. The 2015 American College of Medical Genetics Standards and Genomics (ACMG) was used for the interpretation of sequence variants[11].

RESULTS

In total, 64 patients (33 males/31 females) with suspected CMT were analyzed for molecular etiology. The range of the patient ages was between 3 and 74 years; 33 male cases had a mean age of 26.4 years, and 31 female cases had a mean age of 25.3 years.

First, MLPA was performed for deletion/duplication analysis for all of the patients. In all, 25 (39%) patients were diagnosed by MLPA. *PMP22* duplication was detected in 14 patients, and *PMP22* deletion was detected in 11 patients. An extra 11 patients with normal *PMP22* MLPA results who were consulted to our laboratory for further genetic analysis were also included in the study. Eventually, 50 patients with normal *PMP22* MLPA results underwent NGS for targeted gene panels associated with CMT.

Notably, 18 (36%) patients including 10 males and 8 females had pathogenic/likely pathogenic variants at *INF2*, *EGR2*, *HSPB1*, *GJB1*, *GNB4*, *LITAF*, *GDAP1*, *MFN2*, *IGHMBP2*, *SH3TC2*, *GAN*, *SBF1*, *MRM2*, and *PLA2G6* genes. Nine (50%) patients were under 18 years old. Family history was positive for six patients and consanguinity marriage for seven parents. Nine patients had homozygote pathogenic/likely pathogenic variants for genes (*IGHMBP2*, *SH3TC2[2]*, *GDAP1[3]*, *GAN*[2], and *SBF1*) that have autosomal recessive manner. Eight patients had heterozygote pathogenic/likely pathogenic variants for genes (*INF2[2]*, *EGR2*, *HSPB1*, *GNB4*, *LITAF*, *GDAP1*, and *MFN2*) that have autosomal dominant and X-linked manner (*GJB*1), respectively. In only one patient, a heterozygote pathogenic variant had been detected for an autosomal recessive inherited gene (*GAN*) (Table 2).

A total of 17 (13%) variants on 13 patients were assessed as variants of unknown significance in our study (Table 2). Five (38%) patients were under 18 years old. Pathogenic, likely pathogenic, and variant of uncertain significant variants (VUS) were confirmed by bidirectional Sanger sequencing. Over 99% of the coding exons of all genes in the panel were sequenced to a read depth of 30× or greater in almost all cases. According to these results, the molecular diagnosis rate was 39%.

WES was performed as further examination in 5 patients (3 of them under 18 years old) whose panel results were found to be normal. Pathogenic and likely pathogenic variants had been detected at four different genes in these patients (Table 3). As a result, among 22 pediatric patients, 17 were diagnosed by NGS and WES, and 19 out of 28 adult patients were also diagnosed. So, the diagnosis rates for the pediatric age group and adult groups were 55 and 39%, respectively, excluding VUS.

DISCUSSION

CMT diseases are a very wide spectrum of hereditary neuropathies that are caused by a large number of different genes[12]. There is a genetic heterogeneity in the inheritance of the genes responsible for CMTs. The molecular pathways of these genes related to CMTs are quite complex; thus, the diagnosis is also complicated[12]. At this point, a new approach is needed for the correct diagnosis[6].

PMP22 duplication/deletion test is the first diagnostic method for CMT1. In our study, MLPA was the first method used for the investigation of duplication/deletion analysis for *PMP22* gene. The diagnostic yield for MLPA was 39%, which was nearly compatible with the literature[7].

If the MLPA test is negative or there is another type of CMT, a targeted NGS gene panel should be performed[13]. With these targeted gene panels listing all known disease-causing genes, a large group of genes can be sequenced and analyzed to show the different variants (pathogenic, likely pathogenic, or variants of unknown significance), and this method can be accepted as the most effective genetic testing in CMT. The diagnosis rate is 18–31% for CMT gene panels, related to the sequencing quality and the included genes[14]. Vaeth et al. reported that 6.7% pathogenic/likely pathogenic variants were detected with targeted NGS panel in CMT patients[15]. The higher depth of coverage is an important factor for the higher accuracy of the test[14]. The diagnostic yield for NGS in our study was 36%. We think that the reason why this rate is slightly higher than that reported in the literature is that the right patients were chosen based on their clinical findings, EMG results, and family history. Notably, 18 patients who had undergone to NGS had pathogenic/likely pathogenic variants mostly at autosomal recessive inherited genes (*IGHMBP2*, *SH3TC2*, *GDAP1*, *GAN*, *SBF1*, *EGR2*, *MFN2*) and one at X-linked inherited gene (*GJB1*). The rest of the patients had pathogenic/likely pathogenic variants at autosomal dominant inherited genes (*INF2*, *HSPB1*,

Table 2. Cases with pathogenic/likely pathogenic variants and variant of uncertain significant variants.

Gender	Age	Result	ACMG 2015 criteria	Phenotype (OMIM)
M	14	*INF2* (NM_022489.3):c.218G>A(p.Gly73Asp) Heterozygote	Likely pathogenic (novel)	CMTDIE (614455)
M	44	*EGR2* (NM_000399.4):c.1142G>T(p.Arg381Leu) Heterozygote	Likely pathogenic (novel)	CMT1D (607678)
F	36	*INF2* (NM_022489.3):c.271C>G(p.Arg91Gly) Heterozygote	Likely pathogenic	CMTDIE (614455)
M	74	*HSPB1* (NM_001540.5):c.562C>T(p.R188W) Heterozygote	Likely pathogenic	CMT2F (606595)
F	40	*GJB1* (NM_000166.5):c.581T>C(p.M194T) Heterozygote	Likely pathogenic	CMTX (1302800)
M	28	*GNB4 (NM_021629.4):c.266A>C(p.Lys89Thr)* Heterozygote	Likely pathogenic	CMTDIF (615185)
M	9	*LITAF* (NM_004862.3):c.430G>A(p.Val144Met) Heterozygote	Likely pathogenic	CMT1C (601098)
M	10	*GDAP1* (NM_018972.4): c.836A>G(p.Tyr279Cys) Heterozygote	Pathogenic	CMT2K (607831)
F	3	*MFN2* (NM_001127660.1):c.1090C>T(p.Arg364Trp) Heterozygote	Likely pathogenic	CMT2A2A (609260)
F	5	*IGHMBP2* (NM_002180.2): c.1347G>A(p.Met449Ile) Homozygote	Pathogenic	CMT2S (616155)
M	20	*SH3TC2* (NM_024577.3):c.1896_1897delGGinsA(p.Ala633fs*12) Homozygote	Pathogenic	CMT4B2 (604563)
F	33	*SH3TC2* (NM_024577.3):c.2860C>T(p.R954*) Homozygote	Pathogenic	CMT4C (601596)
M	15	*GDAP1* (NM_018972.3):c.347T>G(p.M116R) Homozygote	Likely pathogenic	CMT2K (607831)
F	12	*GAN* (NM_022041.3):c.1369_1370dupAG(p.R458fs*32) Homozygote	Pathogenic	Giant axonal neuropathy-1
F	19	*SBF1* (NM_002972.4):c.5297G>A(p.Arg1766His) Homozygote	Likely pathogenic	CMT4B2 (604563)
M	13	*GDAP1* (NM_001040875.3):c.278G>A(p.Arg93His) Homozygote	Likely pathogenic	CMT2K (607831)
M	6	*GAN* (NM_022041.4):c.968C>A(p.S323*) Homozygote	Pathogenic	Giant axonal neuropathy-1
F	23	*GDAP1* (NM_018972.3):c.347T>G(p.M116R) Homozygote	Likely pathogenic	CMT2K (607831)
M	18	*MFN2* (NM_014874.3): c.2167G>A(p.p.Val723Ile) Heterozygote	VUS	CMT2A2A (609260)
F	32	*SBF2* (NM_030962.3):c.5014_5016delAAA(p.Lys1672del) Homozygote, *ARHGEF10* (NM_014629.3):c.2881T>G(p.Ser961Ala) Heterozygote	VUS	CMT4B2 (64563) Slowed nerve conduction velocity (608236)
F	32	*VCP* (NM_007126.5):c.34C>A(p.L12I) Heterozygote	VUS	CMT2Y (616687)
M	5	*PMP22* (NM_000304.4):c.103G>A(p.A35T) Heterozygote	VUS	CMT1A (118220) CMT1E (118300)
F	52	*TRPV4* (NM_021625.4):c.133G>A(p.G45S) Heterozygote	VUS	HMSN2C (606071)
M	17	*SBF1* (NM_002972.4):c.1637-4delG Heterozygote, *SH3TC2* (NM_024577.3):c.3293C>T(p.T1098I) Heterozygote	VUS	CMT4B2 (604563) CMT4B2 (604563)
M	16	*REEP1* (NM_022912.2):c.262T>C(p.Y88H) Heterozygote, *FIG4* (NM_014845.5):c.1246T>G(p.W416G) Heterozygote	VUS	HMN5B (614751) CMT4J (611228)
F	25	*INF2* (NM_022489.4):c.1541C>T(p.P514L) Heterozygote	VUS	CMTDIE (614455)
F	29	*PLEKHG5* (NM_020631.5):c.2362_2363TC[2] (p.Leu789fs) Heterozygote	VUS	CMTC (615376)
M	2	*DYNC1H1*:c.10619A>G(p.N3540S) Heterozygote	VUS	CMT2O (614228)
F	38	*DHTKD1* (NM_018706.7):c.857A>G(p.Asn286Ser) Heterozygote, *PLEKHG5* (NM_001042663.2):c.1778G>A(p.Arg593Gln) Heterozygote	VUS	CMT2Q (615025) CMTC (615376)
F	31	*GARS1* (NM_0013166772.1):c.1598C>T(p.T533M) Heterozygote	VUS	CMT2D (601472)
M	19	*EGR2* (NM_001136179.3):c.364_369dupCCTCCT (p.Pro172_Pro173dup) Heterozygote	VUS	CMT1D (607678)

Table 3. Molecular findings of whole-exome sequencing at chosen patients.

Gender	Age	Clinical findings	Gene (RefSeq Transcript)	Mutation nucleotide change/ Amino acid change	Zygosity and inheritance	Database info dbSNP/ HGMD/ Novel/ ClinVar	SIFT	PolyPhen	CADD score*	Frequency
Male	5	Delayed motor development, severe hypotonia, dysmorphic facial features, cleft lip	*VAMP1* (NM_199245.3)	c.202C>T/p.R68*	Homozygous/ AR	CM11716 (DM)/ rs76969393	No prediction	No prediction	36	0.00000795
Male	17	Developmental delay, neurosensory deafness, hepatosplenomegaly, liver enzyme abnormalities, palmoplantar hyperkeratosis	*MRM2* (NM_0133933)	c.638A>C/p.Gln213Pro	Homozygous/ AR	rs372352761	Tolerated	Possibly damaging	21.6	0.00000398
Male	5	Developmental delay, psychomotor regression	*PLA2G6* (NM_001199562.3)	c.1610G>A/p.Arg537Gln	Homozygous/ AR	CM063032 (DM)/ rs776713955	Damaging	Probably damaging	31	0.00000398
Male	19	Gait disturbance, distal muscle weakness, scoliosis	Normal	–	–	–	–	–	–	–
Male	47	Gait instability, distal muscle weakness	*MME* (NM_007289.4)	c.160+1G>C/ splice site	Homozygous/ AR	Novel	No prediction	No prediction	33	–

AR: autosomal recessive. *CADD is a tool for scoring the harmfulness of a variant. A score of 10 indicates that the variant is supposed to be among the top 10% of deleterious variants, a score of 20 indicates the variant is in the top 1%.

GNB4, *LITAF*, *MFN2*). According to the previous studies, autosomal dominant inherited CMTs are more common according to autusomal recessive inherited ones[6]. Due to the prevalence of consanguineous marriages in the Turkish population and the family structure with many children, it is estimated that the autosomal recessive inherited forms of CMTs may have a higher rate, unlike the literature.

By using targeted gene panels, the rate of VUS has been increased. Comments about VUS are still a diagnostic challenge in NGS method. Different laboratories report different comments about the same variant. Hence, it is important to know the effect of VUS for an effective genetic counseling. If there is sufficient data about VUS, it can also be evaluated as benign or likely benign polymorphisms. If there is more than one VUS in a patient, this can affect the disease burden and also explain the variable expressivity at the phenotypes of the patients[16]. VUS variants need to involve a multidisciplinary medical team for phenotype-genotype correlation.

In our study, VUS were identified in 13% of the patients in *MFN2*, *SBF2*, *ARHGEF10*, *VCP*, *PMP22*, *TRPV4*, *SBF1*, SH3TC2, *REEP1*, *FIG4*, *INF2*, *PLEKH5* (2), *DYNC1H1*, *DHTKD1*, *GARS1*, and *EGR2* genes. The single variants in dominant genes associated with CMT were more common (8/14 genes). Only the *PLEKHG5* gene that had autosomal recessive inheritance had two heterozygote VUS, and the other genes had only one VUS. Three patients were co-segregated with the healthy consanguineous obligate carrier parent. Some families of the other patients were not available or could not be reached; hence, family study could not be carried out on these patients, but they are considered to be recalled. Patients were offered an annual follow-up evaluation for VUS. In different CMT-NGS studies, various results were reported for VUS according to population diversities[16-18]. Larger population-based studies could reduce the prevalence of VUS.

WES was also performed in our study, as an advanced examination in five of the male patients whose panel results were found to be normal. The mean age of these patients was 18.6 years. Four patients had homozygote pathogenic/likely pathogenic variants at different genes (*VAMP1*, *MRM2*, *PLA2G6*, and *MME*), which had all autosomal recessive manner. A novel mutation at splice site of *MME* gene was evaluated as likely pathogenic. All of these five patients had neuropathic changes at their EMGs. WES captures and sequences only 1–2% of the entire genome. Over the past decade, WES has been a very

popular research tool and the main driver in the identification of new CMT-related genes. WES also allows sequencing of genes that have never been associated with CMT or other Mendelian diseases. Since neuropathy can accompany other neuromuscular diseases besides CMT, results other than CMT can also be obtained by WES. Different groups report diagnosis rates as 19–45% in people with CMT or complex neuropathy who had negative genetic tests earlier[19]. In our study, WES was performed on molecularly undefined patients with CMT. The diagnosis rate among the patients who underwent WES was 80%. We think that the increased rate is the result of appropriate patient selection and the previous negative genetic tests.

Two novel mutations at *INF2* and *EGR2* genes related to CMTDIE and CMT1D, respectively, were detected by targeted NGS gene panel. Another novel variant at *MME* gene causing CMT2T was found by WES analysis. The variants were classified as "likely pathogenic" according to ACMG criteria[11].

For our study, the diagnosis rate was 39% (25 of 64 patients) with PMP22 MLPA. The molecular diagnosis of 18 (36%) among the 50 patients was confirmed with targeted NGS panels. Four of five patients had molecular diagnosis who underwent WES analysis. The diagnostic yield was compatible with literature[20,21]. The diagnostic rate in pediatric age group (54%) was higher than adult age group (39%). Especially, pediatric patient group that targeted NGS did not diagnose were surprisingly diagnosed by WES.

Out of 64 patients who first applied to our clinic for neuropathy, 25 were diagnosed by MLPA, 18 by targeted NGS panel (out of a total of 50 patients with a normal *PMP22* MLPA result who were consulted to our laboratory for further genetic analysis), and 4 by WES. As a result, the diagnostic yield of our study was 73% (47 patients). Thus, it can be said that the algorithmic molecular approach increases the diagnosis rate in hereditary neuropathies.

CONCLUSION

Gene panels provide excellent capture of intended CMT-associated gene regions, so they minimize false negatives with uniform coverage and high reading depths. The diagnostic rate for CMT gene panels ranges between 18 and 31% in the literature, depending on the CMT cohort, demographic background, sequencing platform, and number of genes included. The most important point is to evaluate the bioinformatics analysis of the variants obtained by NGS in correlation with the clinics of the patients.

In our study, targeted NGS panel was diagnostic in nearly one-third of the patients with CMT clinics after the exclusion of *PMP22* deletion/duplication analysis. WES is an advanced technique in patients with negative targeted gene panels and *PMP22* gene duplication/deletion. The molecular etiology in CMT patients can be determined according to pre-test evaluation, deciding the inheritance type with pedigree analysis, clinical phenotype, and an algorithmic molecular approach for the genetic analysis. Early onset of the disease, consanguinity marriage, or positive family history is important for a correct genetic diagnosis. An accurate diagnosis is also important for an appropriate genetic counseling for the patients to understand the significance of genetic testing. As in our study, the presence of patients without a molecular diagnosis in all the literature suggests that new genes or mechanisms are needed to be discovered in the etiology of CMT.

ACKNOWLEDGMENTS

All authors thank the patients and their family members for their participation in this study. ÖYK was supported by an MRC strategic award to establish an International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) MR/S005021/1'.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

AUTHORS' CONTRIBUTIONS

GGC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **EH:** Data curation, Formal Analysis, Investigation. **BÇ:** Data curation, Formal Analysis, Methodology, Software, Writing – original draft. **ET:** Data curation, Formal Analysis, Methodology. **CNSG:** Data curation, Writing – review & editing. **ÖYK:** Funding acquisition, Resources, Visualization, Writing – original draft. **SB:** Resources, Visualization, Writing – original draft.

REFERENCES

1. Reilly MM, Murphy SM, Laura M. Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2011;16(1):1-14. https://doi.org/10.1111/j.1529-8027.2011.00324.x
2. Barreto LC, Oliveira FS, Nunes PS, de França Costa IM, Garcez CA, Goes GM, et al. Epidemiologic study of Charcot-Marie-Tooth disease: a systematic review. Neuroepidemiology. 2016;46(3):157-65. https://doi.org/10.1159/000443706
3. Pipis M, Rossor AM, Laura M, Reilly MM. Next-generation sequencing in Charcot-Marie-Tooth disease: opportunities and challenges. Nat Rev Neurol. 2019;15(11):644-56. https://doi.org/10.1038/s41582-019-0254-5
4. Nagappa M, Sharma S, Taly AB. Charcot Marie Tooth. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
5. Fridman V, Saporta MA. Mechanisms and treatments in demyelinating CMT. Neurotherapeutics. 2021;18(4):2236-68. https://doi.org/10.1007/s13311-021-01145-z
6. Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME. Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Ann Neurol. 2011;69(1):22-33. https://doi.org/10.1002/ana.22166
7. Rossor AM, Tomaselli PJ, Reilly MM. Recent advances in the genetic neuropathies. Curr Opin Neurol. 2016;29(5):537-48. https://doi.org/10.1097/WCO.0000000000000373
8. Feely SM, Laura M, Siskind CE, Sottile S, Davis M, Gibbons VS, et al. MFN2 mutations cause severe phenotypes in most patients with CMT2A. Neurology. 2011;76(20):1690-6. https://doi.org/10.1212/WNL.0b013e31821a441e
9. Rossor AM, Polke JM, Houlden H, Reilly MM. Clinical implications of genetic advances in Charcot-Marie-Tooth disease. Nat Rev Neurol. 2013;9(10):562-71. https://doi.org/10.1038/nrneurol.2013.179
10. Wang W, Wang C, Dawson DB, Thorland EC, Lundquist PA, Eckloff BW, et al. Target-enrichment sequencing and copy number evaluation in inherited polyneuropathy. Neurology. 2016;86(19):1762-71. https://doi.org/10.1212/WNL.0000000000002659
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. ACMG Laboratory Quality Assurance Committee. 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
12. Stojkovic T. Hereditary neuropathies: an update. Rev Neurol (Paris). 2016;172(12):775-8. https://doi.org/10.1016/j.neurol.2016.06.007
13. Klein CJ. Charcot-Marie-Tooth disease and other hereditary neuropathies. Continuum (Minneap Minn). 2020;26(5):1224-56. https://doi.org/10.1212/CON.0000000000000927
14. -Antoniadi T, Buxton C, Dennis G, Forrester N, Smith D, Lunt P, et al. Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. BMC Med Genet. 2015;16:84. https://doi.org/10.1186/s12881-015-0224-8
15. Vaeth S, Christensen R, Dunø M, Lildballe DL, Thorsen K, Vissing J, et al. Genetic analysis of Charcot-Marie-Tooth disease in Denmark and the implementation of a next generation sequencing platform. Eur J Med Genet. 2019;62(1):1-8. https://doi.org/10.1016/j.ejmg.2018.04.003
16. Cortese A, Wilcox JE, Polke JM, Poh R, Skorupinska M, Rossor AM, et al. Targeted next-generation sequencing panels in the diagnosis of Charcot-Marie-Tooth disease. Neurology. 2020;94(1):e51-61. https://doi.org/10.1212/WNL.0000000000008672
17. Yalcintepe S, Gurkan H, Dogan IG, Demir S, Sag SO, Kabayegit ZM, Atli EI, et al. The importance of multiple gene analysis for diagnosis and differential diagnosis in Charcot-Marie-Tooth disease. Turk Neurosurg. 2021;31(6):888-95. https://doi.org/10.5137/1019-5149.JTN.33661-21.3
18. Chen CX, Dong HL, Wei Q, Li LX, Yu H, Li JQ, et al. Genetic spectrum and clinical profiles in a southeast Chinese cohort of Charcot-Marie-Tooth disease. Clin Genet. 2019;96(5):439-48. https://doi.org/10.1111/cge.13616
19. Hartley T, Wagner JD, Warman-Chardon J, Tétreault M, Brady L, Baker S, et al. Whole-exome sequencing is a valuable diagnostic tool for inherited peripheral neuropathies: outcomes from a cohort of 50 families. Clin Genet. 2018;93(2):301-9. https://doi.org/10.1111/cge.13101
20. Felice KJ, Whitaker CH, Khorasanizadeh S. Diagnostic yield of advanced genetic testing in patients with hereditary neuropathies: a retrospective single-site study. Muscle Nerve. 2021;64(4):454-61. https://doi.org/10.1002/mus.27368
21. Morena J, Gupta A, Hoyle JC. Charcot-Marie-Tooth: from molecules to therapy. Int J Mol Sci. 2019;20(14):E3419. https://doi.org/10.3390/ijms20143419

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220934

Urbanization and kidney dysfunction in Brazilian indigenous people: a burden for the youth

Orlando Vieira Gomes[1,2*], Manoel Pereira Guimarães[2], Jandir Mendonça Nicacio[1,2], Leela Morena[2], Antônio Marconi Leandro da Silva[2], Jeová Cordeiro de Morais Junior[2], Carlos Dornels Freire de Souza[2], Manoel Barral-Netto[3], João Augusto Costa Lima[4], Anderson da Costa Armstrong[1,2]

SUMMARY

OBJECTIVE: The aim of this study was to investigate whether the degree of urbanization influences the prevalence of chronic kidney disease in Brazilian indigenous people.

METHODS: This is a cross-sectional study conducted between 2016 and 2017 in northeastern Brazil and includes individuals aged between 30 and 70 years from two specific indigenous groups who volunteered to participate in the study: the Fulni-ô people (lowest degree of urbanization) and the Truká group (greater degree of urbanization). Cultural and geographical parameters were used to characterize and measure the magnitude of urbanization. We excluded individuals with known cardiovascular disease or renal failure who required hemodialysis. Chronic kidney disease was defined as a single measurement of an estimated glomerular filtration rate <60 mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation.

RESULTS: A total of 184 indigenous people from the Fulni-ô group and 96 from the Truká group with a median age of 46 years (interquartile range: 15.2) were included. We found a chronic kidney disease rate of 4.3% in the total indigenous population, generally affecting an older population: 41.7% over 60 years old (p<0.001). The Truká people had a chronic kidney disease prevalence of 6.2%, with no differences in kidney dysfunction across age groups. The Fulni-ô participants had a chronic kidney disease prevalence of 3.3%, with a higher proportion of kidney dysfunction in older participants (of the six Fulni-ô indigenous people with chronic kidney disease, five were older).

CONCLUSION: Our results suggest that a higher degree of urbanization seems to negatively influence the prevalence of chronic kidney disease in Brazilian indigenous people.

KEYWORDS: Chronic kidney disease. Urbanization. Indigenous peoples.

INTRODUCTION

Chronic kidney disease (CKD) is one of the most important public health concerns of the century, and it is known to be associated with high rates of mortality and social costs[1]. It is characterized by severe, irreversible kidney damage with a reduction in glomerular filtration rate of <60 mL/min/1.73 m^2 or a urinary albumin-to-creatinine ratio of ³30 mg/g^2. Previous studies have shown an increasing prevalence of CKD among indigenous people. When comparing outcomes with the general population, indigenous communities present higher mortality rates[3].

Similar to other colonized indigenous population[3], Brazilian indigenous people have undergone an accelerated process of nutritional and epidemiological transition characterized by reduced physical activity and incorporation of new cultural habits. These factors have promoted the emergence of chronic diseases, such as CKD, and risk factors, such as obesity, hypertension, hyperglycemia, dyslipidemia, and diabetes, among indigenous people[4-6]. However, the literature describing the prevalence and determinants of CKD in Brazilian indigenous people is still scarce.

The Project of Atherosclerosis among Indigenous Populations (PAI) is a population-based study conducted in the Northeast Region of Brazil. The aim of this project was to assess cardiovascular health in indigenous groups with different degrees

[1]Universidade do Estado da Bahia, Postgraduation Program in Human Ecology and Socio-Environmental Management – Juazeiro (BA), Brazil.
[2]Universidade Federal do Vale do São Francisco, School of Medicine – Petrolina (PE), Brazil.
[3]Oswaldo Cruz Foundation, Instituto Gonçalo Muniz – Salvador (BA), Brazil.
[4]John Hopkins University – Baltimore (MD), USA.
*Corresponding author: orlandopetro@msn.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 20, 2022. Accepted on November 14, 2022.

of urbanization. Between 2016 and 2017, the PAI study recruited 999 individuals, with no known previous cardiovascular event, who were inhabitants of the following three communities in the São Francisco River basin: two indigenous tribes (the less urbanized Fulni-ô and the more urbanized Truká people) and an urbanized non-indigenous control group from the same area[4].

For this report, we exclusively assessed indigenous participants in the PAI study with available estimated glomerular filtration rate (eGFR) and clinical data to describe the prevalence of CKD and associated risk factors in both Brazilian indigenous communities living in different degrees of urbanization. Our hypothesis was that the group with a high degree of urbanization would have the highest prevalence of CKD.

METHODS

The PAI study was approved by the National Research Ethics Council (CONEP number 1.488.268), the National Indigenous Foundation (Fundação Nacional do Índio [FUNAI]; process number 08620.028965/2015-66), and the indigenous leaders of both participating groups. All participants provided written informed consent before enrollment in the study.

Study design and recruiting

The PAI study has been described previously[4]. Briefly, it is a descriptive, cross-sectional study composed of two specific indigenous groups from the Sao Francisco Valley in the northeast of Brazil (Figure 1). These groups were assessed between 2016 and 2017, and then stratified by degree of urbanization: the Fulni-ô people with a low level of urbanization and the Truká group with a high level of urbanization. The classification of the degree of urbanization was based on the following group characteristics: geographical location, maintenance of traditional culture, proximity to and contact with cities, and influence of the city on the group's dynamics[7,8].

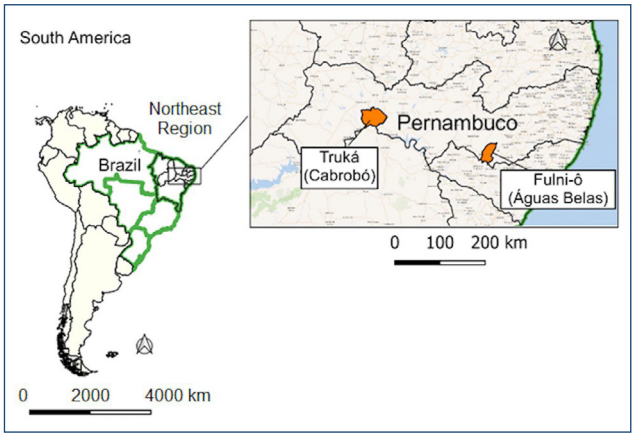

Figure 1. Geographical locations of the Truká and Fulni-ô groups.

The PAI study included individuals aged between 30 and 70 years who voluntarily agreed to participate in the study. Those with clinically manifested heart failure, history of coronary or cerebrovascular vascular diseases requiring hospitalization, renal failure on dialysis, or a history of surgery for peripheral arterial disease or heart disease were excluded.

The current analysis was carried out as an ancillary study of the PAI study, assessing participants with complete data on kidney function. In total, we analyzed 280 individuals: 184 (65.7%) from the Fulni-ô group and 96 (34.3%) from the Truká group.

Sociodemographic and anthropometric parameters

We registered sex as a binary variable (male/female). Age was computed as a continuous variable in years, as well as categorized within four proportional groups (30–39, 40–49, 50–59, and 60–70 years). Individuals were classified according to body mass index as underweight (<18.5), normal (≥18.5 and <25), overweight (≥25 and <30), and obese (≥30). Obesity was subdivided into categories: class 1 (30 to < 35), class 2 (35 to <40), and class 3 "severe" obesity (>40)[9].

Clinical parameters and laboratory testing

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or taking hypertension medications[10]. Diabetes was diagnosed when HbA1c was ³6.5% or using diabetes medications[11]. Dyslipidemia was established if the participant was using hypolipidemic medication or if at least one of the following criteria was met: reduced high-density lipoprotein cholesterol, a level <40 mg/dL in men or 50 mg/dL in women; hypertriglyceridemia, a triglyceride level >150 mg/dL; and hypercholesterolemia, an low-density lipoprotein cholesterol >160 mg/dL[12].

Estimated glomerular filtration rate was calculated using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation without correction for race. According to the 2012 KDIGO criteria[2], we classified the participants into three categories: normal/high (G1) (eGFR: ≥90 mL/min/1.73 m^2), mildly decreased excretory renal function (G2) (eGFR: 60–89 mL/min/1.73 m^2), and substantially reduced (G3) (eGFR: <60 mL/min/1.73 m^2). We defined CKD as a single measurement of eGFR <60 mL/min/1.73 m^2.

Statistical analysis

The following statistical tests were used: the Shapiro-Wilk test for data distribution evaluation and analysis of variance to compare age distribution according to different grades of eGFR (and Tukey's post hoc test, when necessary). In this analyses, confidence intervals of 95% and a significance level of 5% were used. Continuous quantitative variables were presented through central tendency and dispersion (mean±standard deviation) and qualitative variables through frequencies (absolute and relative). Significant associations were considered when $p<0.05$.

RESULTS

A total of 280 indigenous participants were included: 184 (65.7%) from the Fulni-ô group and 96 (34.3%) from the Truká group, with a median age of 46 (interquartile range: 15.2) years in the entire cohort. According to the 2012 KDIGO criteria[2], 59.9% of all participants had normal/high eGFR; 37.8% had mildly decreased excretory renal function (eGFR: 60–89 mL/min/1.73 m^2); and 4.3% had substantially reduced eGFR (<60 mL/min/1.73 m^2), which generally affected a higher age population ($p<0.001$) (Figure 2A).

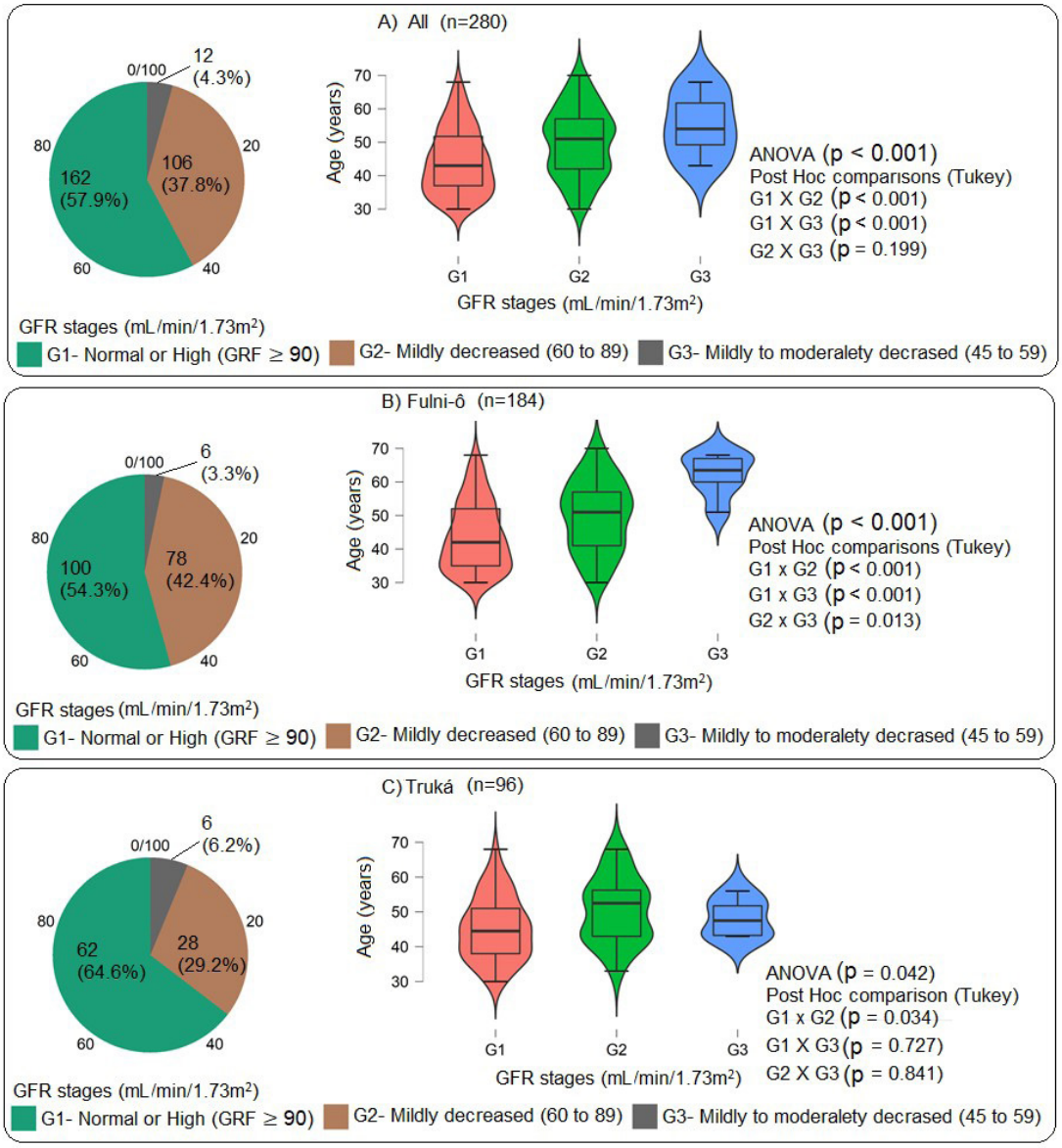

Figure 2. Glomerular filtration rate stage (Chronic Kidney Disease Epidemiology Collaboration, without race correction) of the study population: (A) total population; (B) Fulni-ô; and (C) Truká.

In a more advanced degree of urbanization, the Truká people had a CKD prevalence of 6.2%, with no differences in kidney dysfunction across age groups. On the contrary, the Fulni-ô participants had a CKD prevalence of 3.3%, with a higher proportion of kidney dysfunction in older participants (of the six Fulni-ô indigenous people with CKD, five were older), when compared to young people from the same ethnicity (Figures 2B and 2C). In the prevalence of CKD between the two indigenous groups, no statistically significant difference was found (p=0.068) (Table 1). The Truká people presented a younger population with mildly to moderately decreased kidney function, with a median age of 47.5 years, contrasting with the median age of 63.5 years in the G3 Fulni-ô subgroup (Figures 2B and 2C).

The prevalence of hypertension and diabetes was 24.6 and 9.3%, respectively. Regarding the prevalence of hypertension, no association was found between the groups according to the eGFR. As for diabetes, the prevalence in the indigenous with an eGFR <60 mL/min/1.73 m^2 was 25% (3/12) compared to 8.6% (23/268) in the group with an eGFR ≥60 mL/min/1.73 m^2 (p=0.0453) (Table 1).

Notably, 95 (33.4%) individuals were classified as obese and 109 (38.9%) as overweight. No association was found between the prevalence of obesity in the groups according to estimated eGFR (p=0.327). However, among traditional risk factors for developing CKD, obesity was the only factor that showed a significant difference between the two indigenous groups, with a higher prevalence in the group with the highest degree of urbanization: 43.7% among the Truka (42/96) and 28.8% among the Fulni-ô (53/184) (p=0.0124) (Table 1).

DISCUSSION

We identified a tendency of worse kidney function among the more urbanized Truka ethnicity when compared to the Fulni-ô people, suggesting that a more urbanized setting might be associated with worse kidney function. Additionally, our results also suggest that younger individuals are affected with intensity similar to the elderly among indigenous population with advanced urbanization levels.

The ELSA-Brazil cohort reported that 4.8% of the overall Brazilian population (n=14,636) had an eGFR below 60 mL/min/1.73 m^2, compared to 7.2% of the 153 self-declared indigenous participants[1]. Socioeconomic disadvantages do not seem to fully explain the higher prevalence of CKD among indigenous participants in the ELSA study, as the entire cohort had stable employment and a high level of education. In accordance with our results, the ELSA study findings might, at least in part, be explained by the fact that the indigenous participants have experienced acculturation in a highly urbanized setting.

In non-indigenous Brazilian adults, the prevalence of systemic arterial hypertension is 21.4%[13]. In our study population, the prevalence of systemic arterial hypertension was slightly higher. However, this prevalence is slightly lower than in other ethnic groups that have been studied previously[5,6]. Other traditional risk factors related to CKD, such as diabetes mellitus and obesity, also showed considerable prevalence.

In relation to diabetes mellitus, according to a recently conducted survey[14], the prevalence in the study groups was similar to that found in the Brazilian population and associated with CKD. This result may be closely linked to the high prevalence of obesity in this population, with an alarming prevalence of 33.4%. This number is higher than that found in a population-based survey conducted in Brazil (prevalence of 16.8% for men and 24.4% for women)[15], and much higher than that found in the Brazilian Amazon Region, i.e., 14.4% in the Parkatêjê people and 15% in the Aruák people[16]. Among the risk factors for developing CKD, obesity was the only factor for which there was a statistical difference between the two indigenous groups.

Our results are likely due to the greater proximity and integration of the study groups with neighboring non-indigenous population and, consequently, the incorporation of an urban lifestyle. Consequently, changing dietary habits, especially the increased consumption of industrial foods, lead to an increase in chronic noncommunicable diseases and cardiovascular risk[4,7]. In this perspective study, it is likely that younger generations of indigenous people come in contact sooner and have more contact with these aspects of urban life than their ancestors.

Our study has limitations for generalizing the results due to the small sample size and its cross-sectional nature, which does not allow for the establishment of the causality of the association. Another limitation stems from the ethnic and cultural diversity of Brazilian indigenous people, which makes the final analysis difficult. Nevertheless, our results are relevant because they present unpublished data on a theme that has been less studied among Brazilian indigenous people. Furthermore, they suggest the influential role of urbanization in the prevalence of CKD and warn of its high prevalence in indigenous communities, which is a situation that occurs with indigenous people in other countries[17]. Finally, when considering the exclusion criteria of the PAI study (a study designed for a group of generally healthy participants), the low percentage of elderly in the sample (14.3%), the use of only GFR to estimate CKD, the percentage of indigenous people with CKD, and mildly decreased excretory renal function (G2) are significant and cause concern.

Table 1. Characterization of the study population, according to estimated glomerular filtration rate (estimated glomerular filtration rate <60 and ≥60 mL/min/1.73 m²) and ethnic group (Fulni-ô and Truká indigenous people) (n=280).

Variables	eGFR				Ethnic group		
	Total n=280 (100%)	eGFR <60 n=12 (4.3%)	eGFR ≥60 n=268 (95.7%)	p-value	Fulni-ô n=184 (65.7%)	Truká n=96 (34.3%)	p-value
Ethnic group							
Fulni-ô	184 (65.7%)	6 (3.3%)	178 (96.7%)	0.389[c]	-	-	-
Truká	96 (34.3%)	6 (6.2%)	90 (93.8%)		-	-	-
Gender							
Female	182 (65%)	11 (6.1%)	171 (93.9%)	0.095[c]	121 (65.7%)	61 (63.5%)	0.712[c]
Male	98 (35%)	1 (1.0%)	97 (99.0%)		63 (34.3%)	35 (36.5%)	
Age (years)							
(Md; IQR)	46.0; 15.2	54.0; 12.5	45.5; 16.0	0.008[d]	46.0; 17	45.5; 9.2	0.791[d]
30–39 years	77 (27.5%)	0 (0.0%)	77 (100.0%)	0.015[d]	55 (29.9%)	22 (22.9%)	0.051[c]
40–49 years	90 (32.1%)	3 (3.3%)	87 (96.7%)		52 (28.2%)	38 (39.6%)	
50–59 years	73 (26.1%)	4 (5.5%)	69 (94.5%)		45 (24.5%)	28 (29.2%)	
60–70 years	40 (14.3%)	5 (12.5%)	35 (87.5%)		32 (17.4%)	8 (8.3%)	
Body mass index							
(Md; IQR)	27.5; 6.7	28.0; 2.2	27.3; 6.7	0.761[d]	27.0; 6.2	29.3; 7.0	0.028[d]
Low weight	1 (0.4%)	0 (0.0%)	1 (100.0%)	0.442[c]	1 (0.6%)	0 (0.0%)	0.238[c]
Normal	75 (26.8%)	2 (2.6%)	73 (97.3%)		54 (29.4%)	21 (21.9%)	
Overweight	109 (38.9%)	8 (7.3%)	101 (92.7%)		76 (41.3%)	33 (34.4%)	
Obesity I	68 (24.3%)	1 (1.5%)	67 (98.5%)		38 (20.6%)	30 (31.2%)	
Obesity II	18 (6.4%)	1 (5.5%)	17 (94.4%)		10 (5.4%)	8 (8.3%)	
Obesity III	9 (3.2%)	0 (0.0%)	9 (100.0%)		5 (2.7%)	4 (4.2%)	
Obesity (I, I, II)	95 (33.4%)	2/12 (16.7%)	93/268 (34.7%)	0.327[c]	53 (28.8%)	42 (43.7%)	0.012[c]
Presence of comorbidity							
No	184 (65.7%)	7 (3.8%)	178 (96.2%)	0.811[c]	125 (67.9%)	59 (61.5%)	0.278[c]
Yes	96 (34.3%)	5 (5.6%)	84 (94.4%)		59 (32.1%)	37 (38.5%)	
Type of comorbidity present							
Hypertension	69 (24.6%)	5 (7.2%)	64 (92.8%)	0.162[c]	41 (22.2%	28 (29.2%)	0.205[c]
Diabetes	26 (9.3%)	3 (11.5%)	23 (88.5%)	0.045[c]	20 (10.9%)	6 (6.3%)	0.206[c]
Dyslipidemia	12 (4.3%)	0 (0.0%)	12 (100.0%)	0454[c]	9 (4.9%)	3 (3.1%)	0.489[c]
Smokinga							
Active smoking	168 (79.6%)	5 (3.0%)	163 (97.0%)	0.491[c]	149 (93.2%)	19 (37.2%)	<0.001[c]
Never smoked	12 (5.7%)	0	12 (100.0%)		9 (5.6%)	3 (5.9%)	
Stopped smoking	31 (14.7%)	2 (6.4%)	29 (94.6%)		2 (1.2%)	29 (56.8%)	
Alcoholismb							
Active drinking	63 (26.2%)	3 (4.8%)	60 (95.2%)	0.532[c]	34 (21.1%)	29 (36.7%)	0.015[c]
Never drank	134 (55.9%)	5 (3.7%)	129 (96.3%)		101 (62.7%)	33 (41.8%)	
Stopped drinking	43 (17.9%)	4 (9.3%)	39 (90.7%)		26 (16.2)	17 (21.5%)	
eGFR							
≥90 mL/min/1.73 m²	-	-	-	-	100	62	0.068
60–89 mL/min/1.73 m²	-	-	-	-	78	28	
<60 mL/min/1.73 m²	-	-	-	-	6	6	

[a]Assessed in 211 individuals. [b]Data from 240 individuals. [c]Chi-squared continuity correction. [d]Mann-Whitney U test. Md: median; IQR: interquartile range.

CONCLUSION

A higher degree of urbanization seems to negatively influence the prevalence of kidney disease in Brazilian indigenous people, which is an important concern in assessing the youth in indigenous communities.

AUTHORS' CONTRIBUTIONS

OVG: Conceptualization, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **CDFS:** Conceptualization, Formal Analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **ACA:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **MPG:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **JMN:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **LM:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **AMLS:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **JCMJ:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **MBN:** Project administration, Supervision, Visualization. **JACL:** Project administration, Supervision, Visualization.

REFERENCES

1. Barreto SM, Ladeira RM, Duncan BB, Schmidt MI, Lopes AA, Benseñor IM, et al. Chronic kidney disease among adult participants of the ELSA-Brasil cohort: association with race and socioeconomic position. J Epidemiol Community Health. 2016;70(4):380-9. https://doi.org/10.1136/jech-2015-205834
2. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3(1):1-150. Available from: https://kdigo.org/wp-content/uploads/2017/02/KDIGO_2012_CKD_GL.pdf
3. Huria T, Pitama SG, Beckert L, Hughes J, Monk N, Lacey C, et al. Reported sources of health inequities in Indigenous Peoples with chronic kidney disease: a systematic review of quantitative studies. BMC Public Health. 2021;21(1):1447. https://doi.org/10.1186/s12889-021-11180-2
4. Patriota PVAM, Ladeia AMT, Marques J, Khoury R, Barral A, Cruz AA, et al. Echocardiography and analysis of subclinical cardiovascular diseases in indigenous people living in different degrees of urbanization: project of atherosclerosis among indigenous populations (Pai). Arq Bras Cardiol: Imagem Cardiovasc. 2020;33(4):1-8. https://doi.org/10.47593/2675-312X/20203304eabc78
5. Souza Filho ZA, Ferreira AA, Dos Santos J, Meira KC, Pierin AMG. Cardiovascular risk factors with an emphasis on hypertension in the Mura Indians from Amazonia. BMC Public Health. 2018;18(1):1251. https://doi.org/10.1186/s12889-018-6160-8
6. Chagas CA, Castro TG, Leite MS, Viana MACBM, Beinner MA, Pimenta AM. Estimated prevalence of hypertension and associated factors in Krenak indigenous adults in the state of Minas Gerais, Brazil. Cad Saúde Pública. 2020;36(1):e00206818. https://doi.org/10.1590/0102-311X00206818
7. Armstrong AC, Ladeia AMT, Marques J, Armstrong DMFO, Silva AML, Morais Junior JC, et al. Urbanization is associated with increased trends in cardiovascular mortality among indigenous populations: the PAI study. Arq Bras Cardiol. 2018;110(3):240-5. https://doi.org/10.5935/abc.20180026
8. Armstrong A, Marques J, Patriota P, Armstrong D, Negreiro GH, Saad PF, et al. Urbanization and indigenous health in northeast Brazil—concepts of the project of atherosclerosis among indigenous populations (PAI). Navigating complexity: human–environmental solutions for a challenging future [Internet]. 2018. [cited on December 6, 2021]. Available from: https://sheconference2018.weebly.com/uploads/1/6/2/3/16236920/she_5.pdf
9. World Health Organization. World Health Organization: body mass index-BMI. 2021. [cited on June 23, 2021]. Available from: https://www.euro.who.int/en/health-topics/disease-prevention/%0Anutrition/a-healthy-lifestyle/body-mass-index-bmi
10. Fernanda M. Consolim-Colombo, José Francisco Kerr Saraiva, Maria Cristina Oliveira Izar. *Tratado de Cardiologia SOCESP*. 4a. SOCESP, editor. 2019.
11. Chaila MZ, Viniegra M, Gagliardino JJ, Martínez A, Simesen Bielke MG, Frusti M, et al. Glycated hemoglobin measurement: comparison of three methods versus high performance liquid chromatography. J Diabetes Sci Technol. 2022;16(3):724-31. https://doi.org/10.1177/1932296821997179
12. Faludi AA, Izar MCO, Saraiva JFK, Chacra APM, Bianco HT, Neto AA, et al. Atualização da diretriz Brasileira de dislipidemias e prevenção da aterosclerose – 2017. Arq Bras Cardiol. 2017;109(2 Suppl 1):1-76. https://doi.org/10.5935/abc.20170121
13. Instituto Brasileiro de Geografia e Estatística. Pesquisa nacional de saúde: percepção do estado de saúde, estilo de vida e doenças crônicas. Rio de Janeiro (BR): Instituto Brasileiro de Geografia e Estatística; 2014. Available from: https://ibge.gov.br/
14. Muzy J, Campos MR, Emmerick I, da Silva RS, Schramm JMA. Prevalence of diabetes mellitus and its complications and characterization of healthcare gaps based on triangulation of studies. Cad Saúde Pública. 2021;37(5):e00076120. https://doi.org/10.1590/0102-311X00076120
15. Ferreira APS, Szwarcwald CL, Damacena, GN. Prevalence of obesity and associated factors in the Brazilian population: a study of data from the 2013 National Health Survey. Rev Bras Epidemiol. 2019;22:e190024. https://doi.org/10.1590/1980-549720190024
16. Ingaramo RA. Obesity, diabetes, and other cardiovascular risk factors in native populations of South America. Curr Hypertens Rep. 2016;18(1):9. https://doi.org/10.1007/s11906-015-0613-6
17. Yeates KE, Cass A, Sequist TD, McDonald SP, Jardine MJ, Trpeski L, et al. Indigenous people in Australia, Canada, New Zealand and the United States are less likely to receive renal transplantation. Kidney Int. 2009;76(6):659-64. https://doi.org/10.1038/ki.2009.236

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220936

Cardiovascular disease risk prediction in scleroderma

Aliye Çelikkol[1]*, Rıdvan Mercan[2], Savaş Güzel[1], Ahsen Yılmaz[1]

SUMMARY

OBJECTIVE: Cardiovascular disease risk prediction in scleroderma is important. In this study of scleroderma patients, the aim was to investigate the relationship between cardiac myosin-binding protein-C, sensitive troponin T, and trimethylamine N-oxide and cardiovascular disease risk with the Systematic COronary Risk Evaluation 2 model of the European Society of Cardiology.

METHODS: Systematic COronary Risk Evaluation 2 risk groups of 38 healthy controls and 52 women with scleroderma were evaluated. Cardiac myosin-binding protein-C, sensitive troponin T, and trimethylamine N-oxide levels were analyzed with commercial ELISA kits.

RESULTS: In scleroderma patients, cardiac myosin-binding protein-C and trimethylamine N-oxide levels were higher than healthy controls but sensitive troponin T was not ($p<0.001$, $p<0.001$, and $p=0.274$, respectively). Out of 52 patients, 36 (69.2%) were at low risk, and the other 16 (30.8%) patients were at high-moderate risk with the Systematic COronary Risk Evaluation 2 model. At the optimal cutoff values, trimethylamine N-oxide could discriminate high-moderate risk with sensitivity 76%, specificity 86% and cardiac myosin-binding protein-C with sensitivity 75%, specificity 83%. Patients with high trimethylamine N-oxide levels (≥10.28 ng/mL) could predict high-moderate- Systematic COronary Risk Evaluation 2 risk 15 times higher than those with low trimethylamine N-oxide (<10.28 ng/mL) levels (odds ratio [OR]: 15.00, 95%CI 3.585–62.765, $p<0.001$). Similarly, high cardiac myosin-binding protein-C (≥8.29 ng/mL) levels could predict significantly higher Systematic COronary Risk Evaluation 2 risk than low cardiac myosin-binding protein-C (<8.29 ng/mL) levels (OR: 11.00, 95%CI 2.786–43.430).

CONCLUSION: Noninvasive cardiovascular disease risk prediction indicators in scleroderma, cardiac myosin-binding protein-C, and trimethylamine N-oxide could be recommended to distinguish between high-moderate risk and low risk with the Systematic COronary Risk Evaluation 2 model.

KEYWORDS: Heart disease risk factors. Myosin-binding protein C. Troponin T. Trimethyloxamine. Scleroderma, systemic.

INTRODUCTION

Scleroderma (SSc) is a rare connective tissue disease characterized by endothelial dysfunction, dysregulation of innate and adaptive immunity, and diffuse fibrosis[1]. Cardiopulmonary complications such as heart failure, pulmonary fibrosis, and hypertension are the leading causes of death in SSc[2].

Troponins as cardiac biomarkers emerged as an indicator of myocyte necrosis and damage[3]. Except for cardiac ischemia, cardiac troponin-T (cTrT) levels are known to be an important marker for mortality in other heart diseases[4]. Cardiac myosin-binding protein-C (cMBPC) is a sarcomeric thick filament protein which is crucial in regulating sarcomere structure and function in the heart[5]. The increases and decreases in serum levels of cMBPC following defined myocardial injury are faster than those of sensitive troponin T (sTrT)[6]. cMBPC is degraded after myocardial infarcts, and its fragments cause disturbances in calcium transitions and heart failure in cardiomyocyte cultures[7].

The gut microbiota metabolizes dietary choline, phosphatidylcholine, L-carnitine, and betaine to produce trimethylamine N-oxide (TMAO). High levels of TMAO increase the risk of kidney failure, diabetes mellitus, heart failure, atherosclerosis, hypertension, and cancer and can lead to serious cardiovascular events including death[8,9].

This study investigated the differences in sTrT, cMBPC, and TMAO levels between the low-risk group and the high-moderate-risk group of CVDs with the Systematic COronary Risk Evaluation 2 (SCORE2) risk model in SSc.

METHODS

The study protocol was endorsed by the relevant ethics committee with protocol number 2021.232.09.18. All study participants

[1]Tekirdağ Namık Kemal University, Faculty of Medicine, Department of Medical Biochemistry – Tekirdağ, Turkey.
[2]Tekirdağ Namık Kemal University, Faculty of Medicine, Department of İnternal Medicine, Section of Rheumatology – Tekirdağ, Turkey.
*Corresponding author: acelikkol@nku.edu.tr

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on August 29, 2022. Accepted on October 30, 2022.

gave written informed consent before sample collection or the questionnaire interview.

Participants

This study included 52 female patients with SSc and 38 healthy women aged 18–65 years who applied to the rheumatology outpatient clinic of the research hospital. All the patients fulfilled the new American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2013 classification criteria for SSc[10] and were classified in a limited or diffuse subset according to LeRoy classification[11]. The extension of cutaneous involvement was evaluated with a modified Rodnan skin score[12]. Hypertension was defined by antihypertensive medication or systolic blood pressure (BP) of 140 mm Hg or diastolic BP of 90 mm Hg on at least two occasions. Ejection fraction and pulmonary arterial pressure were evaluated using echocardiography. Patients were evaluated using the Medsger SSc Severity Scale[13] and the SCORE2 model.

Exclusion criteria were pregnancy, symptoms of heart failure, including dyspnea; venous swelling and recent major lower extremity edema; kidney involvement; or serious disease complications, such as cancer or gangrene.

Systematic coronary risk evaluation 2 risk prediction of cardiovascular disease

According to the most recent reports of the World Health Organization, European countries were grouped into four risk regions per 100,000 population, age, and sex standardized overall CVD mortality rates (ICD sections 9, I00-I99). SCORE2 risk models are designed for use in people aged 40–69 years. Since our country was in the high-risk group, it was evaluated using the table used for high-risk countries (C). SCORE2 risk was evaluated according to age, gender, systolic BP, and non-HDL-C, and a value of <10 was defined as low risk, between ≥10 and 20 as moderate risk, ≥20–30 as high risk, and ≥30 as very high risk of CVD[14].

Laboratory assessments

The serum routine biochemical tests were analyzed using Cobas c8000 (Roche Diagnostics; Geneva, Switzerland). Investigated tests were studied with commercial ELISA kits (cMBP-C, catalog no: E3757Hu; sTrT, catalog no: E4862Hu; TMAO, catalog no: E4733Hu).

Statistical analysis

Mean and standard deviation values were given for normal distribution, and the Student's t-test and one-way analysis of variance test were applied. The Mann-Whitney U test was used for the variables that did not have normal distribution. The best cutoff points were calculated using the receiver operating characteristic (ROC) curve for the prediction of CVD risk with the SCORE2 risk model. Univariate and multivariate analyses were performed using a logistic regression model. The odds ratio (OR) was reported with the corresponding 95%CI, and a $p<0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS Statistic version 22.0 (SPSS Inc., Chicago, IL) software.

RESULTS

The study cohort consisted of 52 SSc patients (11 diffuse and 29 limited), Anti-SCL-70 antibody was positive in 14 (26.92%), and the anti-centromere antibody was positive in 16 (30.77%) patients. There were 28 (54%) patients with a disease duration of 6–10 years. There was no significant difference between the SSc patients and healthy groups in age distribution, and body mass index (BMI) ($p=0.096$ and $p=0.074$, respectively). Routine parameters of CRP, TChol, TG, HDL-C, and LDL-C levels were significantly higher in SSc patients compared to those in healthy subjects ($p=0.003$, $p<0.001$, $p<0.001$, $p<0.001$, and $p=0.007$, respectively).

Healthy subjects had low risk with the SCORE2 model (3.58 (1.0–10.0)) but SSc patients were in low-, moderate-, and high-risk groups (9.5 (1.0–29.0)). There was a significant difference between healthy subjects and SSc patients ($p<0.001$) (Table 1). While there was no significant difference in the sTrT levels between the SSc patient and control groups, cMBPC and TMAO were significantly higher in SSc patients ($p=0.274$, $p<0.001$, and $p<0.001$, respectively).

In the CVD risk prediction of SSc patients with the SCORE2 model, 36 (69.2%) of 52 patients were at low risk, and the other 16 (30.8%) patients were at high-moderate risk (Table 1). In this study, cMBPC and TMAO levels in the low-risk group were lower than those in the high-moderate-risk group (both $p<0.001$) (Figure 1), but there was no significant change in sTrT levels ($p=0.297$).

The ideal cutoff value for predicting SCORE2 high-moderate risk for TMAO, cMBPC, and sTrT in SSc patients was calculated separately by ROC analysis (Figure 1). The optimal cutoff value for TMAO was 10.28 ng/mL (AUC: 0.873, 95%CI 0.773–973, $p<0.001$), and the ideal cutoff value for cMBPC was 8.29 ng/mL (AUC: 0.816, 95%CI 0.684–0.948, $p<0.001$). For sTrT (AUC: 0.595, 95%CI 0.422–0.769, $p=0.276$), the median value was determined as the cutoff value. In terms of diagnostic value, sensitivity was 75% and specificity was 83% for cMBPC, and sensitivity was 76% and specificity was 86% for TMAO.

Table 1. Demographic and laboratory characteristics of participants.

	Healthy group (n=38)	SSc patients (n=52)
	Mean±SD/ (min-max)	Mean±SD/ (min-max)/n (%)
Demographic characteristics		
Age (year)	51.263±8.83	54.35±8.28
BMI (kg/m²)	28.78±2.21	29.19±3.45
Laboratory characteristics		
Glucose (mg/dl)	93.21±6.52	103.87±17.08***
Creatinine (mg/dl)	0.65±0.09	0.67±0.18
CRP (mg/l)	1.72 (0.2–5.0)	2.24 (0.15–5.93)
TChol (mg/dl)	156.02±15.22	236.23±47.03***
TG (mg/dl)	101.74±29.07	186.86±58.94***
HDL-C (mg/dl)	53.39±11.04	46.83±11.39**
LDL-C (mg/dl)	80.70±13.40	148.57±43.17***
Non-HDL-C (mmol/l)	3.35±0.73	4.96±1.12***
SCORE2	3.58 (1.0–10.0)	9.5 (1.0–29.0)***
cMBPC (ng/mL)	4.92±1.18	8.03±3.29***
sTrT (ng/L)	55.94±20.41	60.13±15.66
TMAO (ng/mL)	6.90±1.23	10.45±3.01***
Clinical characteristics		
Current smoking		22 (42.30)
Disease duration		
1–5 years		14 (27)
6–10 years		28 (54)
>10 years		10 (19)
Diffuse SSc (positive)		11 (21.15)
Localized SSc (positive)		29 (55.77)
Digital ulcer (positive)		11 (21.15)
Digital gangrene (positive)		2 (3.85)
Telangiectasia (positive)		12 (23.08)
Proximal muscle weakness (positive)		14 (26.92)
Gastrointestinal symptoms (positive)		22 (42.31)
Pulmonary symptoms (positive)		4 (7.69)
Cardiovascular symptoms (hypertension)		23 (44.23)
Antibody characteristics		
Anti-cyclic citrullinated peptide (positive)		4 (7.69)
Rheumatoid factor (positive)		8 (15.38)
ANA cytoplasmic speckled (positive)		10 (19.23)
ANA anti-centromere (positive)		16 (30.77)
ANA SCL-70 (positive)		14 (26.92)
ENA anti-Ro (SSA) (positive)		10 (19.23)
ENA anti-centromere B (positive)		16 (30.77)
ENA anti-SCL-70 (positive)		17 (32.69)
ENA Ro-52 recombinant (positive)		11 (21.15)

p<0.05*, p<0.01**, p<0.001***. SSc: scleroderma; SD: standard deviation; min: minimum; max: maximum; BMI: body mass index; cMBPC: cardiac myosin-binding protein-C; sTrT: sensitive troponin T; TMAO: trimethylamine N-oxide; CRP: c-reactive protein; TChol: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SCORE2: the Systematic COronary Risk Evaluation 2 model; ANA: antinuclear antibody; ENA: extractable nuclear antigens; ANA SCL-70: anti-topoisomerase I antibodies (Anti-Scleroderma); ENA anti-SCL 70: extractable nuclear antigens anti-scleroderma antibodies; Ro-52: tripartite motif-containing protein 21.

In SSc patients, SCORE2 model levels were correlated with age (r=638, p<0.001, disease duration (r=-0.406, p=0.032), cMBPC (r=0.319, p=0.021), sTrT (r=0.346, p=0.012), and TMAO (r=0.383, p=0.005) but not correlated with none-HDL-C and BMI.

While cMBPC and sTrT were not correlated in SSc patients, TMAO was correlated with both cMBPC and sTrT (with cMBPC r=0.689, p<0.001; with sTrT r=0.355, p=0.010).

The univariate analysis was established to predict high-moderate SCORE2 risk. Those with high TMAO levels (≥10.28 ng/mL) predicted high-moderate risk 15 times higher than those with low (<10.28 ng/mL) levels (OR: 15.00, 95%CI 3.585–62.765, p<0.001). Similarly, high cMBPC (≥8.29 ng/mL) levels predicted significantly higher SCORE2 risk than low cMBPC (<8.29 ng/mL) levels (OR: 11.00, 95%CI 2.786–43.430, p=0.001) (Table 2).

When high TMAO (OR 9.405, 95%CI 2.020–43.791, p=0.003) and high cMBPC (OR 6.236, 95%CI 1.329–29.256, p=0.020) were evaluated together in the multivariate analysis, SCORE2 showed the predictive model feature in estimating high risk (Table 2).

DISCUSSION

Since the risk of CVD is high in SSc, it is necessary to focus on early risk prediction and to identify risk factors[5]. This study presented two main findings: (1) SSc patients could be distinguished for CVD risk prediction with the SCORE2 model and (2) TMAO and cMBPC were associated with SCORE2 risk. Recent studies have reported the evaluation of sTrT levels as the best indicator and risk marker of CVD in SSc. Avouac et al. and Bosello et al. reported higher sTrT level in patients with SSc than in the control group[3,4]. Both studies investigated sTrT levels in patients with SSc who had CVD complications. Contrary to these findings, in our study, sTrT levels were similar in SSc patients compared to those in healthy controls (p=0.274) (Table 1). However, in terms of the role of cardiac biomarkers in detecting early cardiac disease, there was no significant relationship in studies with cardiac-MRI[15]. Although studies reported hs-cTnT association with both systolic and diastolic function abnormalities, Ross et al. reported that there was a lack of definitive data to support their use[16]. The reason why our and similar studies did not find a significant difference regarding sTrT is that it would not be valuable in CVD risk prediction because of the increase in acute myocardial injury.

Due to these disadvantages in sTrT, the research for alternative CVD risk markers continues in SSc. One of these markers was cMBPC, which was reported in cardiac myocytes in greater

Table 2. Univariate and multivariate analysis of parameters for systematic coronary risk evaluation 2 high-risk prediction.

Variable	Category	Univariate analysis		Multivariate analysis	
		OR (95%CI)	p-value	OR (95%CI)	p-value
TMAO	<10.28/≥10.28	15.000 (3.585–62.765)	**<0.001**	9.405 (2.020–43.791)	**0.004**
cMBPC	8.29/≥8.29	11.000 (2.786–43.430)	**0.001**	6.236 (1.329–29.256)	**0.020**
sTrT	52.24/≥52.24	1.437 (0.439–4.699)	0.549		
CRP	Continuous	0.989 (0.849–1.152)	0.883		
LDL-C	Continuous	1.003 (0.989–1.017)	0.656		
ALB	Continuous	1.965 (0.423–9.140)	0.389		
BMI	Continuous	1.301 (1.033–1.640)	**0.025**		
C3	Continuous	0.695 (0.068–7.098)	0.759		
C4	Continuous	0.004 (<0.001–6.824)	0.147		
IgM	Continuous	0.754 (0.384–1.484)	0.414		
IgG	Continuous	0.938 (0.779–1.131)	0.505		
IgA	Continuous	0.782 (0.433–1.412)	0.414		

Statistically significant p-values are marked in bold. Multivariate analysis was created using the Forward-LR model. TMAO: trimethylamine N-oxide; cMBPC: cardiac myosin-binding protein-C; sTrT: sensitive troponin T; CRP: C-reactive protein; LDL-C: low-density lipoprotein; ALB: albumin; BMI: body mass index; C3: complement 3; C4: complement 4; IgM: immunoglobulin M; IgG: immunoglobulin G; IgA: immunoglobulin A.

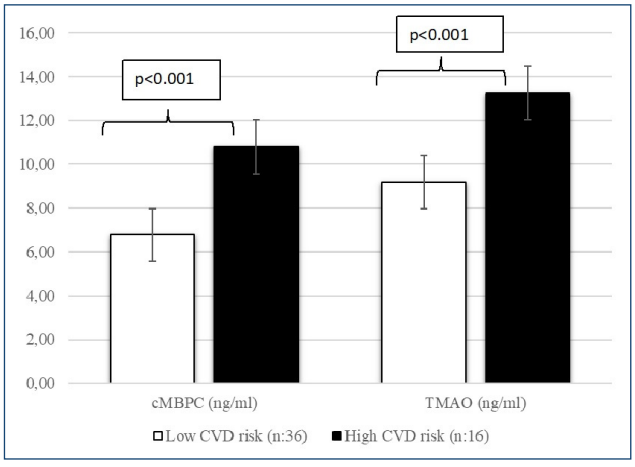

Figure 1. Changes of parameters with low-risk and high-moderate risk in systematic coronary risk evaluation 2 model.

amounts than sTrT. cMBPC was released more rapidly after acute myocardial infarction (AMI) and has been studied as a new cardiac protein of CVD indicator. In literature, cMBPC concentrations in AMI were significantly higher than those without AMI[17]. After a myocardial injury, cMBPC could be detected earlier in the blood, and its concentration has been reported to rise faster and more sensitively than cTnT/I or the new RNA biomarkers[17,18]. To the best of our knowledge, this study was the first to investigate cMBPC levels in SSc. Significantly higher cMBPC levels were found in the SSs patients than in healthy controls (p<0.001) (Table 1).

Trimethylamine N-oxide, an indicator for CVD risk in recent years, is a molecule produced by intestinal bacteria, which is thought to have a powerful effect on human life[14]. TMAO was strongly associated with atherosclerosis[19,20]. However, no study was found in the literature related to TMAO levels in SSc patients. In this study, TMAO levels in SSc patients were significantly higher than those in healthy subjects (p<0.001) (Table 1). It would be better to evaluate TMAO and cMBPC levels in determining cardiac risk in SSc. At the same time, the presence of a positive correlation between sTrT and TMAO levels in our study supports the notion that TMAO is associated with CVD risk.

In this study, we evaluated SSc patients according to the new SCORE2 risk estimation algorithm. Only limited studies are available on this subject. Ozen et al. underestimated the risk of subclinical atherosclerosis in SSc patients using the previous SCORE risk estimation model[21]. Along with the SCORE, the 2013 American College of Cardiology and American Heart Association (ACC/AHA) risk indexes were also reported as insufficient[22]. Similarly, Kurman et al. revealed the inadequacy of the Framingham risk score and ACC/AHA risk indexes in estimating CVD risk[23]. In this study, although there was an increase in serum sTrT levels in the group with high CVD risk in SSc patients separated according to SCORE2, no significant difference was found (p=0.297). Contrary to our findings, Barsotti et al. reported higher sTrT levels in high-risk SSc patients according to the heart involvement index, with the presence of conditions such as unexplained hypertension,

ischemic heart disease, smoking, congenital heart disease, and diastolic dysfunction[24]. Similarly, De Luca et al. reported high sTrT values in 58.1% of SSc patients with arrhythmia and ventricular ectopic beats[25]. However, the performance of sTrT levels was not sufficient in both studies.

In this study, cMBPC and TMAO levels were significantly higher in the high-moderate-risk group than in the low-risk group (both p<0.001) (Figure 1). For SCORE2, high-moderate-risk prediction with optimal cutoff values determined in the ROC curves of TMAO and cMBPC could discriminate cMBPC with 75% sensitivity and 83% specificity and TMAO with 76% sensitivity and 86% specificity. Both parameters gave better results than values for cTrT in a study by Barsotti et al.[24]. cMBPC and TMAO could be used as risk indicators and are compatible with SCORE2 in the evaluation of CVD risk.

In the univariate regression analysis, those with a high TMAO had a 15 times higher efficiency in predicting patients with a high SCORE2 value compared to those with a low value and, similarly, with high cMBPC levels compared to low levels. In the multivariate analysis, high TMAO and high cMBPC were determined as a predictive model feature for estimating high SCORE2 risk. Our findings are a unique and important addition to the literature, and to the best of our knowledge, this is the first study in this evolving field.

Our study should be interpreted with its limitations. The main limitation is the small sample size. Although TMAO levels could be affected by diet, geographical region characteristics and dietary habits were not detailed. Interferences with the tests were ignored.

CONCLUSION

While sTrT did not increase significantly in SSc patients, cMBPC and TMAO levels were higher than those in healthy controls. In addition, cMBPC and TMAO distinguished between high-moderate-risk and low-risk SCORE2 groups as noninvasive CVD risk estimation indicators. Further research is warranted to develop better CVD risk prediction tools in SSc. It is recommended that TMAO and cMBPC levels could be evaluated in order to estimate the 10-year risk of CVD death with SCORE2 in SSc patients.

AUTHORS' CONTRIBUTIONS

AÇ: Conceptualization, Data curation, Formal Analysis, İnvestigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **RM:** Conceptualization, Data curation, Formal Analysis, İnvestigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. **SG:** Conceptualization, Data curation, Formal Analysis, İnvestigation, Resources, Supervision, Writing – review & editing. **AY:** Conceptualization, Data curation, Formal Analysis, İnvestigation, Methodology, Project administration, Writing – original draft.

REFERENCES

1. Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. Curr Opin Rheumatol. 2012;24(2):165-70. https://doi.org/10.1097/BOR.0b013e32834ff2e8
2. Simeón-Aznar CP, Fonollosa-Plá V, Tolosa-Vilella C, Espinosa-Garriga G, Campillo-Grau M, Ramos-Casals M, et al. Registry of the Spanish network for systemic sclerosis: survival, prognostic factors, and causes of death. Medicine (Baltimore). 2015;94(43):e1728. https://doi.org/10.1097/MD.0000000000001728
3. Avouac J, Meune C, Chenevier-Gobeaux C, Borderie D, Lefevre G, Kahan A, et al. Cardiac biomarkers in systemic sclerosis: contribution of high-sensitivity cardiac troponin in addition to N-terminal pro-brain natriuretic peptide. Arthritis Care Res. 2015;67(7):1022-30. https://doi.org/10.1002/acr.22547
4. Bosello S, De Luca G, Berardi G, Canestrari G, de Waure C, Gabrielli FA, et al. Cardiac troponin T and NT-proBNP as diagnostic and prognostic biomarkers of primary cardiac involvement and disease severity in systemic sclerosis: a prospective study. Eur J Intern Med. 2019;60:46-53. https://doi.org/10.1016/j.ejim.2018.10.013
5. Offer G, Moos C, Starr R. A new protein of the thick filaments of vertebrate skeletal myofibrils. Extractions, purification and characterization. J Mol Biol. 1973;74(4):653-76. https://doi.org/10.1016/0022-2836(73)90055-7
6. Tong CW, Nair NA, Doersch KM, Liu Y, Rosas PC. Cardiac myosin-binding protein-C is a critical mediator of diastolic function. Pflugers Arch Eur J Physiol. 2014;466(3):451-7. https://doi.org/10.1007/s00424-014-1442-1
7. Witayavanitkul N, Mou YA, Kuster DWD, Khairallah RJ, Sarkey J, Govindan S, et al. Myocardial infarction-induced N-terminal fragment of cardiac myosin-binding protein C (cMyBP-C) impairs myofilament function in human myocardium. J Biol Chem. 2014;289(13):8818-27. https://doi.org/10.1074/jbc.M113.541128
8. Gątarek P, Kałużna-Czaplińska J. Trimethylamine n-oxide (TMAO) in human health. EXCLI J. 2021;20:301-19. https://doi.org/10.17179/excli2020-3239
9. Lippi G, Danese E, Mattiuzzi C, Favaloro EJ. The intriguing link between the intestinal microbiota and cardiovascular disease. Semin Thromb Hemost. 2017;43(6):609-13. https://doi.org/10.1055/s-0036-1597903
10. Van Den 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

11. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol. 1988;15(2):202-5. PMID: 3361530
12. Khanna D, Furst DE, Clements PJ, Allanore Y, Baron M, Czirjak L, et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. J Scleroderma Relat Disord. 2017;2(1):11-8. https://doi.org/10.5301/jsrd.5000231
13. Medsger TA, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, Bencivelli W, et al. Assessment of disease severity and prognosis. Clin Exp Rheumatol. 2003;21(Suppl. 29):S42-6. PMID: 12889222
14. SCORE2 Working Group and ESC Cardiovascular Risk Collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021;42(25):2439-54. https://doi.org/10.1093/eurheartj/ehab309
15. Sugiyama K, Kobayashi H, Kobayashi Y, Yokoe I, Takei M, Kitamura N, et al. Association of cardiac magnetic resonance-detected myocardial abnormalities with disease characteristics and brain natriuretic peptide levels in systemic sclerosis without cardiac symptoms. Int J Rheum Dis. 2019;22(6):1016-22. https://doi.org/10.1111/1756-185X.13540
16. Ross L, Moxey J, Nikpour M. Are troponin and B-type natriuretic peptides useful biomarkers for the diagnosis of systemic sclerosis heart involvement? A systematic literature review. Semin Arthritis Rheum. 2021;51(1):299-309.https://doi.org/10.1016/j.semarthrit.2020.10.016
17. Kaier TE, Alaour B, Marber M. Cardiac myosin-binding protein C—from bench to improved diagnosis of acute myocardial infarction. Cardiovasc Drugs Ther. 2019;33(2):221-30. https://doi.org/10.1007/s10557-018-6845-3
18. Barefield D, Sadayappan S. Phosphorylation and function of cardiac myosin binding protein-C in health and disease. J Mol Cell Cardiol. 2010;48(5):866-75. https://doi.org/10.1016/j.yjmcc.2009.11.014
19. Yang W, Zhang S, Zhu J, Jiang H, Jia D, Ou T, et al. Gut microbe-derived metabolite trimethylamine N-oxide accelerates fibroblast-myofibroblast differentiation and induces cardiac fibrosis. J Mol Cell Cardiol. 2019;134:119-30. https://doi.org/10.1016/j.yjmcc.2019.07.004
20. Dalla Via A, Gargari G, Taverniti V, Rondini G, Velardi I, Gambaro V, et al. Urinary TMAO levels are associated with the taxonomic composition of the gut microbiota and with the choline TMA-lyase gene (cutC) harbored by enterobacteriaceae. Nutrients. 2020;12(1):62. https://doi.org/10.3390/nu12010062
21. Ozen G, Inanc N, Unal AU, Korkmaz F, Sunbul M, Ozmen M, et al. Subclinical atherosclerosis in systemic sclerosis: not less frequent than rheumatoid arthritis and not detected with cardiovascular risk indices. Arthritis Care Res. 2016;68(1):1538-46. https://doi.org/10.1002/acr.22852
22. Sanz Pérez I, Martínez Valle F, Guillén-del-Castillo A, Roque Pérez A, Cuéllar Calàbria H, Pizzi MN, et al. Subclinical cardiovascular disease and systemic sclerosis: a comparison between risk charts, quantification of coronary calcium and carotid ultrasonography. Autoimmun Rev. 2018;17(9):900-5. https://doi.org/10.1016/j.autrev.2018.03.015
23. Kurmann RD, Sandhu AS, Crowson CS, Matteson EL, Osborn TG, Warrington KJ, et al. Cardiovascular risk factors and atherosclerotic cardiovascular events among incident cases of systemic sclerosis: results from a population-based cohort (1980-2016). Mayo Clin Proc. 2020;95(7):1369-78. https://doi.org/10.1016/j.mayocp.2019.12.015
24. Barsotti S, Bruni C, Orlandi M, Della Rossa A, Marasco E, Codullo V, Guiducci S. One year in review 2017: systemic sclerosis. Clin Exp Rheumatol. 2017;35(Suppl. 106)(4):3-20. PMID: 29035173
25. De Luca G, Bosello SL, Gabrielli FA, Berardi G, Parisi F, Rucco M, et al. Prognostic role of ventricular ectopic beats in systemic sclerosis: a prospective cohort study shows ECG indexes predicting the worse outcome. PLoS One. 2016;11(4):e0153012. https://doi.org/10.1371/journal.pone.0153012

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220945

Perceptions of the Brazilian obstetrics physicians about the term obstetric violence: a cross-sectional study

Diogo Coutinho Terribile[1], Carlos Izaias Sartorao Filho[1,2*]

SUMMARY

INTRODUCTION: We observe a growing global discussion about the practices considered "obstetric violence" against women during pregnancy and childbirth. Otherwise, the indiscriminate subjective and lay interpretation of the term "obstetric violence" can lead to a misunderstanding among medical professionals.

OBJECTIVE: This study aimed to describe the obstetrician's perceptions about the term "obstetric violence" and the medical groups affected negatively by the topic.

METHODS: A cross-sectional study applied to Brazilian obstetrics physicians regarding their perceptions of "obstetric violence."

RESULTS: From January to April 2022, we sent about 14,000 direct mail nationwide. A total of 506 participants responded. We observed that 374 (73.9%) participants consider the term obstetric violence nocive or harmful to professional practice. Furthermore, after Poisson regression, we described that the respondents who graduated before 2000 and from a private institution were significant and independent groups for the full or partial agreement that the term is nocive for the obstetricians in Brazil.

CONCLUSION: We observed that almost three in four obstetrician participants consider the term "obstetric violence" nocive or harmful to professional practice, particularly in those who graduated before 2000 and from a private institution. The findings are relevant to propose further debates and strategies to mitigate the possible harms caused to the obstetrician team by the indiscriminate use of the term obstetric violence.

KEYWORDS: Gender-based violence. Physician-patient relations. Professional–-patient relations. Obstetrics. Pregnancy. Parturition.

INTRODUCTION

We observe a growing global discussion about the practices considered "obstetric violence" against women during pregnancy and childbirth[1]. Any interactions that offend the dignity and autonomy of pregnant women to achieve a desired or imagined result by obstetricians and staff can lead to a rupture in the doctor–-patient relationship and can be considered "obstetric violence[2]." The World Health Organization is concerned with the issues involving disrespect and abuse during childbirth, prioritizing evaluation, prevention, and elimination of these practices[3]. There are many efforts to recognize and change this condition, avoiding medical hospital cultures that can perpetuate practices and behaviors harmful to human beings[4]. In contrast, the indiscriminate use of subjective and lay interpretation of the term "obstetric violence" can lead to a misunderstanding that negatively affects the doctor–-patient relationship.

The Spanish Society of Gynecology and Obstetrics recently issued an official web communication on the subject: "We find the term 'obstetric violence' inappropriate, biased and unfair because of its malicious legal meaning, as an intention to cause harm, to injure, use force or threat, criminally liable, which we must reject completely." Furthermore, we consider that the widespread use of the term "obstetric violence" can compromise the behavior of the professional team involved in the care of pregnant women. Therefore, the topic is very relevant to be studied to understand the interpretation and attitudes of the professionals involved in obstetric care. The study can guide future interventions to minimize the effects of compromise in the doctor–-patient relationship in obstetrics care. The need for good care and compliance with good practices in obstetrics care is undeniable. However, we hypothesize that obstetricians disagree with using the generalized form of the term "obstetric violence" as it has usually been propagated.

OBJECTIVE

The aim of this study was to describe the perceptions of the obstetrics medical professional on the term "obstetric

[1]Fundação Educacional do Município de Assis, Faculty of Medicine – Assis (SP), Brazil.
[2]Universidade Estadual Paulista "Júlio de Mesquita Filho, Botucatu Medical School – São Paulo (SP), Brazil.
*Corresponding author: carlos.sartorao@unesp.br
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 15, 2022. Accepted on October 12, 2022.

violence." From the obstetrician's point of view, to describe whether the term "obstetric violence" in Brazil may be nocive to the doctor–-patient relation. To estimate the percentage of Brazilian obstetricians who agree and those who disagree with the term "obstetric violence" besides the medical groups more affected.

METHODS

We developed a cross-sectional study at the Faculty of Medicine of Educational Foundation of Assis Municipality, São Paulo, Brazil. The recruitment period was from January to April 2022. We collected data from an anonymous web-based questionnaire sent by email to Brazilian obstetricians and gynecologists. According to the latest publication in 2018 by the Federal Medicine Council, there are more than 30,000 gynecologists in Brazil. In addition, we obtained direct mail contact from about 14,000 obstetricians and gynecologists provided by the Brazilian Federation of Ob/Gyn societies and the Regional Councils of Medicine from each state nationwide. For this estimated population, using a 5% sampling error, 95% confidence level, and more heterogeneous population distribution (50/50), the estimated sample size was 380 respondents.

Inclusion criteria were as follows: physicians registered with the Regional Councils of Medicine of Brazil who work in obstetrics. Exclusion criteria included responses to questionnaires with inconsistent answers or missing data. Study variables included the sociodemographic profile; whether or not to agree with the term obstetric violence; whether the regulation of laws or regulations on obstetric violence is beneficial or harmful for the doctor–-patient relation; and working or not in obstetrics for the last 5 years. Therefore, participation in this research does not violate legal and ethical standards. Data were collected using an online email questionnaire sent to Brazilian physicians working in obstetrics nationwide. The invitation letter was sent through direct mail, endorsed, and posted by The Obstetrics and Gynecology Society of Sao Paulo State (SOGESP) to all the physicians included in the association. The research obtained the official authorization from the Ethics Committee Institution under number 51946721.3.0000.8547. The participants who agreed obtained access after agreeing to the informed consent form. The questionnaire contains sociodemographic profile (age, sex, state location), professional profile (year of graduation, the period in professional activity, graduation from a public or private medical school, the highest graduation, if worked as an obstetrician in the last 5 years, work institution public or not, hospital or not), and the guiding questions of the research objectives, as published in the supplementary file. According to the Likert scale, the number and percentage of physicians who agree or disagree with the term "obstetric violence" (disagree entirely, partially, neither disagree nor agree, partially agree, or totally agree). The number and percentage of physicians agree with the possibility that the term "obstetric violence" harms the obstetrician's practice of medicine. Data collected were analyzed according to the outcomes using statistical methods to determine the proposed objectives. The sample characteristics were presented in number and percentage rates. Then, the bivariate associations were estimated between each independent variable with the outcome of full or partial agreement about the assertion that the term obstetric violence may be harmful/nocive to professional practice. The dependent variables that showed bivariate association with $p \leq 0.20$ were taken to a multiple regression model with Poisson response. The associations were considered statistically significant in the final model if $p \leq 0.05$. The analysis was done using the SPSS v21 software (IBM, New York).

RESULTS

From January to April 2022, we sent approximately 14,000 direct mail nationwide with the invitation, consent form, and questionnaire for the obstetrician physicians in Brazil. A total of 510 participants responded, and 4 questionnaires were excluded due to inconsistent data on the association between age and the year of graduation. Thus, 506 participants were included (Table 1).

The number of participants who agreed that the term "obstetric violence" may be nocive for the professional practice was 374 (73.9%). In addition, 349 participants (68.9%) agreed that the term "obstetric violence" seems inappropriate, tendentious, and unfair. Moreover, 354 (70.5%) considered that the media placement of the term "obstetric violence" may be malefic to the doctor–-patient relation. Finally, 127 (25.1%) respondents disagreed that the "obstetric violence" term may be nocive.

We performed the bivariate association to investigate the population-stratified-dependent variables and the prevalence of agreement that the term use is nocive for professional practice.

The bivariate association for the prevalence of participants in full or partial agreement that the term "obstetric violence" may be nocive to the doctor–-patient relation demonstrates that male participants, graduation before the year 2000, and graduation from a private institution were significant statistically ($p<0.20$). Thus, we performed the Poisson regression to obtain the independent variables (Table 2).

Table 1. Participant characteristics (n=506).

Variable	n	%
Gender		
Female	293	57.9
Male	213	42.1
Median age in years (min–max)	47 (25–100)	
18–29	39	7.7
30–59	348	68.8
60–69	73	14.4
70 +	49	9.1
Graduation conclusion year		
1950–1959	3	0.6
1960–1969	10	2
1970–1979	50	9.9
1980–1989	86	17
1990–1999	106	20.9
2000–2009	100	19.7
2010–2019	144	28.5
2020–2022	7	1.4
Graduation conclusion period		
Before 2000	255.0	50.4
After 2000	251.0	49.6
Graduation institution		
Public	290	57.3
Private	216	42.7
Highest academic degree		
Medical graduation	13	2.6
Specialization course	43	8.5
Residence	310	61.3
Master's degree	67	13.2
Doctoral degree	57	11.3
Postdoctoral or highest	16	3.2
Private office working		
No	180	35.4
Yes	326	64.6
Private hospital working		
No	231	45.5
Yes	275	54.5
Public office working		
No	408	80.6
Yes	98	19.4
Public hospital working		
No	185	36.6
Yes	321	63.4
Preceptor working		
No	393	77.6
Yes	113	22.4
Public--private hospital		
No	399	78.8
Yes	107	21.2
Primary healthcare office		
No	425	84.0
Yes	81	16.0
Number of institutions areworking		
1	114	22.5
2	176	34.8
3	88	17.4
≥4	128	25.3
Primary Brazilian geographical region		
South	89	17.5
Southeast	332	65.5
North	18	3.6
Northeast	36	7.2
Midwest	31	6.2
Working in obstetrics in the last 5 years		
No	31	6.1
Yes	475	93.9
The context: it seems inappropriate, tendentiously, and unfair to use "obstetric violence"		
Fully disagree	122	24.1
Partially disagree	30	6.0
Neutral	5	1.0
Partially agree	69	13.6
Fully agree	280	55.3
Please give your opinion: "Using the term obstetric violence may be nocive for the professional practice, from the point of view of the self-judged qualified worker that ever practiced considered violent acts during your lifespan"		
Fully disagree	115	22.7
Partially disagree	12	2,4
Neutral	5	1.0
Partially agree	49	9.7
Fully agree	325	64.2
Is the "obstetric violence" media placement beneficial or malefic for the medical--patient relationship?		
Absolutely malefic	351	69.9
Relatively malefic	3	0.6
Indifferent	51	10.2
Absolutely beneficial	90	17.9
Other responses	7	1.4
I prefer not to respond	4	

Continue...

n: number of participants.

Table 2. Poisson regression to explain the prevalence of full or partial agreement about the harmful or nocive consideration of the term violence obstetric for the professional practice.

Variable	β	95%CI		PR	95%CI		p
(Intercept)	-0.332	-0.545	-0.119	0.72	0.58	0.89	0.002
Male gender	0.174	-0.045	0.394	1.19	0.96	1.48	0.119
Graduation after 2000	-0.361	-0.585	-0.136	0.70	0.56	0.87	0.002
Private medical school	0.241	0.037	0.444	1.27	1.04	1.56	0.020

β: beta coefficient; 95%CI: 95% confidence interval; PR: predictive risk. p<0.05.

After a Poisson regression, those respondents who graduated from private medical institutions remain statistically significant (β: 0.241; p: 0.020) to the full or partial agreement concerning the sentence that obstetric violence may be nocive to the professional practice. The respondents who graduated after 2000 had a significant negative β-coefficient (β: -0.361; p: 0.002); thus, we considered that graduation before 2000 was statistically significant.

DISCUSSION

We observed that almost three in four obstetrician respondents consider the term obstetric violence nocive or harmful to the professional practice in Brazil. Moreover, more than 70% responded that the media placement of obstetric violence is malefic to the medical–-patient relationship. Besides, the context seems inappropriate, tendentious, and unfair for more than 70%. The Spanish Gynecology and Obstetrics Society recently published a bulletin entitled "comunicado SEGO: "*violencia obstétrica*"" on their website (www.sego.es), positioning the same perception against the indiscriminate use of the term obstetric violence by the institution[5-7].

Furthermore, after Poisson regression, we described that the respondents who graduated before 2000 and graduated from a private medicine institution were significant and independent groups for the full or partial agreement that the term is nocive for the obstetricians in Brazil. However, we did not find similarities in the current literature to provide discussion concerning the graduation period and institution type.

The primary limitation is due to the cross-sectional study design, with no evidence of a temporal relationship between exposure and outcome. Second, the cross-sectional study may be prone to nonresponse bias when those who consent to participate in the study differ from those who do not, resulting in a sample not representative of the population. Third, we used a nonvalidated questionnaire. We did not find similar studies and valid questionnaires about the topic in the current literature.

Considering our objectives, limitations, analyses, and the lack of similar studies in the literature, we provide a relevant inference that the perception of the majority of obstetricians in Brazil concerning the term obstetric violence may be nocive and may cause damage to the professional behavior and doctor–-patient relationship.

Our study evaluated a nationwide proportion of obstetricians, and we consider the results consistent with validating externally, significantly nationwide in Brazil.

CONCLUSION

We described that more than 70% of Brazilian obstetricians in the survey considered the term obstetric violence harmful to professional practice. Physicians who graduated before 2000 and from a private medicine institution were the most influential groups following the perception that the term may be nocive for the doctor–-patient relation. Besides, the topic is considered very controversial. Nevertheless, it is evident that the abuse and disrespect during obstetric or female care need to be recognized and dealt with firmly. Therefore, the results of our study are relevant to propose further debates and strategies to mitigate the possible harms caused by the indiscriminate use of the term obstetric violence in Brazil.

ACKNOWLEDGMENTS

The authors thank Miss Cristiane Muniz from the Obstetrics and Gynecology Society of Sao Paulo State; Prof. Dr. Luiz Fernando Ferrari Neto from SINDHOSP; and Prof. Dr. Hélio Nunes for the statistical analysis.

AUTHORS' CONTRIBUTIONS

DCT: Conceptualization, Data curation, Format Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **CISF:** Conceptualization, Data curation, Format Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Scambia G, Viora E, Chiantera A, Colacurci N, Vicario M. "Obstetric violence": between misunderstanding and mystification. Eur J Obstet Gynecol Reprod Biol. 2018;228:331. https://doi.org/10.1016/j.ejogrb.2018.06.012
2. Garcia LM. A concept analysis of obstetric violence in the United States of America. Nurs Forum. 2020;55(4):654-63. https://doi.org/10.1111/nuf.12482
3. Shakibazadeh E, Namadian M, Bohren M, Vogel J, Rashidian A, Nogueira Pileggi V, et al. Respectful care during childbirth in health facilities globally: a qualitative evidence synthesis. BJOG Int J Obstet Gynaecol. 2018;125(8):932-42. https://doi.org/10.1111/1471-0528.15015
4. Denny E. Respectful maternity care needs to be the standard for all women worldwide. BJOG Int J Obstet Gynaecol. 2018;125(8):943. https://doi.org/10.1111/1471-0528.15055
5. Mena-Tudela D, Iglesias-Casás S, González-Chordá VM, Cervera-Gasch Á, Andreu-Pejó L, Valero-Chilleron MJ. Obstetric violence in Spain (Part I): women's perception and interterritorial differences. Int J Environ Res Public Health. 2020;17(21):7726. https://doi.org/10.3390/ijerph17217726
6. Mena-Tudela D, Iglesias-Casás S, González-Chordá VM, Cervera-Gasch Á, Andreu-Pejó L, Valero-Chilleron MJ. Obstetric violence in Spain (Part II): interventionism and medicalization during birth. Int J Environ Res Public Health. 2020;18(1):199. https://doi.org/10.3390/ijerph18010199
7. Mena-Tudela D, Iglesias-Casás S, González-Chordá VM, Valero-Chillerón MJ, Andreu-Pejó L, Cervera-Gasch Á. Obstetric violence in Spain (Part III): healthcare professionals, times, and areas. Int J Environ Res Public Health. 2021;18(7):3359. https://doi.org/10.3390/ijerph18073359

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220955

Genomic monitoring unveils a high prevalence of severe acute respiratory syndrome coronavirus 2 Omicron variant in vaccine breakthrough cases in Bahia, Brazil

Gúbio Soares Campos[1], Marta Giovanetti[2], Laíse de Moraes[3], Helena Souza da Hora[1], Antônio Carlos de Albuquerque Bandeira[4], Keila Veronica Oliveira Motta De Alcantara[1], Silvia Ines Sardi[1]*

SUMMARY

OBJECTIVE: Genome sequencing has been proved to be an excellent tool to monitor the molecular epidemiology of the disease caused by severe acute respiratory syndrome coronavirus 2, i.e., coronavirus disease 2019. Some reports of infected, vaccinated individuals have aroused great interest because they are primarily being infected with circulating variants of concern. To investigate the cases of infected, vaccinated individuals in Salvador, Bahia, Brazil, we performed genomic monitoring to estimate the magnitude of the different variants of concern in these cases.

METHODS: Nasopharyngeal swabs from infected (symptomatic and asymptomatic), vaccinated or unvaccinated individuals (n=29), and quantitative reverse transcription polymerase chain reaction cycle threshold value (Ct values) of ≤30 were subjected to viral sequencing using nanopore technology.

RESULTS: Our analysis revealed that the Omicron variant was found in 99% of cases and the Delta variant was found in only one case. Infected, fully vaccinated patients have a favorable clinical prognosis; however, within the community, they become viral carriers with the aggravating factor of viral dissemination of variants of concern not neutralized by the currently available vaccines.

CONCLUSION: It is important to acknowledge the limitations of these vaccines and to develop new vaccines to emergent variants of concern, as is the case of influenza vaccine; going through new doses of the same coronavirus vaccines is "more of the same."

KEYWORDS: COVID-19. SARS-CoV-2. Genome. Vaccines.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), a disease that emerged in December 2019 in Wuhan, China, fueled worldwide efforts to develop vaccines to control the rapid spread of infection.

Currently, there are several vaccines against SARS-CoV-2 approved by the World Health Organization (WHO)[1]. However, the viral replication of this RNA virus has general characteristics common to other RNA viruses; the high mutation rate, principally in the spike (S) glycoprotein, allows it to generate new viral variants to improve its chances of survival in the host and escape to immune detection[2].

It has been shown that SARS-CoV-2 vaccines do not protect against viral infection, although they significantly reduce morbidity and mortality in infected patients. Moreover, the neutralizing antibodies persist for no more than 4 or 5 months, even with a full two-dose regimen[3]. These factors may contribute to the fact that vaccinated individuals (two doses), as well as those administered booster doses, may acquire the SARS-CoV-2 infection. Although the clinical situation of the vaccinated (two-dose regimen) and infected patients is associated with a significant reduction in COVID-19 symptoms and, protection against severe disease, they become a possible viral carrier and can trigger viral dissemination within the community[4,5].

Recent studies of infected, vaccinated individuals have generated notable interest because they show that these individuals can primarily be infected with the circulating variants of concern (VOCs), such as Omicron (B.1.529). Omicron variant is more aggressive, with greater transmissibility and infectivity than the Delta variant[6].

We conducted a genomic study to estimate the magnitude and range of SARS-CoV-2 VOCs in cases of infected (symptomatic and asymptomatic), fully vaccinated, or unvaccinated individuals.

[1]Universidade Federal da Bahia, Health Institute of Science, Laboratory of Virology – Salvador (BA), Brazil.
[2]Oswaldo Cruz Foundation, Laboratory of Flavivirus – Rio de Janeiro (RJ), Brazil.
[3]Oswaldo Cruz Foundation, Gonçalo Moniz Institute, Vector-Borne Infectious Diseases Laboratory – Salvador (BA), Brazil.
[4]Hospital Aeroporto – Salvador (BA), Brazil.
*Corresponding author: sissardi@yahoo.com.br

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: This study was supported by the Fundação de Apoio a Extensão e Pesquisa, Bahia, Brazil (FAPESB) grant 2020/COVID-19; MG was supported by the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E-26/202.248/2018(238504) and the CRP-ICGEB Research Grant 2020 Project CRP/BRA20-03, Contract no. CRP/20/03.
Received on November 16, 2022. Accepted on November 18, 2022.

METHODS

Study design and participants

From December 2021 to January 2022, the Laboratory of Virology, Institute of Health Science, Federal University of Bahia, confirmed the SARS-CoV-2-positive quantitative reverse transcription polymerase chain reaction (RT-qPCR) in a total of 29 vaccinated or unvaccinated individuals. These individuals visited a public health unit to confirm whether they were infected with SARS-CoV-2 or not. According to their vaccination status, the individuals were classified into three groups: fully vaccinated (two-dose regimen), fully vaccinated with three doses, and unvaccinated. The fully vaccinated individuals reported being vaccinated with Coronavac (Butantan, Brazil) Moderna AstraZeneca (Oxford, UK), or Pfizer (Pfizer, USA), and only the elderly people (>60 years) reported having received the third dose of Moderna AstraZeneca or Pfizer. The unvaccinated individuals were defined as those who had not received any vaccine. Inclusion criteria were as follows:

(1) any gender or age with COVID-19 infection;
(2) without comorbidity;
(3) symptomatic or asymptomatic;
(4) not hospitalized, and
(5) vaccinated or unvaccinated.

Exclusion criteria included individuals with SARS-CoV-2-negative RT-qPCR results.

Molecular detection of severe acute respiratory syndrome coronavirus 2

Detection of SARS-CoV-2 was done from nasopharyngeal and oropharyngeal swabs (n=29) pooled together at the time of sample collection. The samples were submitted to RNA extraction (Maxwell® RSC Viral Total Nucleic Acid Purification Kit, Promega, USA) and subsequent to RT-qPCR (GoTaq® Probe 1-Step qRT-PCR System, Promega, USA) assay following the CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)–PCR Diagnostic Panel[7] (Table 1). Samples with cycle threshold (Ct) values ≤39 were considered SARS-CoV-2-positive.

Ethics statement

This research was reviewed and approved by the Ethical Committee of the Federal University of Bahia (CAAE 30687320.9.0000.5662), and informed consent of all participants or their legal guardians have been obtained.

cDNA synthesis and whole-genome sequencing

Samples (n=29) were selected for sequencing based on the Ct value (≤30) and availability of epidemiological metadata (sex, age, residence in Salvador, symptoms, etc.) (Table 2). The preparation of SARS-CoV-2 genomic libraries was done using the nanopore sequencing technology[8]. The SuperScript IV Reverse Transcriptase kit (Invitrogen, USA) was initially used

Table 1. Overview of the primers and probes* used for severe acute respiratory syndrome coronavirus 2 detection by real-time polymerase chain reaction assay.

2019-nCoV_N1-F	GAC CCC AAA ATC AGC GAA AT
2019-nCoV_N1-R	TCT GGT TAC TGC CAG TTG AAT CTG
2019-nCoV_N1-P	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
2019-nCoV_N1-P	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ
2019-nCoV_N2-F	TTA CAA ACA TTG GCC GCA AA
2019-nCoV_N2-R	GCG CGA CAT TCC GAA GAA
2019-nCoV_N2-P	FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
2019-nCoV_N2-P	FAM-ACA ATT TGC /ZEN/ CCC CAG CGC TTC AG-3IABkF

*CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)–qPCR Diagnostic Panel.

Table 2. Demographic and clinical characteristics in the study group of vaccinated or unvaccinated participants during severe acute respiratory syndrome coronavirus 2 vaccine outbreaks, Bahia, Brazil, between December 2021 and January 2022.

Participants	Variable	Number/total
	Groups (years)	
Age	6–19	5/29
	20–30	8/29
	31–45	7/29
	46–60	5/29
	> 61	4/29
Gender	F	15/29
	M	14/29
COVID-19 Immunization: (Oxford/AstraZeneca; Pfizer BioNTech, or Coronavac)	2 doses	18/25
	3 doses	6/25
No COVID-19 Immunization		4/29
Symptomatic (self-referred by vaccinated with two or three doses and unvaccinated individuals)	Fever	18/29
	Sore throat**	
	Cough**	
	Headache	
	Body pain	
Asymptomatic (vaccinated individuals)	2 doses or 3 doses	11/25

**Most referred by the participants.

for cDNA synthesis as per the manufacturer's instructions. The cDNA generated was subjected to multiplex PCR sequencing using the Q5 High Fidelity Hot-Start DNA Polymerase (New England Biolabs, UK) and a set of specific primers designed by the ARTIC Network for sequencing the complete SARS-CoV-2 genome (version 4)[9]. All experiments were performed in a biosafety level 2 cabinet. Amplicons were purified using 1´ AMPure XP Beads (Beckman Coulter, USA) and quantified on a Qubit 3.0 fluorimeter (Thermo Fisher Scientific, USA) using Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). DNA library preparation was performed using the Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies, UK) and the Native Barcoding Kit (EXP-NBD104 and EXP-NBD114, Oxford Nanopore Technologies, UK). Sequencing libraries were loaded into an R9.4 flow cell (Oxford Nanopore Technologies, UK). In each sequencing run, we used negative controls to prevent and check for possible contamination with <2% mean coverage.

Generation of consensus sequences from nanopore

The fast5 files generated during sequencing were basecalled under the high-accuracy model performed using Guppy v.6.0.1 (Oxford Nanopore Technologies, UK). The basecalled fastQ files with a minimum Q score of 7 were selected for subsequent trim adaptor and demultiplex processes performed using Guppy v.6.0.1. Furthermore, the fastQ files were submitted to the ARTIC Network's field bioinformatics pipeline v.1.2.1[9]. Briefly, a length filtering was performed to remove additional chimeras using artic guppyplex. The assembly was performed by Minimap2 v.2.17[10] using GenBank accession no. MN908947.3 as genome reference. The primer sequences were trimmed using align_trim.py, the assembly was then polished, and the variant calling was performed using Medaka v.1.0.3 (Oxford Nanopore Technologies, UK) and evaluated using LongShot v.0.4.1[11]. The consensus sequences were then masked with "N" at regions with coverage depth <20, and the variant candidates were incorporated into the consensus genome using BCFtools v.1.10.2[12]. The alignment statistics were calculated using SAMtools v.1.10 (using htslib 1.10.2)[12], exonerate v.2.4.0 (using glib version v.2.68.0),[13] and Seqtk v.1.3-r106[14]. This entire workflow is available at https://github.com/khourious/vgapONT.

Phylogenetic inference

Lineage assignment was performed using the Pangolin lineage classification software tool[15]. The newly identified isolates were compared with a diverse pool of genome sequences (n=3,441) sampled worldwide collected up to October 28, 2021. All sequences were aligned using the ViralMSA tool v.1.1.20[10,16], and phylogenetic analysis using the maximum likelihood approach was done using IQ-TREE2 v.2.2.0[17]. TreeTime v.0.8.5[18] was used to transform this ML tree topology into a dated tree using a constant mean rate of 8.0×10^{-4} nucleotide substitutions per site per year after the exclusion of outlier sequences.

Data availability statement

Newly generated SARS-CoV-2 sequences have been deposited in GISAID under the following accession numbers: EPI_ISL_9265729, EPI_ISL_9265745, EPI_ISL_9265746, EPI_ISL_9265743, EPI_ISL_9265744, EPI_ISL_9265749, EPI_ISL_9265728, EPI_ISL_9265747, EPI_ISL_9265748, EPI_ISL_9265730, EPI_ISL_9265752, EPI_ISL_9265731, EPI_ISL_9265753, EPI_ISL_9265750, EPI_ISL_9265751, EPI_ISL_9265734, EPI_ISL_9265756, EPI_ISL_9265735, EPI_ISL_9265732, EPI_ISL_9265754, EPI_ISL_9265733, EPI_ISL_9265755, EPI_ISL_9265738, EPI_ISL_9265739, EPI_ISL_9265736, EPI_ISL_9265737, EPI_ISL_9265741, EPI_ISL_9265742, EPI_ISL_9265740.

RESULTS

In this study, vaccinated individuals (25/29) were immunized with a complete two-dose regimen; elderly people >60 years (6/25) were immunized with three doses, and 7- to 13-year-old children (4/29) were unvaccinated (Table 2). The symptoms self-referred by participants were mild (fever, cough, sore throat, or headache) in individuals in the vaccinated and unvaccinated groups. Vaccinated individuals self-reported to be asymptomatic (11/25). In all cases, there was no need for hospitalization.

The RT-qPCR Ct values varied between 14.6 and 27.2, even in asymptomatic individuals (Figure 1). Of the vaccinated individuals (n=25), 24 confirmed the presence of Omicron variant and only one Delta variant. The unvaccinated individuals (n=4) confirmed to be infected by the Omicron variant. Our genomic

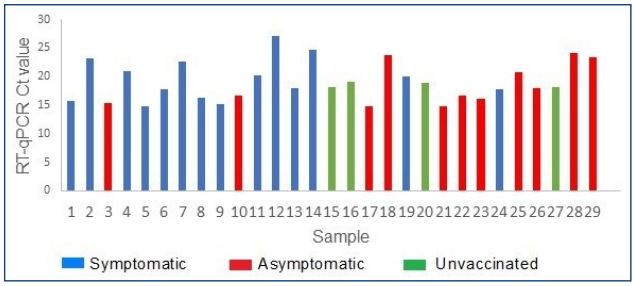

Figure 1. Distribution of RT-qPCR cycle threshold values in the clinical samples from vaccinated or unvaccinated individuals infected by severe acute respiratory syndrome coronavirus 2 variants. Cycle threshold values from vaccinated symptomatic individuals (blue), vaccinated asymptomatic individuals (red), and unvaccinated individuals (green). RT-qPCR: quantitative real-time polymerase chain reaction.

surveillance analysis revealed that in Bahia, three VOCs primarily dominated the epidemiology in the state. The Gamma variant (P.1), which was prominent during the second wave of the pandemic, persisted until August 2021, when it was replaced by the Delta variant (B.1.617.2) (Figure 2A), which in turn was replaced by the emerging Omicron variant in December 2021. The Alpha variant was also detected in Bahia at the end of December 2021, but it remained at a very low frequency (<1%). To explore the relationship between the sequenced genomes, we also constructed a phylogenetic tree with these VOCs and those from the other parts of the world. Our time-stamped phylogeny revealed that the Omicron isolates in Bahia are scattered throughout the phylogeny, suggesting that multiple independent introductions have occurred over time (Figure 2B).

DISCUSSION

Since the start of the COVID-19 pandemic in December 2019, efforts have been directed to the development of vaccines against SARS-CoV-2. Given the global situation, the WHO has authorized the use of several types of vaccines using mRNA technology, adenovirus vector vaccine, and inactivated viruses[1].

Recent reports confirm that the currently used vaccines do not protect against infection but do reduce the severity of cases. Indeed, countries that have vaccinated at least 50% of their population have managed to reduce the mortality rate. However, the current scenario shows the tendency of vaccinated individuals getting infected with emerging VOCs that cannot be neutralized by the immunity generated from these vaccines[2]. The new variants have emerged as a result of increased viral circulation, primarily in countries where vaccine coverage is low, which quickly spread worldwide. Examples of these variant leaks include Delta in India and Omicron in South Africa[19].

Our results reinforce the notion that virus migration generally follows national and international patterns of human mobility, facilitating the spread of emerging VOCs not only within countries but also globally. The identification of SARS-CoV-2 suspected cases through genome-wide sequencing in Bahia also revealed this, and as of 2020, co-circulation of three different VOCs, such as Gamma (P.1), Delta, and Omicron, appears to have dominated the epidemiological history in the state. The Alpha variant currently remains at a very low frequency (<1%) in the state, consistent with the effectiveness of SARS-CoV-2 vaccines against it while being less effective against new VOCs[5].

Initially, it was considered in the literature that reaching herd immunity (≥70%) would prevent the circulation of the virus in the population; this fact is now being questioned. The countries that have reached or surpassed this percentage (England, Israel, and Brazil) have high infection rates since the emergence of Omicron[20,21]. In Bahia, 74.9% of the vaccination coverage was recently achieved with two doses, but our work demonstrated breakthrough infection by Omicron with a high viral load even in double or triple vaccinated individuals. Moreover, asymptomatic vaccinated individuals can still spread the virus to other people.

One limitation of our work is that the level of protective immunity of those who are currently vaccinated is not

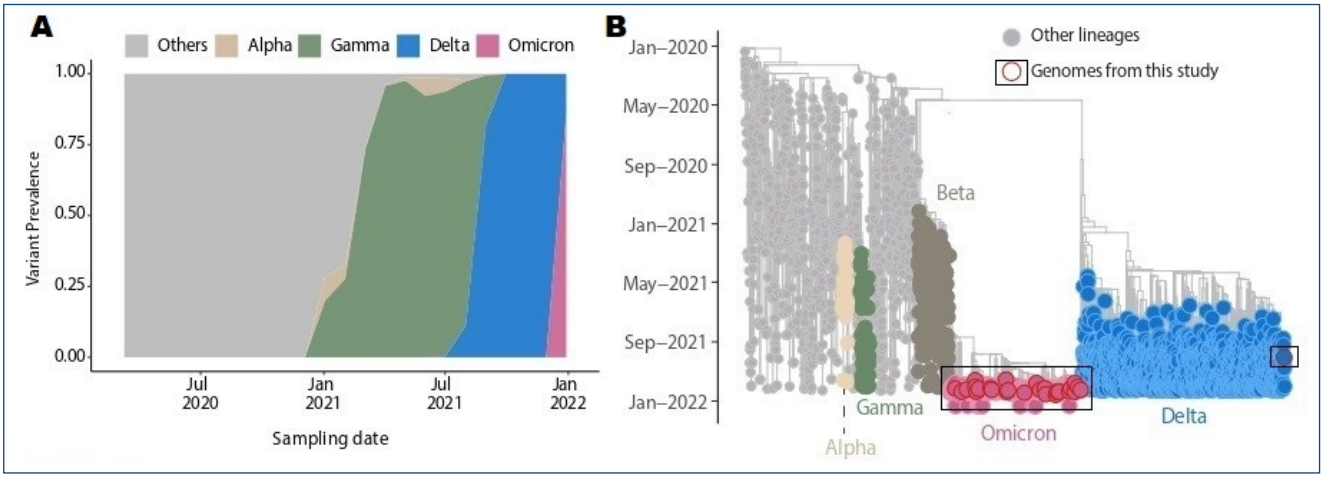

Figure 2. Genomic epidemiology of the severe acute respiratory syndrome coronavirus 2. Omicron variant in Salvador, Bahia, Northeast Brazil. (A) Dynamics of the severe acute respiratory syndrome coronavirus 2 epidemic in Bahia showing the progression in the proportion of circulating variants in the state over time, with the rapid replacement of the Delta by the Omicron variant. (B) Time-resolved maximum likelihood phylogenetic tree including the new Omicron and Delta isolates obtained in this study and n=4,249 representative severe acute respiratory syndrome coronavirus 2 genomes collected up to January 16, 2021. Alpha (brown), Beta (dark gray), Gamma (green), Delta (blue), and Omicron (red) variants of concern are highlighted in the tree. New Omicron (n=28) and Delta (n=1) genomes obtained in this study are highlighted with a black box in an each of these genomes. Genomes of the other lineages are shown in light gray.

known; however, even with three doses, there have been cases of viral infection. Dose reinforcements in countries such as England and Israel with a four-dose regimen raise doubts as to whether they will solve viral recrudescence for new VOCs in circulation[22,23].

It is important to acknowledge the limitations of these vaccines and urgently develop new vaccines to emergent VOCs, similar to the case of influenza vaccine; going through new doses of the same vaccines is "more of the same."

Measures to control the spread of the virus, such as the use of face masks, social distancing, and crowd avoidance, continue to be effective, but these need to be combined with new immunogens that prevent viral infection (an elementary concept for the approval of vaccines for widespread use).

AUTHORS' CONTRIBUTIONS

GSC: Conceptualization, Formal Analysis, Supervision, Writing – review & editing. **SIS:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing. **MG:** Formal Analysis, Methodology, Visualization, Writing – review & editing. **LM:** Formal Analysis, Validation. **HSH:** Methodology. **KVOMDA:** Methodology. **ACAB:** Methodology, Writing – review & editing.

REFERENCES

1. World Health Organization. WHO coronavirus disease (COVID-19). [cited on 2022 Feb 2]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines
2. Mengist HM, Kombe Kombe AJ, Mekonnen D, Abebaw A, Getachew M, Jin T. Mutations of SARS-CoV-2 spike protein: implications on immune evasion and vaccine-induced immunity. Semin Immunol. 2021;55:101533. https://doi.org/10.1016/j.smim.2021.101533
3. Mizrahi B, Lotan R, Kalkstein N, Peretz A, Perez G, Ben-Tov A, et al. Correlation of SARS-CoV-2-breakthrough infections to time-from-vaccine. Nat Commun. 2021;12(1):6379. https://doi.org/10.1038/s41467-021-26672-3
4. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021;373:n1088. https://doi.org/10.1136/bmj.n1088
5. Hacisuleyman E, Hale C, Saito Y, Blachere NE, Bergh M, Conlon EG, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med. 2021;384(23):2212-18. https://doi.org/10.1056/NEJMoa2105000
6. Chen J, Wang R, Gilby NB, Wei GW. Omicron variant (B.1.1.529): infectivity, vaccine breakthrough, and antibody resistance. J Chem Inf Model. 2022;62(2):412-22. https://doi.org/10.1021/acs.jcim.1c01451
7. National Center for Immunization and Respiratory Diseases (NCIRD) D of VD. Novel coronavirus (2019-nCoV) real-time RT-PCR primers and probes. [cited on 2022 Jan 24]. Available from: https://www.cdc.gov/Coronavirus/2019-Ncov/Lab/Rt-Pcr-Panel-Primer-Probes.Html
8. Giovanetti M, Slavov SN, Fonseca V, Wilkinson E, Tegally H, Patané JSL, et al. Genomic epidemiology reveals how restriction measures shaped the SARS-CoV-2 epidemic in Brazil. MedRxiv. 2021. https://doi.org/10.1101/2021.10.07.21264644
9. ARTICnetwork. ARTIC field bioinformatics pipeline [Internet]. GitHub. [cited on 2022 Feb 2]. Available from: https://github.com/artic-network/fieldbioinformatics
10. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34(18):3094-100. https://doi.org/10.1093/bioinformatics/bty191
11. Edge P, Bansal V. Longshot enables accurate variant calling in diploid genomes from single-molecule long read sequencing. Nat Commun. 2019;10:4660:1-10. https://doi.org/10.1038/s41467-019-12493-y
12. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10(2):giab008. https://doi.org/10.1093/gigascience/giab008
13. Slater GSC, Birney E. Automated generation of heuristics for biological sequence comparison. BMC Bioinform. 2005;6:31. https://doi.org/10.1186/1471-2105-6-31
14. Li H. Seqtk [Internet]. GitHub [cited on 2022 Jan 20]. Available from: https://github.com/lh3/seqtk
15. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. 2020;5:1403-7. https://doi.org/10.1038/s41564-020-0770-5
16. Moshiri N. ViralMSA: massively scalable reference-guided multiple sequence alignment of viral genomes. Bioinformatics. 2021;37(5):714-16. https://doi.org/10.1093/bioinformatics/btaa743
17. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 2020;37(5):1530-34. https://doi.org/10.1093/molbev/msaa015
18. Sagulenko P, Puller V, Neher RA. TreeTime: maximum-likelihood phylodynamic analysis. Virus Evol. 2018;4(1):1-9. https://doi.org/10.1093/ve/vex042
19. Hirabara SM, Serdan TDA, Gorjao R, Masi LN, Pithon-Curi TC, Covas DT, et al. SARS-COV-2 variants: differences and potential of immune evasion. Front Cell Infect Microbiol. 2022;11:781429. https://doi.org/10.3389/fcimb.2021.781429
20. Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med. 2021;385(24):1-10. https://doi.org/10.1056/NEJMoa2114228
21. Rössler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 Omicron variant neutralization in serum from vaccinated and convalescent persons. N Engl J Med. 2022;386(7):698-700. https://doi.org/10.1056/NEJMc2119236
22. World Health Organization. WHO coronavirus disease (COVID-19). [cited on 2022 Jan 24]. Available from: https://www.who.int/countries/isr; https://www.who.int/countries/gbr/
23. Eggink D, Andeweg SP, Vennema H, van Maarseveen N, Vermaas K, Vlaemynck B, et al. Increased risk of infection with SARS-CoV-2 Omicron BA.1 compared with delta in vaccinated and previously infected individuals, the Netherlands, 22 November 2021 to 19 January 2022. Euro Surveill. 2022;27(4):1-6. https://doi.org/10.2807/1560-7917.ES.2022.27.4.2101196

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220960

The clinical value of lung ultrasound in premature infants with bronchopulmonary dysplasia

Jingyi Xu[1], Yikang Fu[1], Fang Wang[1], Wen Zhou[1], Lan Chen[1], Ling Liu[1]*

SUMMARY

OBJECTIVE: This study aimed to explore the risk factors of bronchopulmonary dysplasia in premature infants and the clinical application value of lung ultrasound in the diagnosis of bronchopulmonary dysplasia.

METHODS: A total of 80 premature infants with a gestational age of <32 weeks or a birth weight of <1,500 g who were treated in our hospital from January to August 2021 were randomly divided into a bronchopulmonary dysplasia group (n=12) and a non-bronchopulmonary dysplasia group (n=62). The clinical data, lung ultrasound, and X-ray image characteristics of the two groups were compared.

RESULTS: Among the 74 preterm infants, 12 preterm infants were diagnosed with bronchopulmonary dysplasia, and 62 preterm infants were determined not to have bronchopulmonary dysplasia. There were significant differences in sex, severe asphyxia, invasive mechanical ventilation, premature membrane ruptures, and intrauterine infection between the two groups ($p<0.05$). Lung ultrasound showed abnormal pleural lines and alveolar-interstitial syndrome in all 12 patients with bronchopulmonary dysplasia and vesicle inflatable signs in 3 patients. Before clinical diagnosis, the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of lung ultrasound in the diagnosis of bronchopulmonary dysplasia were 98.65, 100, 98.39, 92.31, and 100%, respectively. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of X-rays in the diagnosis of bronchopulmonary dysplasia were 85.14, 75.00, 87.10, 52.94, and 94.74%, respectively.

CONCLUSION: The diagnostic efficiency of lung ultrasound for premature bronchopulmonary dysplasia is better than that of X-rays. The application of lung ultrasound can screen patients with bronchopulmonary dysplasia early for timely intervention.

KEYWORDS: Premature birth. Bronchopulmonary dysplasia. Lung. Ultrasonography.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is one of the most common and serious respiratory diseases in premature infants[1] and is life-threatening[2]. In recent years, the incidence of BPD has increased[3]. Since the pathogenesis of BPD is not yet clear, there is still no effective clinical treatment for BPD, and only supportive care is used. To further improve the survival rate of premature infants and life quality in later growth and development, early and effective diagnosis of BPD is particularly important. It is necessary to identify the occurrence of BPD in premature infants early and take preventive and control measures. The diagnostic criteria formulated by the National Institute of Child and Human Development (NICHD) are not of great value for early diagnosis (within 28 days). Although X-rays are applied in early diagnosis, they are not convenient for detection and cannot be used at the bedside. Moreover, due to radiation, X-rays cannot be used for continuous monitoring. With the rapid development of ultrasound technology, lung ultrasound (LUS) has been widely used in the diagnosis of paediatric lung diseases. As a method for the diagnosis and treatment of pulmonary diseases in children, LUS has a high probability of producing a correct diagnosis and a high sensitivity and specificity[4]. At present, the application of LUS in the diagnosis of neonatal diseases is gradually being promoted and recognised by many clinicians. In this study, the NICHD's standard was used as the gold standard for BPD diagnosis, and the predictive values of LUS and X-ray examinations for BPD in premature infants were compared.

METHODS

Research objects

Preterm infants under 32 weeks who were admitted to our hospital from January 2021 to August 2021 were selected as the research subjects (a total of 248 neonates under 32 weeks were admitted during the period). Inclusion criteria include (1) admission within 24 h after birth and (2) a gestational

[1]Guiyang Maternal and Child Health Care Hospital, Department of Neonates – Guiyang, China.
*Corresponding author: liulingxll@163.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: This study was funded by the 2020 Guiyang City High-level Innovative Young Health Talent Training Program and the Project of Guiyang Science and Technology Bureau.
Received on August 19, 2022. Accepted on October 14, 2022.

age score at birth of <32 weeks or a birth weight of <1500 g. Exclusion criteria include (1) the mother received an abnormal non-invasive DNA test or amniocentesis during her obstetric examination; (2) severe congenital malformations were suggested before birth; (3) imaging showed congenital pulmonary dysplasia, such as a congenital diaphragmatic hernia or isolated lung; or (4) corrected gestational age before 36 weeks of death. A total of 80 neonates were randomly enrolled. Treatment was ceased for three of these patients due to financial difficulties, and three died during treatment. The final sample size was 74.

The participants were divided into a BPD group (n=12) and a non-BPD group (n=62). The diagnostic criteria of BPD follow the consensus of the NICHD: children with a gestational age of <32 weeks should be diagnosed with BPD at 36 weeks of corrected gestational age or 28 days after birth (56 days at the latest). The diagnostic criterion includes any neonate who is oxygen-dependent [inspired oxygen concentration (FiO_2) >21%] for more than 28 days[5].

Research methods

Lung ultrasound inspection

Lung ultrasound examination method: Neonates were placed in supine and lateral positions, and GE LOGIQ P6 Pro and GE high-frequency line shock probe (15 Hz) ultrasonic diagnostic apparatuses were used for ultrasonography in a quiet state. The parasternal line, the anterior axillary line, the posterior axillary line, and the double nipple line were divided into six lung areas, namely, anterior superior, anterior inferior, superior axillary, axillary, posterior superior, and posterior inferior. A total of 12 lung areas on both sides were scanned, and the images were saved. The first LUS examination was performed within 24 h after the neonate was admitted to the hospital, and thereafter, it was reviewed twice a week until discharge.

Observation indicators for the diagnosis of BPD by LUS include the following: ① abnormal pleural line, ② B-line, ③ alveolar-interstitial syndrome, and ④ signs of vesicular effusion or bronchial fluid filling.

Chest X-ray examination

A MobileDiagnost wDR (Philips Medical DMC GmbH) machine was selected, and the supine position and anterior-posterior views were taken. X-ray examinations were performed within 24 h of the neonate's admission, and chest X-ray examinations were performed at 4 weeks after birth or at 36 weeks of corrected gestational age.

Observational indicators of the X-ray diagnosis of BPD: ① Stage I (1–3 days): ground-glass-like changes in both lungs, ② Stage II (4–10 days): complete opacity of both lungs, ③ Stage III (11–30 days): small transparent cysts with restarting between linear or patchy shadows in both lung fields, and ④ Stage IV (after 1 month): the luminal areas of both lung fields enlarged to form vesicles, with hyperinflation and atelectasis.

Statistical analysis

The SPSS version 22.0 software was used for statistical analysis. The measurement data were analysed with an independent sample t-test, and the count data were analysed with a χ^2 test. A $p<0.05$ indicated a statistically significant difference.

RESULTS

Comparison of clinical data between bronchopulmonary dysplasia and non-bronchopulmonary dysplasia

Among the 74 premature infants, 12 were patients with BPD, and 62 were patients without BPD. There were 11 males and 1 female in the BPD group, with an average age of 29.29±2.79 weeks and a weight of 1240±75.41 g. There were 31 males and 31 females in the non-BPD group, with an average age of 30.43±1.71 weeks and a weight of 1439±40.65 g. There were significant differences in birth weight, gender, severe asphyxia, use of invasive mechanical ventilation, premature rupture of membranes, and intrauterine infection between the two groups ($p<0.05$) (see Table 1).

Image features of lung ultrasound and X-ray diagnosis of bronchopulmonary dysplasia

A total of 12 children with BPD underwent dynamic LUS examination (Figure 1A). All of them showed abnormal pleural lines and alveolar-interstitial syndrome changes, and three patients showed signs of air vesicles.

A total of 12 children with BPD were examined using X-rays (Figure 1B). There were three cases with no obvious abnormal features, nine cases with bilateral lung ground-glass-like changes, five cases with linear or patchy shadows, and three cases with cystic translucent shadows.

Comparison of bronchopulmonary dysplasia prediction results between lung ultrasound and X-rays

Before clinical diagnosis, the LUS results were consistent with the confirmed cases in 73 children, and the diagnostic

Table 1. Comparison of clinical data between the bronchopulmonary dysplasia group and non-bronchopulmonary dysplasia group.

Group	Gestational age (week)	Sex (male/female)	Born body (g)	Small for gestational age (yes/no)	Severe asphyxia (yes/no)	Invasive mechanical ventilation (yes/no)
Non-BPD group (n=62)	30.43±1.71	31/31	1439±40.65	7/55	1/61	20/42
BPD group (n=12)	29.29±2.79	11/1	1240±75.41	3/9	3/9	8/4
t/χ^2 value	1.91	7.11	2.09	1.62	10.75	5.06
p	0.06	0.01	0.04	0.20	<0.01	0.02
Group	**Premature rupture of membranes (yes/no)**	**Intrauterine infection (yes/No)**	**Preeclampsia (yes/no)**	**Gestational diabetes (yes/no)**	**Prenatal use of antibiotics (yes/no)**	**Prenatal hormone use (yes/no)**
Non-BPD group (n=62)	46/16	5/57	4/58	6/56	26/36	46/16
BPD group (n=12)	4/8	5/7	1/11	0/12	2/10	6/6
t/χ^2 value	7.66	9.71	0.06	1.26	2.73	2.82
p	<0.01	<0.01	0.81	0.26	0.10	0.09

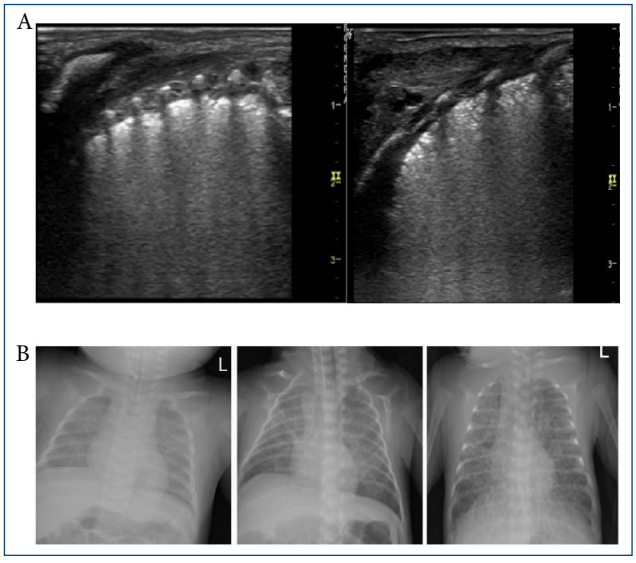

Figure 1. (A) Lung ultrasound image features. (B) X-ray image features.

accuracy was 98.65%. When LUS was used to predict BPD, its sensitivity, specificity, positive predictive value, and negative predictive value were 100, 98.39, 92.31, and 100%, respectively. The X-ray results were consistent with the confirmed cases in 63 children, and the diagnostic accuracy rate was 85.14%. When X-rays were used to predict BPD, their sensitivity, specificity, positive predictive value, and negative predictive value were 75.00, 87.10, 52.94, and 94.74%, respectively (see Table 2).

DISCUSSION

Prolonged use of supplemental oxygen for BPD in preterm infants, high mortality, residual airway hyperresponsiveness in surviving infants, repeated lower respiratory tract infections, feeding difficulties, and growth retardation[6-8] make the prevention and treatment of BPD a key research topic in the Department of Neonatology. Compared with the non-BPD group, the BPD group had lower birth weights and a higher proportion of male infants, severe asphyxia, invasive mechanical ventilation, premature membrane ruptures, and intrauterine infections. This is generally consistent with previous reports of risk factors for BPD in preterm infants[9,10]. There was an inverse linear relationship between low birth weight and the incidence of BPD. Neonates with a birth weight of <1500 g have a 20% chance of developing BPD[11], and those with a birth weight of <1000 g have an even higher chance of developing BPD[12]. The high risk of BPD in premature infants with very low birth weights is mainly related to the defective development of their organs[13]. The high incidence of BPD in male infants is mainly due to the relationship between oestrogen and pulmonary surfactant production. Costeloe et al. reported that the incidence of BPD in male infants was twice that of female infants[14]. Invasive mechanical ventilation produces a large amount of toxic peroxide substances under high concentrations of oxygen, causing lung damage and increasing the incidence of BPD[15]. Infection is a key link in the occurrence of BPD. Intrauterine infection can induce the premature rupturing of membranes. Relevant

Table 2. Clinical value of lung ultrasound and X-rays in predicting bronchopulmonary dysplasia (%).

Inspection method	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Diagnostic accuracy (%)
LUS	100	98.39	92.31	100	98.65
X-rays	75.00	87.10	52.94	94.74	85.14

literature reports that when the mother has an intrauterine infection, the inflammatory factors that are produced can mediate foetal lung injury in preterm infants[16]. Birth weight, asphyxia, invasive mechanical ventilation, premature membrane rupture, and intrauterine infection can be used as important indicators for predicting BPD in preterm infants.

In recent years, LUS examination has been widely used in the diagnosis of respiratory diseases in premature infants. Compared with computed tomography (CT) and X-ray examinations, LUS has the advantages of being able to be carried out at the bedside, without radiation, and being able to be dynamically monitored[17]. Some studies of ultrasound for the diagnosis of BPD have been reported. Alonso-Ojebarrena et al. performed a dynamic LUS examination on 59 premature infants with a gestational age of ≤32 weeks and/or birth weight of ≤1,500 g[18]. Their results show that the sensitivity and specificity of LUS for diagnosing BPD were 71 and 80%, respectively, at 1 week after birth; 74 and 100%, respectively, at 2 weeks after birth; and 100 and 100%, respectively, at 4 weeks after birth. The experimental results of Balany et al. indicated that LUS can be used as an auxiliary examination method to predict the risk of BPD in premature infants[19]. In this study, before the clinical diagnosis of BPD, the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of LUS in diagnosing BPD were 98.65, 100, 98.39, 92.31, and 100%, respectively, which are significantly higher than the clinical efficacy index of X-rays for the diagnosis of BPD. The characteristic images of LUS in children with BPD +are mainly abnormal pleural lines, alveolar-interstitial syndrome, and changes in air vesicle signs. The results of this study are basically consistent with the previous reports of Liu et al.[20]. Liu et al. observed ultrasound signs such as pleural line abnormalities (insect-eaten changes), interstitial pulmonary syndrome, and air vesicle signs, and the sensitivity and specificity for diagnosing BPD were both 100%. It is completely feasible to use LUS to replace X-rays in neonatal wards for the early diagnosis of neonatal BPD[20].

However, this study has certain limitations. First, when using ultrasound to diagnose BPD, although physicians in the ultrasound department took the pictures and read the reports independently of each other, due to the subjectivity of interpretation and the influence of clinical information, the results may be biased in selection and measurement. Second, this study discussed only the diagnostic value of LUS for BPD but not its correlation with clinical features (such as the degree of lung lesions). Third, the sample size of this study was small, and an experiment with a large sample should be conducted in the future to confirm the generalisability of the results of this study.

CONCLUSION

In the process of clinical diagnosis and treatment, intervention strategies should be formulated according to their risk factors to improve the survival rate and prognosis of premature infants. As an effective detection method to predict the occurrence of BPD in premature infants, LUS can replace X-ray examination to guide the clinical formulation of accurate treatment plans, track the progress and outcome of the disease, and reduce the incidence of BPD. Therefore, LUS is recommended for clinical use.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Guiyang Maternal and Child Health Care Hospital.

AUTHORS' CONTRIBUTIONS

JX: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **YF:** Data curation, Investigation, Software. **LL:** Data curation, Methodology, Project administration, Writing – review & editing. **FW:** Data curation, Software. **WZ:** Data curation, Validation. **LC:** Data curation, Validation.

REFERENCES

1. Yu HK, Liu X, Chen JK, Li ZH, Wang S, Huang JH, et al. Clinical value of lung ultrasound score in diagnosis and prognosis of children with severe pneumonia. Chin J Ultrasound Med. 2019;35(3):229-31.
2. Neonatal Group of Pediatrics Branch of Chinese Medical Association, Editorial Board of Chinese Journal of Pediatrics. Expert consensus on clinical management of premature infants with brochopulmonary dysplasia. Chin J Pediatrics. 2020;58(5):358-9. https://doi.org/10.3760/cma.j.cn112140-20200317-00254
3. Yin X, Zong SY, Tang YL, Liu Y, Jiang LH. The value of pulmonary ultrasound scoring in predicting bronchopulmonary dysplasia in premature infants. Chin J Ultrasound Med. 2019;3(12):1076-8.
4. Kiciński P, Kęsiak M, Nowiczewski M, Gulczyńska E. Bronchopulmonary dysplasia in very and extremely low birth weight infants – analysis of selected risk factors. Pol Merkur Lekarski. 2017;42(248):71-5. PMID: 28258680
5. Li RW. Research progress of diagnostic criteria for bronchopulmonary dysplasia. J Clin Pediatrics. 2021;39(4):308-12.
6. Li JF, Zhu XP. Analysis of risk factors for bronchopulmonary dysplasia in very low birth weight infants. Matern Child Health Care China. 2018;33(23):5565-9.
7. Villamor-Martínez E, Pierro M, Cavallaro G, Mosca F, Kramer B, Villamor E. Probiotic supplementation in preterm infants does not affect the risk of bronchopulmonary dysplasia: a meta-analysis of randomized controlled trials. Nutrients. 2017;9(11):1197. https://doi.org/10.3390/nu9111197
8. Kim JK, Chang YS, Sung S, Ahn SY, Yoo HS, Park WS. Trends in survival and incidence of bronchopulmonary dysplasia in extremely preterm infants at 23–26 weeks gestation. J Korean Med Sci. 2016;31(3):423-9. https://doi.org/10.3346/jkms.2016.31.3.423
9. Daurat A, Millet I, Roustan JP, Maury C, Taourel P, Jaber S. Thoracic trauma severity score on admission allows to determine the risk of delayed ARDS in trauma patients with pulmonary contusion. Injury. 2016;47(1):147-53. https://doi.org/10.1016/j.injury.2015.08.031
10. Mojoli F, Bouhemad B, Mongodi S, Lichtenstein D. Lung ultrasound for critically Ill patients. Am J Respir Crit Care Med. 2019;199(6):701-14. https://doi.org/10.1164/rccm.201802-0236CI
11. Lardón-Fernández M, Uberos J, Molina-Oya M, Narbona-López E. Epidemiological factors involved in the development of bronchopulmonary dysplasia in very low birth-weight preterm infants. Minerva Pediatr. 2017;69(1):42-9. https://doi.org/10.23736/S0026-4946.16.04215-8
12. Maturu P, Wei-Liang Y, Jiang W, Wang L, Lingappan K, Barrios R, et al. Newborn mice lacking the gene for cyp1a1 are more susceptible to oxygen-mediated lung injury, and are rescued by postnatal β-naphthoflavone administration: implications for bronchopulmonary dysplasia in premature infants. Toxicol Sci. 2017;157(1):260-71. https://doi.org/10.1093/toxsci/kfx036
13. Chen JR. Risk factors analysis and early prediction of bronchopulmonary dysplasia in premature infants. Shantou University. 2021. https://doi.org/10.27295/d.cnki.gstou.2021.000482
14. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ. 2012;345:e7976. https://doi.org/10.1136/bmj.e7976
15. Principi N, Di Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. J Transl Med. 2018;16(1):36. https://doi.org/10.1186/s12967-018-1417-7
16. Voynow JA. "New" bronchopulmonary dysplasia and chronic lung disease. Paediatr Respir Rev. 2017;24:17-8. https://doi.org/10.1016/j.prrv.2017.06.006
17. Davidson LM, Berkelhamer SK. Bronchopulmonary dysplasia: chronic lung disease of infancy and long-term pulmonary outcomes. J Clin Med. 2017;6(1):4. https://doi.org/10.3390/jcm6010004
18. Alonso-Ojembarrena A, Lubián-López SP. Lung ultrasound score as early predictor of bronchopulmonary dysplasia in very low birth weight infants. Pediatr Pulmonol. 2019;54(9):1404-9. https://doi.org/10.1002/ppul.24410
19. Balany J, Bhandari V. Understanding the impact of infection, inflammation, and their persistence in the pathogenesis of bronchopulmonary dysplasia. Front Med (Lausanne). 2015;2:90. https://doi.org/10.3389/fmed.2015.00090
20. Liu J. Diagnosis of common neonatal lung diseases: the necessity and feasibility of ultrasound instead of X-ray. China Pediatr Emerg Med. 2019;26(8):565-70.

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220962

Does cryptogenic organizing pneumonia change seasonal?

Tugce Sahin Ozdemirel[1*], Sertan Bulut[1], Esma Sevil Akkurt[1], Zeynep Erayman Ozen[1], Mahmut Hamdi Erdogdu[1], Funda Demirag[2], Berna Akinci Ozyurek[1]

SUMMARY

BACKGROUND AND AIM: Meteorological factors affect the respiratory system, and the most important factor is the change in ambient temperature and humidity. We aimed to investigate the seasonal characteristics of patients diagnosed with cryptogenic organizing pneumonia.

METHODS: The study included 84 cryptogenic organizing pneumonia, 55 chronic obstructive pulmonary disease, and 42 asthma patients. To determine the characteristics of the disease according to the seasons, the number of attacks and admissions was grouped according to the seasonal characteristics and analyzed for three groups.

RESULTS: Among cryptogenic organizing pneumonia and chronic obstructive pulmonary disease patients, males significantly predominated ($p<0.001$). The hospitalization rate was highest in chronic obstructive pulmonary disease patients but similar to cryptogenic organizing pneumonia and asthma patients ($p<0.001$). The highest admission rate in cryptogenic organizing pneumonia patients was observed in spring (39.3% in spring, 26.2% in fall, 22.6% in winter, and 11.9% in summer). In winter, cryptogenic organizing pneumonia patients were admitted less frequently than chronic obstructive pulmonary disease and asthma patients. The neutrophil-to-lymphocyte ratio was higher in cryptogenic organizing pneumonia patients than in asthma patients and similar to chronic obstructive pulmonary disease patients.

CONCLUSION: As a result of our study, the high rate of diagnosis and admission in the spring in cryptogenic organizing pneumonia suggested that the effect of allergens on the formation of cryptogenic organizing pneumonia should be investigated. In contrast, it should be kept in mind that cryptogenic organizing pneumonia may develop as a prolonged finding of involvement that may occur in the lung parenchyma due to lung infections and/or cold weather triggering during the winter months. In this regard, further studies can be conducted in which allergens and/or the history of infection in patients and meteorological variables are also evaluated.

KEYWORDS: Organizing pneumonia. COPD. Asthma. Infection. Weather.

INTRODUCTION

Cryptogenic organizing pneumonia (COP) is a rare but very characteristic clinicopathological picture among pulmonary diseases. When no underlying cause is found, it is referred to as "idiopathic/cryptogenic" organizing pneumonia. In contrast, if it occurs as a result of another disease or drug use, it is called secondary organizing pneumonia. The incidence of COP in men and women is similar. Although the age range is from 20 to 80 years, it occurs most frequently between the ages of 50 and 60 years[1,2]. It is more common in nonsmokers and former smokers[3]. The diagnosis of COP is made histopathologically by the presence of granulation tissue composed of fibroblasts, collagen, and fibrinous exudate in the alveolar structure and by demonstrating a specific finding, the Masonic bodies[1]. The basic radiological appearance is peripherally located multifocal airspace consolidation. The consolidations are rarely unilateral, may be recurrent and migratory, and range from a ground-glass appearance to consolidation with air bronchograms[4]. The clinical presentation of patients may resemble upper respiratory tract infection or pneumonia[5].

Chronic obstructive pulmonary disease (COPD) is an increasing cause of morbidity and mortality worldwide. Acute exacerbations negatively impact health status, hospitalization rates, disease progression, and mortality in COPD[6]. In COPD, an acute worsening of respiratory symptoms requiring additional treatment is defined as an exacerbation. Triggering factors for an acute exacerbation are infections and noninfectious conditions. However, the etiology is unknown in more than 30% of exacerbations[7].

Asthma is a disease resulting from the interaction of environmental and genetic factors. While genetic factors play an important role in the development of asthma, environmental factors are important in both the onset and exacerbation of the disease[8,9].

[1]University of Health Sciences, Atatürk Sanatorium Training and Research Hospital, Department of Chest Disease – Ankara, Turkey.
[2] University of Health Sciences, Atatürk Sanatorium Training and Research Hospital, Department of Pathology – Ankara, Turkey.
*Corresponding author: drtugcesahin@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on July 15, 2022. Accepted on November 14, 2022.

Meteorological factors affect the respiratory system, and the most important factor is the change in ambient temperature and humidity[10-12]. It has been reported that the increase in outdoor temperature, in which atmospheric pressure increases, leads to decreased peak expiratory flow rate and morning dyspnea in COPD patients[13]. Asthma attacks such as COPD increase during the winter months[14].

Our study aimed to investigate the seasonal characteristics of our patients diagnosed with COP.

METHODS

Between January 1, 2012, and December 31, 2020, 84 patients diagnosed clinically, radiologically, and pathologically with COP in our hospital, and 55 COPD and 42 asthma patients diagnosed according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) and Global Initiative for Asthma (GINA) guidelines were included in our study[6,9]. The demographic and clinical characteristics of our patients, who were divided into three groups, and the laboratory data (white blood cell, neutrophils, lymphocytes, eosinophils, thrombocytes, hemoglobin, C-reactive protein, erythrocyte sedimentation rate, and albümin values) at the time of initial diagnosis were retrospectively collected. To determine the characteristics of the disease according to the seasons, the number of attacks and admissions in our study was grouped according to the seasonal characteristics and analyzed for three groups. Patients with comorbid malignant disease, collagen tissue disease, and secondary organizing pneumonia were excluded from the study.

The study was approved by the Ethics Committee of the University of Health Sciences, Atatürk Sanatorium Training and Research Hospital (2012-KAEK-15/2535). The study was conducted according to the Declaration of Helsinki.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) version 21.0 was used for statistical analysis. Categorical variables are presented as frequency and percentage. Continuous variables were evaluated using the Kolmogorov-Smirnov test and histograms to determine whether their distribution was normal. Normally distributed numeric parameters were compared in groups using Student's t-test or one-way analysis of variance (ANOVA), whereas those without normal distribution were analyzed using the Mann-Whitney U test or the Kruskal-Wallis test. As appropriate, categorical variables were compared with the chi-square test or Fisher's exact test.

RESULTS

Our study evaluated 122 COP patients diagnosed in our hospital between January 1, 2012, and December 31, 2020. Of the patients with COP, 27 were excluded due to concomitant malignancy and 11 were excluded due to infection. Our study group consisted of 84 COP, 55 COPD, and 42 asthma patients. The mean age of patients with COP was 59.4±11.7 years, and 55% were male. Age was similar in patients with COP and asthma and younger than in patients with COPD ($p<0.001$). Among patients diagnosed with COP and COPD, males significantly predominated ($p<0.001$). In the COP group, 30 (35.7%) individuals had never smoked, 36 (42.9%) were former smokers, and 18 (21.4%) were active smokers. Regarding pulmonary function test results, FEV1 was higher in patients with COP than in patients with COPD and asthma ($p<0.001$ and $p<0.001$, respectively). The hospitalization rate was highest in patients with COPD but similar in patients with COP and asthma ($p<0.001$). The highest admission rate in COP patients was observed in spring (39.3% in spring, 26.2% in fall, 22.6% in winter, and 11.9% in summer). In winter, COP patients were admitted less frequently than COPD and asthma patients. The main characteristics of the study participants are shown in Table 1.

While 78 (92.9%) COP patients had a pathological diagnosis, 6 of them were diagnosed by clinical-radiological examination. The most common pattern on thorax computed tomography (CT) in patients diagnosed with COP was a nodule (31%). This finding was observed as focal infiltration in 28.6%, ground-glass consolidation in 25%, air bronchograms in 13.1%, and cavity in 2.4%. The clinicopathological characteristics of patients with COP are summarized in Table 2.

As can be seen in Table 3, albumin and hemoglobin levels were lower and erythrocyte sedimentation rate was higher in patients with COP than in the other two groups ($p=0.001$, $p=0.003$, and $p=0.001$, respectively). The neutrophil-to-lymphocyte rate was higher in COP patients than in asthma patients and similar to COPD patients.

DISCUSSION

The aim of this study was to see if there is a seasonal variation in COP patients. It was found that the highest admission rate in COP patients was observed in spring. In winter, COP patients were admitted less frequently than COPD and asthma patients.

It is well known that changes in meteorological parameters increase mortality and morbidity in adults with respiratory diseases (COPD, asthma, bronchiectasis, etc.) and have a triggering function for hospital admissions. While an increase in temperature and humidity leads to clinical worsening in asthmatics, exposure

to cold air and cold increases the risk of infection, especially in COP[15]. Seasonal variations in COP were also noted in our study.

Cryptogenic organizing pneumonia typically occurs in the fifth to sixth decades of life, and both sexes are equally affected[16]. History of smoking is not considered a risk factor for COP, and most patients do not smoke[17]. In our study, the mean age of patients with COP was similar to that reported in the literature, but the male sex was more represented in contrast to the literature. In agreement with the literature, the proportion of patients who were active smokers was also lower in COP.

Open lung biopsy is the gold standard in the diagnosis of COP. However, the chance of obtaining a diagnostic sample is high with high-resolution CT-guided transbronchial lung biopsy[18]. In our study, 78 (92.9%) patients had a pathological diagnosis, while 6 were diagnosed by clinical-radiological evaluation.

Radiological findings in COP usually consist of patchy, diffuse consolidations involving bilateral subzones. Other described findings include migratory, irregular, linear, or nodular opacities[19]. In our study, nodular opacities and focal infiltrations were common and usually unilateral. The reason for this difference may be due to early detection of pulmonary infiltrates.

The results of laboratory tests in patients with COP are not specific. However, inflammatory markers such as erythrocyte sedimentation rate, CRP level, and leukocyte count are often elevated[17,20]. In our study, the erythrocyte sedimentation rate was higher in our patients with COP than in the other two groups, and the white blood cell count was higher than in asthma patients.

Table 2. Main diagnostic characteristics of patients with cryptogenic organizing pneumonia.

	Cryptogenic organizing pneumonia (n=84)
Pathological diagnosis (biopsy-proved)	
Yes, n (%)	78 (92.9)
Type of diagnosis	
Transbronchial	37 (44)
Tru-cut	32 (38.1)
Wedge	9 (10.7)
Clinical/radiological	6 (7.1)
CT findings	
Distribution	
Unilateral, n (%)	50 (59.5)
Bilateral, n (%)	34 (40.5)
Pattern	
Cavity, n (%)	2 (2.4)
Focal infiltration, n (%)	24 (28.6)
Ground-glass opacification, n (%)	21 (25)
Nodule, n (%)	26 (31)
Air bronchograms, n (%)	11 (13.1)
Exacerbation season	
Spring, n (%)	33 (39.3)
Summer, n (%)	10 (11.9)
Autumn, n (%)	22 (26.2)
Winter, n (%)	19 (22.6)

Table 1. Characteristics of study group.

	Cryptogenic organizing pneumonia (n=84)	Chronic obstructive pulmonary disease (n=55)	Asthma (n=42)	p
Age	59.4±11.7*	66.7±8.1*#	56.6±12.9#	**<0.001**
Female, n (%)	29 (34.5)*	5 (9.1)*#	22 (52.4)#	**<0.001**
Tobacco smoking				
Never smoker, n (%)	30 (35.7)*	0 (0)*#	14 (33.3)#	**<0.001**
Past smoker, n (%)	36 (42.9)*	50 (90.9)*#	12 (28.6)#	
Current smoker, n (%)	18 (21.4)	5 (9.1)*	16 (38.1)*	
Pulmonary function test findings				
FEV1, lt, median (min–max)	2.47 (0.65–4.42)*	1.05 (0.45–3.07)*	1.98 (0.85–3.93)*	**<0.001**
FEV1, %, median (min–max)	78.5 (21–123)*	38 (16–94)*	68.5 (33–90)*	**<0.001**
Exacerbation season				
Spring, n (%)	33 (39.3)*	15 (27.3)	6 (14.3)*	**0.009**
Summer, n (%)	10 (11.9)	2 (3.6)	3 (7.1)	
Autumn, n (%)	22 (26.2)	14 (25.5)	11 (26.2)	
Winter, n (%)	19 (22.6)*#	24 (43.6)#	22 (52.4)*	
Admission number, median (min–max)	1 (1–6)*	2 (1–10)*#	1.5 (1–7)#	**<0.001**

*#p-values are significantly different between groups.

Table 3. Laboratory results of the patients.

	Cryptogenic organizing pneumonia (n=84)	Chronic obstructive pulmonary disease (n=55)	Asthma (n=42)	p
White blood cell, median (min–max)	8,910 (1,230–24,000)*	9,120 (4,370–23,000)#	7,605 (3,900–13,310)*#	0.011
Neutrophils, median (min–max)	5,735 (1,100–16,900)*	6,200 (1,480–17,000)#	4,280 (1,000–10,550)*#	0.002
Lymphocytes, median (min–max)	1,945 (300–8,200)	1,860 (396–6,620)	2,265 (1,190–4,550)	0.332
Neutrophils/lymphocytes ratio, median (min–max)	2.97 (0.21–17.83)*	3.48 (0.22–19.45)#	1.95 (0.56–6.17)*#	0.001
Eosinophils, median (min–max)	177 (0–3,700)	150 (0–610)	215 (10–890)	0.237
Hemoglobin±SD	13.1±1.8*#	13.9±1.9*	14.1±1.7#	0.003
Thrombocytes, median (min–max)	288,500 (119,000–1,314,000)*#	247,000 (24,900–497,000)*	254,000 (23,000–409,000)#	0.001
C-reactive protein, median (min–max)	4.90 (0.01–175)	3.70 (0.01–85)	3 (0.3–25.40)	0.607
Erythrocyte sedimentation rate, median (min–max)	45 (7–120)*#	20 (2–120)*	17 (4–29)#	<0.001
Albumin±SD	36.7±5.5*	38.3±5.6#	41±3.1*#	<0.001

*#p-values are significantly different between groups.

The CRP level was higher than the other two groups, but not statistically significant. This may be due to the fact that CRP values in the other groups were measured during the exacerbation phase.

Seasonal factors are known to influence the frequency of COPD and asthma attacks. The number of hospital admissions due to exacerbation of COPD increases when temperatures decrease and/or triggering factors such as viral infections become more common during the winter months[15]. Meteorological factors weaken the body's immunity and favor the spread of pathogens that cause infections[21,22]. The incidence of viral respiratory infections has been shown to increase in cold weather[10].

In general, morbidity and mortality are known to increase in patients with chronic diseases, advanced age, and male gender; however, the number of studies examining the effects of environmental factors is limited. The literature has also found that seasonal factors such as air temperature and humidity increase the incidence of pneumonia[23]. No study was found in the literature investigating COP's seasonal association. In our study, the rate of admissions and diagnoses in COP patients was highest in the spring. In winter, COP patients were admitted less frequently than COPD and asthma patients.

The clinical presentation in COP is quite characteristic. The patient presents with a viral, infection-like clinical picture that has persisted for several weeks. Diagnosing patients with these causes takes several weeks[1,2]. The fact that the diagnosis of COP in our study was made in the spring suggests that patients' symptoms begin in the winter months and the diagnosis may not be made until the spring. In contrast, pollen is known to cause an increase in asthma attacks in spring[24]. This may be related to the high diagnosis and admission rate in the spring in COP.

This study has some limitations. First, it was performed in a single center as a retrospective study. Second, the study included a relatively small number of COP patients. Due to the small number of cases because of missing data, some cases that may have an impact on the results may have been excluded from the study. Another limitation of our study is that we could not access the allergy history of the patients and allergy tests, if any, from the hospital information registry system.

CONCLUSION

As a result of our study, the high rate of diagnosis and admission in the spring in COP suggested that the effect of allergens on the formation of COP should be investigated. In contrast, it should be kept in mind that COP may develop as a prolonged finding of involvement that may occur in the lung parenchyma due to lung infections and/or cold weather triggering during the winter months. In this regard, further studies can be conducted in which allergens and/or the history of infection in patients and meteorological variables are also evaluated.

ETHICS COMMITTEE APPROVAL

This study was approved by the Ethics Committee of the University of Health Sciences, Atatürk Sanatorium Training and Research Hospital (2012-KAEK-15/2535).

INFORMED CONSENT

Written informed consent was obtained from all participants who participated in this study.

AUTHORS' CONTRIBUTIONS

TSO: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **SB:** Data curation, Investigation, Methodology, Writing – original draft. **ESA:** Formal Analysis, Methodology, Writing – review & editing. **ZEO:** Data curation, Formal Analysis. **MHE:** Data curation, Formal Analysis. **FD:** Data curation, Formal Analysis. **BAO:** Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing.

REFERENCES

1. Wells AU. Cryptogenic organizing pneumonia. Semin Respir Crit Care Med. 2001;22(4):449-60. https://doi.org/10.1055/s-2001-17387
2. Cordier J-F. Update on cryptogenic organising pneumonia (idiopathic bronchiolitis obliterans organising pneumonia). Swiss Med Wkly. 2002;132(41-42):588-91. https://doi.org/10.4414/smw.2002.10015
3. Cottin V, Cordier J-F. Cryptogenic organizing pneumonia. Semin Respir Crit Care Med. 2012; 33(5):462-75. https://doi.org/10.1055/s-0032-1325157
4. Oikonomou A, Hansell DM. Organizing pneumonia: the many morphological faces. Eur Radiol. 2002;12(6):1486-96. https://doi.org/10.1007/s00330-001-1211-3
5. Poletti V, Cazzato S, Minicuci N, Zompatori M, Burzi M, Schiattone ML. The diagnostic value of bronchoalveolar lavage and transbronchial lung biopsy in cryptogenic organizing pneumonia. Eur Respir J. 1996;9(12):2513-6. https://doi.org/10.1183/09031936.96.09122513
6. Global Initiative for Chronic Obstructive Lung Disease, Inc. Global Strategy for the Diagnosis, Management and Prevention of COPD (2018 Report). Accessed online, Chapter 5, Management of exacerbations; 2018. p. 98-103.
7. Sapey E, Stockley RA. COPD exacerbations. 2: aetiology. Thorax. 2006;61(3):250-8. https://doi.org/10.1136/thx.2005.041822
8. Toskala E, Kennedy DW. Asthma risk factors. Int Forum Allergy Rhinol. 2015;5(Suppl 1):S11-6. https://doi.org/10.1002/alr.21557
9. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2018. Available from: www.ginasthma.org
10. Gautret P, Gray GC, Charrel RN, Odezulu NG, Al-Tawfiq JA, Zumla A, et al. Emerging viral respiratory tract infections, environmental risk factors and transmission. Lancet Infect Dis. 2014;14(11):1113-22. https://doi.org/10.1016/S1473-3099(14)70831-X
11. Silva DR, Viana VP, Müller AM, Livi FP, Dalcin PTR. Respiratory viral infections and effects of meteorological parameters and air pollution in adults with respiratory symptoms admitted to the emergency room. Influenza Other Respir Viruses. 2014;8(1):42-52. https://doi.org/10.1111/irv.12158
12. du Prel J-B, Puppe W, Gröndahl B, Knuf M, Weigl JAI, Schaaff F, et al. Are meteorological parameters associated with acute respiratory tract infections? Clin Infect Dis. 2009 49(6):861-8. https://doi.org/10.1086/605435
13. Mann M, Patel K, Reardon JZ, Goldstein M, Godar TJ, ZuWallack RL. The influence of spring and summer New England meteorologic conditions on the respiratory status of patients with chronic lung disease. Chest. 1993;103(5):1369-74. https://doi.org/10.1378/chest.103.5.1369
14. López-Campos JL, Abad Arranz M, Calero-Acuña C, Romero-Valero F, Ayerbe-García R, Hidalgo-Molina A, et al. Seasonal variability in clinical care of COPD outpatients: results from the Andalusian COPD audit. Int J Chron Obstruct Pulmon Dis. 2017;12:785-92. https://doi.org/10.2147/COPD.S121885
15. D'Amato G, Cecchi L, D'Amato M, Annesi-Maesano I. Climate change and respiratory diseases. Eur Respir Rev. 2014;23(132):161-9. https://doi.org/10.1183/09059180.00001714
16. Basarakodu KR, Aronow WS, Nair CK, Lakkireddy D, Kondur A, Korlakunta H, et al. Differences in treatment and in outcomes between idiopathic and secondary forms of organizing pneumonia. Am J Ther. 2007;14(5):422-6. https://doi.org/10.1097/01.pap.0000249905.63211.a1
17. Baha A, Yıldırım F, Köktürk N, Galata Z, Akyürek N, Demirci NY, et al. Cryptogenic and secondary organizing pneumonia: clinical presentation, radiological and laboratory findings, treatment, and prognosis in 56 cases. Turk Thorac J. 2018;19(4):201-8. https://doi.org/10.5152/TurkThoracJ.2018.18008
18. Akira M, Yamamoto S, Sakatani M. Bronchiolitis obliterans organizing peumonia manifesting as multiple large nodules and masses. Am J Roentgenol. 1998;170(2):291-5. https://doi.org/10.2214/ajr.170.2.9456931
19. Faria IM, Zanetti G, Barreto MM, Rodrigues RS, Araujo-Neto CA, Silva JLP, et al. Organizing pneumonia: chest HRCT findings. J Bras Pneumol. 2015;41(3):231-7. https://doi.org/10.1590/S1806-37132015000004544
20. Cordier J-F. Cryptogenic organising pneumonia. Eur Respir J. 2006;28(2):422-46. https://doi.org/10.1183/09031936.06.00013505
21. Mourtzoukou EG, Falagas ME. Exposure to cold and respiratory tract infections. Int J Tuberc Lung Dis. 2007;11(9):938-43. PMID: 17705968
22. Liu Y, Liu J, Chen F, Shamsi BH, Wang Q, Jiao F, et al. Impact of meteorological factors on lower respiratory tract infections in children. J Int Med Res. 2016;44(1):30-41. https://doi.org/10.1177/0300060515586007
23. Liu L, Poon R, Chen L, Frescura A-M, Montuschi P, Ciabattoni G, et al. Acute effects of air pollution on pulmonary function, airway inflammation, and oxidative stress in asthmatic children. Environ Health Perspect. 2009;117(4): 668-74. https://doi.org/10.1289/ehp11813
24. Schatz M, Rosenwasser L. The allergic asthma phenotype. J Allergy Clin Immunol Pract. 2014;2(6):645-8. https://doi.org/10.1016/j.jaip.2014.09.004

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220982

The effects of fat graft and platelet-rich fibrin combination after epineurectomy in rats

Menekşe Kastamoni[1], Senem Esin Yavaş[2], Güzin Yesim Ozgenel[3], Semiha Ersoy[2]*

SUMMARY

OBJECTIVE: One of the most important factors that adversely affects the outcome of peripheral nerve surgery is the formation of epineural and extraneural scar tissue after surgery. Many surgical methods and pharmacological and chemical agents have been used to prevent the formation of epineural scar tissue, but satisfactory results have not been achieved in clinical applications. The purpose of this study was to investigate the combined effect of fat graft and platelet-rich fibrin on the formation of epineural scar tissue and on nerve healing in the mature rat model.

METHODS: A total of 24 female Sprague-Dawley rats were used. A circumferential segment of epineurium was excised from both bilateral sciatic nerves. The epineurectomized right nerve segment was wrapped with a combination of fat graft and platelet-rich fibrin (experimental group), while the left nerve segment did not receive any surgical procedure other than the epineurectomy (sham group). Notably, 12 randomly selected rats were sacrificed in the fourth week for histopathological examination of early results. The other 12 rats were sacrificed in the eighth week for late results.

RESULTS: The formation of fibrosis, inflammation, and myelin degeneration were less common in the experimental group, while nerve regeneration was found to be higher at both 4 and 8 weeks.

CONCLUSION: The intraoperative application of a combination of fat graft and platelet-rich fibrin appears to be effective on nerve healing after surgery at both the early and late periods.

KEYWORDS: Sciatic neuropathy. Nerve regeneration. Platelet-rich fibrin. Adipose tissue. Receptor, EphA4.

INTRODUCTION

Epineural and extraneural scar tissue that develops out of control by causing chronic pain and loss of function is an important factor that adversely affects the outcome of peripheral nerve surgery[1]. To prevent perineural scar formation occurring after peripheral nerve surgery, various techniques and agents (wrapping of nerves with various natural materials such as vein, buccal mucosa, fascia tissue, human amniotic membrane, hyaluronic acid injection around the damaged nerve, and 5-fluorouracil) have been tried, but these methods have not solved the problem entirely[2-4]. As the adipose tissue is a rich source of multipotent mesenchymal cells called adipose-derived stem cells[5], the frequency and importance of using fat grafts during surgical procedures are gradually increasing. In vitro studies have demonstrated that platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) activate the adipose-derived stem cells with secreted factors[6].

Remyelination is important in the recovery of axonal function after nerve injury. Schwann cells are responsible for myelination/remyelination in the peripheral nervous system (PNS)[7,8]. The Eph (erythropoietin-producing hepatocellular carcinoma) receptors form the largest family of tyrosine kinase receptors. They are divided into two subfamilies, namely, EphA and EphB, and their ligands are ephrins. It has been reported that the activation of one of these receptors, EphA4, decreases myelination by oligodendrocytes in the central nervous system, while its inhibition increases it[9]. There are few studies showing that EphA4, which is also expressed in intact Schwann cells, negatively regulates myelination by these cells in PNS damage repairs[10,11].

Considering the studies using fat graft alone and PRF alone, in our experimental study, we aimed to reduce the adhesion and scar formation in the nerves by using the stimulating effect of thrombocytes on adipose tissue-originated stem cells and increasing tissue repair. We also attempted to evaluate the efficacy of the applied treatment with EphA4 expression levels.

[1]Niğde Training and Research Hospital, Department of Plastic Reconstructive and Aesthetic Surgery – Niğde, Turkey.
[2]Bursa Uludağ Üniversitesi, School of Medicine, Department of Histology and Embryology – Bursa, Turkey.
[3]Bursa Uludağ Üniversitesi, School of Medicine, Department of Plastic Reconstructive and Aesthetic Surgery – Bursa, Turkey.
*Corresponding author: semihaersoy@uludag.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 30, 2022. Accepted on November 14, 2022.

METHODS

Animals

All animals in this study were used according to a protocol approved by the Bursa Uludag University Experimental Animals and Ethics Committee (approval no.: 2019-05/03). A total of 24 12-week-old female Sprague-Dawley rats weighing 250–300 g were used. Animals were kept in groups of four in cages with free access to food and water under alternating 12-h light and dark periods.

Surgical procedure

All animals were anesthetized by sevoflurane 250 mL (Sojourn, Piramal) via an inhaler. Following the induction of general anaesthesia, rats were placed in a supine position. First, approximately 1 mL of blood was taken from the rat tail artery and placed in the device for centrifugation at the appropriate speed for the preparation of PRF. The rat was then returned to the prone position. After longitudinal skin incisions, bilateral sciatic nerves were accessed via blunt dissection through the gluteus maximus and biceps femoris muscles. Bilateral sciatic nerves, from the sciatic notch to the bifurcation, were exposed. A 0.5-cm-long epineurium segment was circumferentially excised from the main nerve trunk to initiate scar tissue formation. For the right sciatic nerve, the incision was extended to the popliteal region, and approximately 10×10×0.5 mm^3 fat grafts were prepared from the adipose tissue in this area. Meanwhile, the fat graft obtained from the popliteal region was mixed with the PRF, whose centrifuge was finished and ready, and it was wrapped around the epineurectomy area. The left nerve segment did not undergo any surgical procedure other than the epineurectomy and was considered the sham (or control) group. The right sciatic nerve was considered the experimental (or treatment) group. For histopathological examinations, 12 randomly selected rats were euthanized in the fourth week for early results and the remaining 12 rats in the eighth week for late results.

Histopathology

The right (experimental/treatment group) and the left (sham/control group) nerve segments were removed en bloc with the surrounding tissue, fixed in neutral-buffered 10% formalin solution, dehydrated in alcohol series, and embedded in paraffin. Then, 5-µm sections were stained with routine haematoxylin and eosin (H&E) for general morphology and with Masson's trichrome for fibrosis. In addition, one group of sections was stained with the Klüver Barrera method to evaluate myelination, while EphA4 receptor immunohistochemistry was applied to another group of sections. Tissues from a rat without any procedure were used to see the healthy sciatic nerve structure and to compare it with other groups.

Immunohistochemistry

Sections were incubated in citrate buffer (pH=6.0) at 98°C and then cooled and rinsed for 3×5 min in TRIS tampon (pH=7.6). They were incubated in a 3% H_2O_2 blocked endogenous peroxidase activity for 5 min at room temperature and rinsed for 3×5 min in TRIS tampon. Blockage of nonspecific binding protein was done by incubating sections with horse serum (Sigma Aldrich, H1138) for 30 min. Incubation with anti-EphA4 primary antibody (Santa Cruz, sc-365503) was done overnight at 4°C. After rinsed for 3×5 min in TRIS tampon, the sections were incubated with donkey anti-mouse IgM secondary antibody (Jackson Immunoresearch, 715-065-140) for 1 h and ABC solution (VECTOR Laboratories, PK6100) for 2 h. The reactions were visualized with DAB and counterstained with Harris haematoxylin.

Results were independently evaluated by two investigators using a light microscope. Fibrosis, inflammation, and degeneration were evaluated on a three-point scale (mild, moderate, and severe). Regenerative changes around the nerve (remyelination and EphA4 receptor expression) were evaluated on a five-point scale (none, poorly, moderate, well, and perfect). Photographs were taken using an Olympus BX 50 photomicroscope.

Statistical evaluation

To investigate the differences in histopathological values according to the fourth and eighth weeks for each group, an analysis was made with the Mann-Whitney U test. Wilcoxon signed-ranks test was used to evaluate the early and late results of histopathological values separately. Analyses were made using the SPSS 22.0 package program. Differences were considered statistically significant at $p<0.05$.

RESULTS

Degeneration

Myelin degeneration was more prominent in the sham groups than in the experimental groups in both the early and late periods (Figure 1). It was observed that the application of combined fat graft and PRF significantly reduced myelin degeneration in both the early and late periods (Figure 2A).

Fibrosis and inflammation

Less collagenous area and inflammation were observed in the sciatic nerves that were wrapped with combined fat graft and

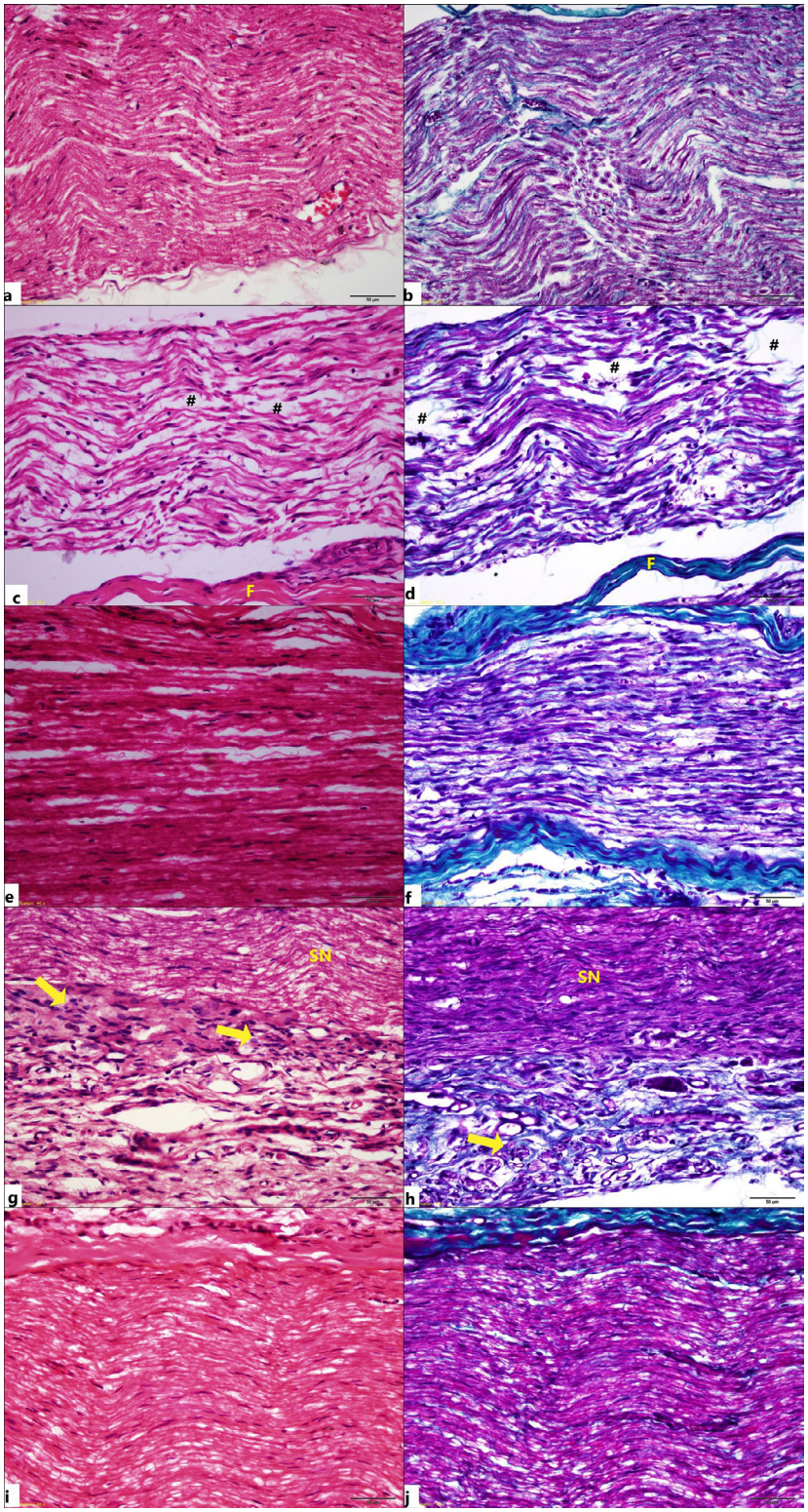

Figure 1. Histological appearance of the all groups. Intact sciatic nerve **(a, b)**, 4-week sham group **(c, d)**, 4-week treatment group **(e, f)**, 8-week sham group **(g, h)**, and 8-week treatment group **(i, j)**. (F: Fibrosis; →: Inflammation; SN: Sciatic nerve; #: Degeneration). (**a, c, e, g, i:** H&E staining, **b, d, f, h, j:** Masson's trichrome staining).

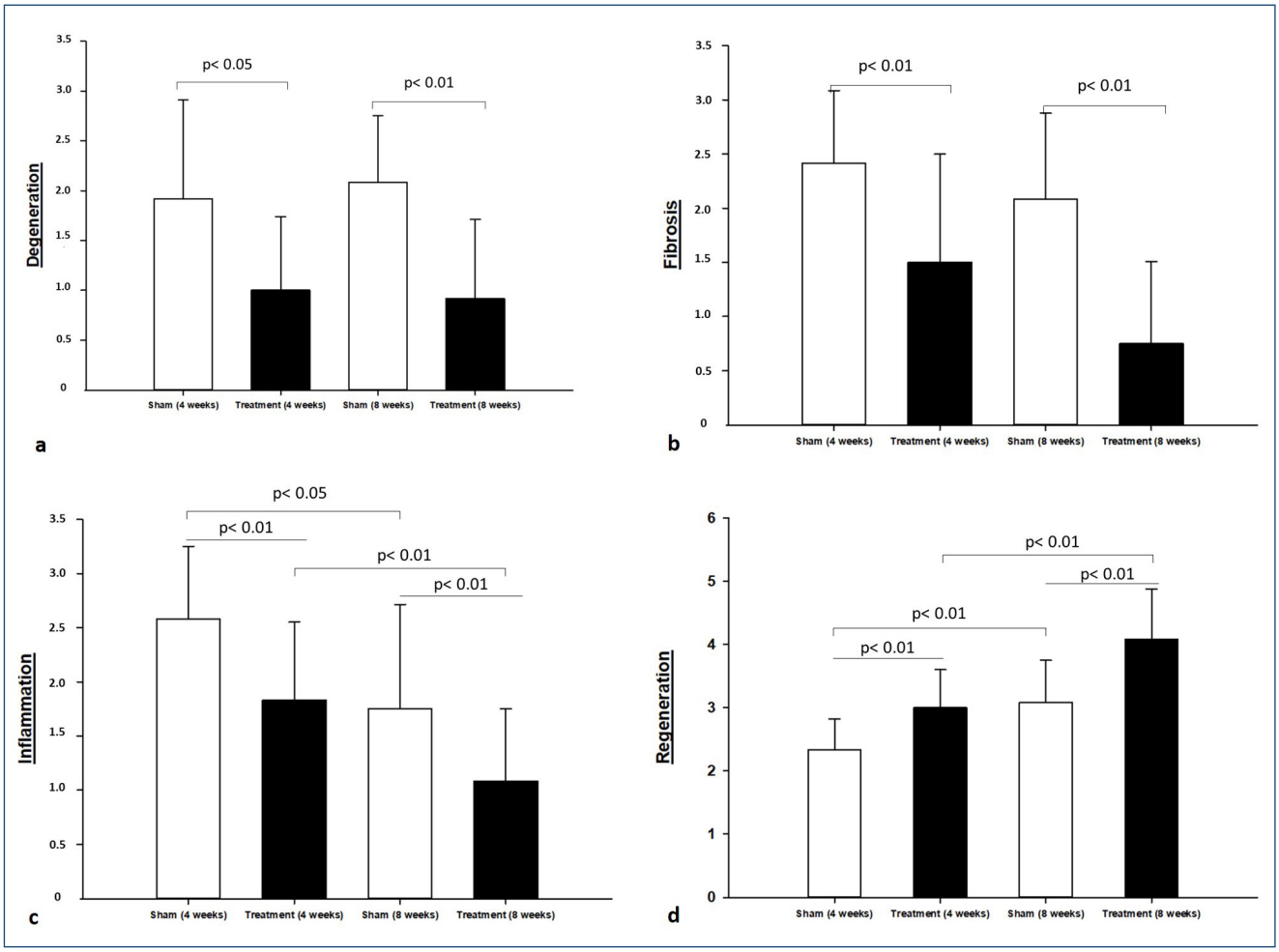

Figure 2. The statistical results.

PRF in both the early and late periods compared to the sham groups (Figure 1). Histopathologically, it was observed that inflammation and fibrosis were statistically significantly reduced in rats treated with 4 and 8 weeks of combined fat grafting and PRF (Figures 2B,C).

Nerve regeneration/remyelination

Regeneration was observed better in the experimental groups than in the sham groups in both the early and late periods (Figure 1). Intact myelin sheaths in control tissues were stained in a characteristic intense blue colour by the Klüver Barrera method (Figure 3A). It was determined that remyelination was not completed and the stained areas and staining intensity decreased in the 8-week sham group (Figure 3B). The sciatic nerves in the 8-week treatment group were observed to have acquired staining and morphology that is almost similar to those of the control sections (Figure 3C). The 4-week treatment and 4-week sham groups were similar to the 8-week sham group (data not shown).

EphA4 receptor expression

It was observed that the expression of EphA4 in Schwann cells according to the control sections (Figure 3D) was relatively increased in the 4-week early treatment group (Figure 3E). EphA4 immunopositivity in the 8-week treatment group (Figure 3F) was relatively decreased compared to the 4-week treatment group (Figure 3E).

The combined fat graft and PRF application was found to have a statistically significant positive effect on nerve regeneration (decrease in EphA4 expression and increase in remyelination) in both the early and late periods in the experimental groups (Figure 2D).

DISCUSSION

Perineural adhesions and scar formation occurring after peripheral nerve surgery are unpredictable and undesirable. Despite advances in microsurgery, functional return after classic methods of nerve repair is unsatisfactory[12]. Various applications, such as ADCON-T/N[13], human amniotic fluid with hyaluronic acid[2], buccal mucosal

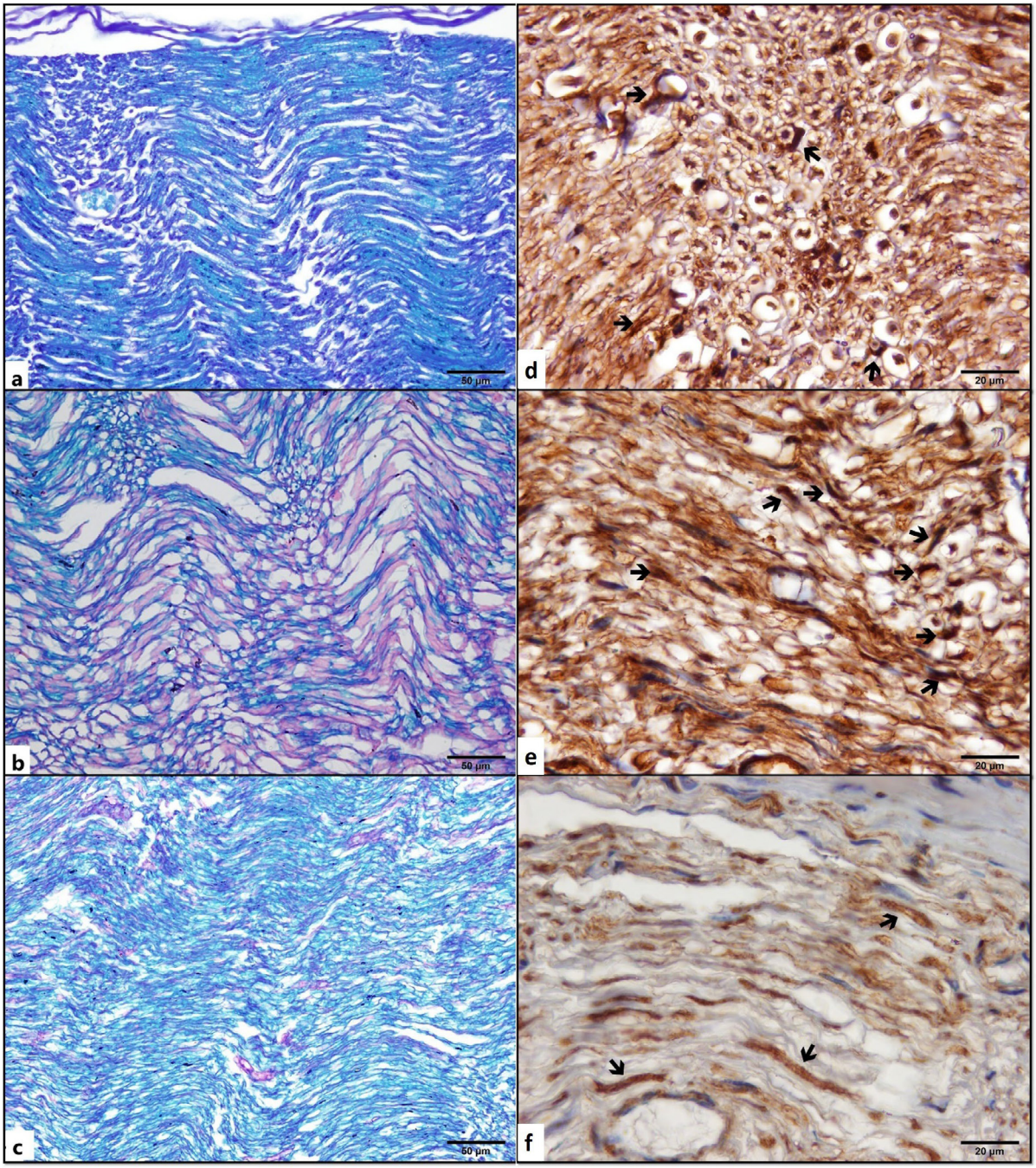

Figure 3. Myelination and EphA4 receptor expression. Intact sciatic nerve **(a)**, 8-week sham group **(b)**, 8-week treatment group **(c)** (Klüver-Barrera staining). Intact sciatic nerve **(d)**, 4-week treatment group **(e)**, and 8-week treatment group **(f)** (EphA4 immunopositivity: →).

graft[3], and topical 5-fluorouracil[4] are some of the methods used to reduce post-surgical adhesions and scar formation.

There has been a recent interest in adipose-derived stem cells. Adipose tissue is a rich source of these multipotent mesenchymal cells and has a unique advantage of being harvested easily, cheaply, and safely[5,6]. Several experimental studies with fat grafts have been tried to improve the results of the peripheral nerve surgery. In one study in rats, free fat grafts were applied around the sciatic nerve with epineurectomy and showed that the fat graft reduced perineural scar tension with unexpected degenerative changes in some

cases[14]. In another study in rats, fat grafts have been shown to positively affect early nerve regeneration but have no significant effect on fibrosis and scar formation[15]. In recent years, studies have been conducted by wrapping fat grafts around the nerves and placing the adipose-derived stem cells in the repair line and found that adipose-derived stem cells have a positive effect on nerve healing. Liu et al.[16] showed that adipose-derived stem cells increase the rates of nerve healing and nerve conduction after repair.

Platelet-rich plasma is prepared by centrifuging one's own blood and contains 4–7 times the dense platelet of normal blood concentration. Alpha granules in platelets contain seven basic growth factors: platelet-derived growth factor (PDGFaa, PDGFbb, and PDGFab), transforming growth factor-beta (TGFβ1 and TGFβ2), epithelial growth factor (EGF), and vascular endothelial growth factor (VEGF). These growth factors have been shown to modulate cell proliferation, differentiation, angiogenesis, and chemotaxis. The most preferred enrichment methods developed to benefit from the mentioned features of platelets are classical PRP and PRF[17]. One study examined the effects of PRF on the sciatic nerve healing model and found that PRF had an accelerating and complementary effect on peripheral nerve healing histologically[18]. *In vitro* studies showed that the factors released by PRP and PRF activate the adipose-derived stem cells and chemotaxis of these cells[6]. This study is the first one in which completely autologous biological products are used and positive results are obtained in all parameters histologically and no complications are observed.

For nerve regeneration to be functional, both axonal elongation and new myelin synthesis should occur[7,8]. In our study, we concluded that remyelination in the 8-week late treatment and control groups was similar and that the long-term treatment applied had a positive effect on regeneration. Harboe et al.[9] showed that the EphrinA1-EphA4 signalling negatively affects myelination in the central nervous system after injury. Similarly, EphA4 has effect on Schwann cell differentiation and regeneration in PNS injuries. It has been reported that remyelination and axonal regeneration are due to Schwann cell differentiation after peripheral nerve damage in EphA4$^{-/-}$ rats[11]. Chen et al.[10] reported that the expression of EphA4 in Schwann cells in the PNS has negative effects on myelination by inhibiting the differentiation of these cells after injury. Similarly, in our study, insufficient myelination in correlation with the increase in EphA4 expression at the end of 4 weeks and remyelination and axonal recovery in correlation with the decrease in EphA4 expression at the end of 8 weeks suggested that EphA4 had a negative effect on myelination and axonal repair in the sciatic nerve.

CONCLUSION

Our results show that the use of the fat graft+PRF combination positively affects nerve recovery after epineurectomy histologically in both the early and late periods. The areas of use of the fat graft+PRF combination should be investigated and increased in order to obtain the expected mutual effects and hence positive results. We believe that this study will be a guide in this regard. However, the limitation of our study is that the degree of functional recovery of the sciatic nerves was not evaluated. We also believe that it would be more meaningful to support our histopathological results with functional results. In addition, it is necessary to investigate through which molecular pathways the graft components are effective in the expression of EphA4 in Schwann cells. In this way, EphA4 can be used as a potential molecular marker for peripheral nerve injuries in the clinics in the future.

ETHICS

This study was conducted at Bursa Uludag University, School of Medicine, Department of Histology and Embryology, Bursa, Turkey, and conducted in accordance with the Declaration of Helsinki. The Ethics Committee approved the study protocol of Bursa Uludag University (approval no.: 2019-05/03).

AUTHORS' CONTRIBUTIONS

MK: Conceptualization, Methodology, Investigation, Resources, Writing – original draft. **SEY:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft. **SE:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft. **GYO:** Conceptualization, Methodology.

REFERENCES

1. Abrams M, Widenfalk J. Emerging strategies to promote improved functional outcome after peripheral nerve injury. Restor Neurol Neurosci. 2005;23(5-6):367-82. PMID: 16477099
2. Ozgenel GY, Filiz G. Combined application of human amniotic membrane wrapping and hyaluronic acid injection in epineurectomized rat sciatic nerve. J Reconstr Microsurg. 2004;20(2):153-7. https://doi.org/10.1055/s-2004-820772
3. Baltu Y, Uzun H, Özgenel GH. The reduction of extraneural scarring with buccal mucosa graft wrapping around the sciatic nerve: an experimental study in a rat model. J Plast Surg Hand Surg. 2017;51(4):259-63. https://doi.org/10.1080/2000656X.2016.1241790

4. Cavus Ozkan M, Tuncel B, Ozgenel GY. The effect of topical 5-fluorouracil application on epineural scar tissue in epineurectomized rat sciatic nerve. Marmara Med J. 2018;31:1-10. https://doi.org/10.5472/marumj.342046
5. Pu LLQ, Coleman SR, Cui X, Ferguson REH, Vasconez HC. Autologous fat grafts harvested and refined by the Coleman technique: a comparative study. Plast Reconstr Surg. 2008;122(3):932-7. https://doi.org/10.1097/PRS.0b013e3181811ff0
6. Lopez JF, Sarkanen JR, Huttala O, Kaartinen IS, Kuokkanen HO, Ylikomi T. Adipose tissue extract shows potential for wound healing: ***in vitro*** proliferation and migration of cell types contributing to wound healing in the presence of adipose tissue preparation and platelet rich plasma. Cytotechnology. 2018;70(4):1193-204. https://doi.org/10.1007/s10616-018-0211-y
7. Jessen KR, Mirsky R. The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci. 2005;6(9):671-82. https://doi.org/10.1038/nrn1746
8. Jessen KR, Mirsky R. The success and failure of the Schwann cell response to nerve injury. Front Cell Neurosci. 2019;13:33. https://doi.org/10.3389/fncel.2019.00033
9. Harboe M, Torvund-Jensen T, Kjaer-Sorensen K, Laursen LS. Ephrin-A1-EphA4 signaling negatively regulates myelination in the central nervous system. Glia. 2018;66(5):934-50. https://doi.org/10.1002/glia.23293
10. Chen R, Yang X, Zhang B, Wang S, Bao S, Gu Y, et al. EphA4 negatively regulates myelination by inhibiting Schwann cell differentiation in the peripheral nervous system. Front Neurosci. 2019;13:1191. https://doi.org/10.3389/fnins.2019.01191
11. Wang Y, Zheng Z, Hu D. Inhibition of EphA4 expression promotes Schwann cell migration and peripheral nerve regeneration. Neuroscience Lett. 2013;548:201-5. https://doi.org/10.1016/j.neulet.2013.05.022
12. Maser BM, Vedder N. Nerve repair and nerve grafting. In: Russell RC, editor. Plastic surgery. Indications, operations, and outcomes. Vol. 4 (Hand Surgery). New York: Mosby; 2000. p. 2103-19.
13. Petersen J, Russel L, Andrus K, MacKinnon M, Silver J, Kliot M. Reduction of extraneural scarring by ADCON-T/N after surgical intervention. Neurosurgery. 1996;38(5):976-83. https://doi.org/10.1097/00006123-199605000-00025
14. Dumanian GA, McClinton MA, Brushart TM. The effect of free fat grafts on stiffness of the rat sciatic nerve and perineural scar. J Hand Surg Am. 1999;24(1):30-6. https://doi.org/10.1053/jhsu.1999.jhsu24a0030
15. Ersen B, Kahveci R, Uğraş N, Ocakoğlu G. Evaluation of effects of fat graft wrapping on early nerve regeneration: an experimental study. Türkiye Klinikleri J Med Sci. 2018;38(4):303-8. https://doi.org/10.5336/medsci.2018-60484
16. Liu GB, Cheng YX, Feng YK, Pang CJ, Li Q, Wang Y, et al. Adipose-derived stem cells promote peripheral nerve repair. Arch Med Sci. 2011;7(4):592-6. https://doi.org/10.5114/aoms.2011.24127
17. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma. Platelets. 2018;29(1):48-55. https://doi.org/10.1080/09537104.2017.1293807
18. Metineren H, Dülgeroğlu TC, Metineren MH. Trombositten zengin fibrinin periferik sinir iyileşmesi üzerindeki histopatolojik etkileri. Dicle Med J. 2017;44(3):243-50. https://doi.org/10.5798/dicletip.338997

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20220986

Serotonin, *ghrelin*, and *motilin* gene/receptor/transporter polymorphisms in childhood functional constipation

Bengu Arslan[1*], Guzide Dogan[2], Seda Orenay-Boyacioglu[3], Metin Caliskan[4], Murat Elevli[5]

SUMMARY

OBJECTIVE: Functional constipation is the most common form of constipation, and its exact aetiology is still unclear. However, it is known that deficiencies in hormonal factors cause constipation by changing physiological mechanisms. Motilin, ghrelin, serotonin acetylcholine, nitric oxide, and vasoactive intestinal polypeptide are factors that play a role in colon motility. There are a limited number of studies in the literature where hormone levels and gene polymorphisms of *serotonin* and *motilin* are examined. Our study aimed to investigate the role of motilin, ghrelin, and serotonin gene/receptor/transporter polymorphisms in constipation pathogenesis in patients diagnosed with functional constipation according to the Rome 4 criteria.

METHODS: Sociodemographic data, symptom duration, accompanying findings, the presence of constipation in the family, Rome 4 criteria, and clinical findings according to Bristol scale of 200 cases (100 constipated patients and 100 healthy control) who applied to Istanbul Haseki Training and Research Hospital, Pediatric Gastroenterology Outpatient Clinic, between March and September 2019 (6-month period) were recorded. Polymorphisms of *motilin-MLN* (rs2281820), *serotonin receptor-HTR3A* (rs1062613), *serotonin transporter-5-HTT* (rs1042173), *ghrelin-GHRL* (rs27647), and *ghrelin receptor-GHSR* (rs572169) were detected by real-time PCR.

RESULTS: There was no difference between the two groups in terms of sociodemographic characteristics. Notably, 40% of the constipated group had a family history of constipation. The number of patients who started to have constipation under 24 months was 78, and the number of patients who started to have constipation after 24 months was 22. There was no significant difference between constipation and control groups in terms of genotype and allele frequencies in *MLN*, *HTR3A*, *5-HTT*, *GHRL*, and *GHSR* polymorphisms ($p>0.05$). Considering only the constipated group, the rates of gene polymorphism were similar among those with/without a positive family history of constipation, constipation onset age, those with/without fissures, those with/without skin tag, and those with type 1/type 2 stool types according to the Bristol stool scale.

CONCLUSION: Our study results showed that gene polymorphisms of these three hormones may not be related to constipation in children.

KEYWORDS: Child. Constipation. *Ghrelin*. *Motilin*. *Serotonin*. Polymorphism, single nucleotide.

INTRODUCTION

Constipation is defined as difficulty in passing stools that may be infrequent (≤2 per week), painful, and associated with stool retention. Chronic constipation is a common health problem, especially in children, and has a great impact on physical/mental health. Chronic constipation is divided into two groups, namely, organically caused and functional constipation. Notably, 95% of constipation is of the functional type in childhood, and the prevalence rates are reported to be 32.2% worldwide. In recent years, research on aetiology and pathogenesis of childhood constipation has focused on environmental factors, behavioural problems, and genetic factors. Various environmental factors are associated with a higher prevalence of childhood constipation which include diet and mobility of the children, low maternal educational level, and social circumstances. The relationship of constipation with behavioural problems is also complex, partly because constipation can be both a cause and product of behavioural problems. Furthermore, constipation has been reported more frequently in children with specific behavioural phenotypes, such as autism spectrum disorder[1,2]. The role of genetics in the aetiology of constipation is still largely unknown. So far, linkage studies, association studies, and direct gene sequencing have yet to find mutations in genes specifically associated with constipation. However, more than 40% of constipated children have a family history of constipation, and a genetic

[1]University of Health Sciences, Basaksehir Cam and Sakura City Hospital, Department of Pediatric Inherited Metabolic Diseases – Istanbul, Turkey.
[2]Bezmialem Vakıf Unıversity, Department of Pediatric Gastroenterology – Istanbul, Turkey.
[3]Aydin Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics – Aydın, Turkey.
[4]Usak University, Faculty of Medicine, Department of Medical Biology – Usak, Turkey.
[5]Istanbul Haseki Training and Research Hospital, Department of Pediatric Gastroenterology – Istanbul, Turkey.
*Corresponding author: benguarslan.ba@gmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: This study was funded by Health Sciences University Research Fund. Project number: 2018\062.
Received on September 07, 2022. Accepted on November 14, 2022.

predisposition is suggested in which monozygotic twins have six times more constipation than dizygotic twins[2].

Deficiencies in hormonal factors cause changes in physiological mechanisms and lead to constipation. Motilin, ghrelin, serotonin, acetylcholine, nitric oxide, and vasoactive intestinal polypeptide play a role in colon motility. The most important task of the motilin hormone is to speed up bowel movements. Motilin binds to motilin receptors (MTLR) located on the cell surface to show its effect[3]. Ghrelin has been shown to have a prokinetic effect on gastrointestinal motility through the vagus and pelvic nerves. Centrally acting GRLN-R agonists stimulate defecation in animals and humans and accelerate gastrointestinal passage[4]. Serotonin plays a role in motility and secretion, slows gastric emptying, and increases colonic motor activity[5].

In the literature, there are limited studies involving only serotonin/motilin hormone levels and gene polymorphisms, and no study have evaluated the polymorphisms of three factors (*motilin*, *ghrelin*, and *serotonin*) together. Our study aimed to clarify the effectiveness of these factors in the pathogenesis of functional constipation and to reveal the underlying genetic cause in many cases monitored by functional constipation.

METHODS

Ethics

Ethical approval was obtained from the Corporate Ethics Committee of Istanbul Haseki Training and Research Hospital in 2019 (document number: 2011-KAEK-50). Informed consent was obtained from the families of all study participants before the study.

Samples and sample size

Our study included 100 children with constipation (constipated group) and 100 children without constipation and gastrointestinal system problems (control group) in Istanbul Haseki Training and Research Hospital, Pediatric Gastroenterology Outpatient Clinic, between March and September 2019 (6-month period). Inclusion criteria of the constipated group were as follows: (1) those with chronic constipation, who meet the criteria for functional constipation according to the Rome 4 criteria; (2) children aged 6 months to 18 years; and (3) those who read the patient informed consent form and agreed to participate in the study. exclusion criteria were as follows: (1) congenital anomalies of the anorectal region and Hirschprung's disease; (2) those with neurological disorders such as cerebral palsy and spina bifida; (3) those diagnosed with hypothyroidism, diabetes mellitus, and diabetes insipidus; (4) those who have had previous abdominal surgery; and (5) those who read the patient informed consent form and did not agree to participate in the study.

Sociodemographic data, duration of symptoms, accompanying findings, presence of constipation in the family, Bristol stool scale findings, complete physical examination findings, and body weight, body length, and weight and height percentiles were recorded in the patient's forms.

The question of encopresis and urinary incontinence evaluation was asked to the families of children older than 3.5 years, who had urinary and stool control and had the ability to urinate and defecate, and the answers were recorded in our case form. When the family answered "yes" to the question of repeated (usually involuntary) passing stool on the child's clothes, encopresis or faecal incontinence was detected and the answers were recorded. The families were asked about the enuresis of the children and the answers were recorded. Anal fissure and skin tag were detected during anal examination in children with constipation and the data were recorded in our case form.

The sample size in the study was determined using G-power. Considering similar studies on the subject in the literature, the minimum sample size was calculated as 200 people, with a statistical power of 89.8%, a confidence level of 95%, and a type 1 error rate of 0.05[6].

Selection of *MLN*, *HTR3A*, *5-HTT*, *GHRL*, and *GHSR* polymorphisms

In the study, polymorphisms [*motilin-MLN* (rs2281820), *serotonin receptor-HTR3A* (rs1062613), *serotonin transporter-5-HTT* (rs1042173), *ghrelin-GHRL* (rs27647), and *ghrelin receptor-GHSR* (rs572169)] that are thought to be related to patients diagnosed with functional constipation and are found to be associated with some gastrointestinal diseases were selected.

DNA isolation

DNA isolation from blood samples was performed using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the kit usage procedures of the commercial company. A volume of 200 µl of blood sample was placed in a 1.5-ml tube, and 200 µl of binding buffer and 40 µl of proteinase K were added, mixed, and incubated at 70°C for 10 min. Then, 100 µl of isopropanol and 200 µl of elution buffer were added, mixed, and incubated at 70°C. All of this prepared mixture was transferred to a tube and centrifuged at 8,000 *g* for 1 min. Then, 500 µl of inhibitor removal buffer was added to the filter tube and centrifuged at 8,000 *g* for 1 min. A volume of 500 µl wash buffer was added and centrifuged at 8,000 *g* for 1 min. This process was repeated 2 times. The tubes were emptied and centrifuged

at 13,000 *g* for 1 min. Elution buffer kept at 70°C was added to the filter tube by placing an Eppendorf tube under the filter tube. Pure DNA was obtained by centrifugation at 8,000 *g* for 1 min.

DNA concentration and purity measurement

DNA purity and concentration were measured using NanoDrop 1000 Spectrophotometer version 3.7 (Thermo Scientific, USA). The concentration and purity of the obtained DNA samples were observed at wavelengths of 260 and 280 nm determined by measuring their absorbance. Generally, 5–50 ng of DNA per reaction was considered sufficient to detect a single-nucleotide polymorphism (SNP).

Determination of genotypes

SNPs were detected in LightCycler 480 (Roche) using a LightSNiP assay (TIBMOLBIOL GmbH, Berlin, Germany) panel based on SimpleProbe® (Roche). Probes provided by the manufacturer detect single-base mismatches and polymorphisms, making the analysis possible. SNPs were observed by a melting curve analysis at the end of amplification.

For each SNP, there is a tube of primer and a tube containing the simple probes in lyophilized form. A volume of 105 µl of water was added to each tube separately and vortexed.

It was prepared according to real-time mix separately for each SNP. After 7.5 µl per mix plate, the final volume of 10 µl was reached by distributing the sample and adding 2.5 µl of DNA to it. The device protocol was entered and worked on the LightCycler 480 device.

Data analysis

Data analysis was done by melting curve genotyping using the LightCycler 480 software.

Statistical analysis

Differences between the patients and controls in terms of categorical variables, such as demographic and clinical data, were analyzed using chi-square (χ^2) tests, while continuous variables were analyzed with Student's t-test. Differences in allelic distribution of the SNPs were also examined using χ^2 test. The statistical significance level was accepted as $p<0.05$.

RESULTS

The demographic characteristics of the groups are shown in Table 1. The average duration of constipation was 19.5±19.3 months (3–120 months), and the median duration of constipation was 12 months in the constipated group. The mean duration of defecation time was 4.4±1.9 days (2 days–11 days) in the constipated group. According to the Bristol stool scale evaluations of the patient group, 56 patients had type 1 stool and 44 patients had type 2 stool. Those with and without encopresis, urinary incontinence, anal fissure, and skin tag were compared with the duration of constipation. There was no statistically significant difference between the groups ($p>0.05$).

Genotype and allele frequency comparisons of constipation and control groups are given in Table 2. According to these comparisons, there was no significant difference in genotype and allele frequencies in terms of gene polymorphisms investigated between constipation and control groups ($p>0.05$). No statistically significant difference was found in the families of children with constipation according to the history of constipation and the onset age of symptoms compared to the allele and genotype frequencies of gene polymorphisms (Table 3) ($p>0.005$).

Table 1. Demographic features of groups.

Demographic features	Constipated group n=100	Control group n=100	p-value
Average age (months)	70.9±54.9	68.9±54.0	0.799
Gender (male/female)	45/55	53/47	0.258
Weight percentile	49.4±32.5	49.6±27.0	0.955
Height percentile	48.1±30.0	48.9±27.8	0.835
Faecal incontinence (yes/no %)	17/83		
Urinary incontinence (yes/no %)	9/91		
Recurrent urinary tractinfection (yes/no %)	14/86		
Family history of constipation (yes/no %)	40/60		
Anal fissure (yes/no %)	33/67		
Skin tag (yes/no %)	28/72		

Table 2. Genotype and allele frequency of constipation and control groups.

Gene and SNP	Constipated group (n=100)	Control group (n=100)	p-value
5HTT-rs1042173			
Genotype GG	31	28	
Genotype GT	45	46	0.885
Genotype TT	24	26	
Allele G	107	102	0.689
Allele T	93	98	
HTR3A-rs1062613			
Genotype CC	70	68	
Genotype CT	27	27	0.768
Genotype TT	3	5	
Allele C	167	163	0.693
Allele T	33	37	
MLN-rs2281820			
Genotype CC	46	43	
Genotype CT	14	9	0.384
Genotype TT	17	8	
Allele C	126	139	0.204
Allele T	74	61	
GHRL-rs27647			
Genotype CC	17	8	
Genotype CT	35	36	0.133
Genotype TT	48	56	
Allele C	71	52	0.070
Allele T	129	148	
GHSR-rs572169			
Genotype GG	59	65	
Genotype GA	38	27	0.109
Genotype AA	3	8	
Allele G	156	157	0.070
Allele A	44	43	

DISCUSSION

Although studies have concluded that constipation develops mainly between the ages of 2–4 years, in 17–40% of children, symptoms begin within the first year[7]. The data obtained in our study show that the average age of the patient group is 70.9±54.9 months, similar to the literature. In our study, the mean constipation time was 19.5±19.3 months (3–120 months), and the median constipation time was 12 months. Misra et al. in their study on 101 constipated cases reported the average duration of complaints of the patients to be 32.2–40.7 months[8]. In our study, the duration of constipation was found to be shorter than in the literature. This may be due to the fact that the patient's family awareness is high and that the family receives polyclinic service in a shorter time.

Functional urinary incontinence is the most frequently investigated clinical finding accompanying constipation[9,10]. There are studies in the literature showing that recurrent urinary infections and the urge to urinate in children with constipation are significantly increased, but bladder functions are positively affected after constipation treatment[11,12]. Benninga et al. reported the rate of urinary incontinence as 41% in children with constipation[9]. In our study, urinary incontinence, urinary tract infections, and urinary system symptoms were found at lower rates compared to the literature. This may be due to ethnic origin or the low number of cases. In our study, it was observed that the complaints of enuresis decreased in children who participated in the study and treated for constipation, similar to the literature.

Table 3. Comparison of genotype distributions of constipation patients with/without a positive family history of constipation and symptom onset age.

Gene and SNP Genotype		With constipation positive family history n=60	Without constipation positive family history n=40	P-value	Symptom onset age <24 months n=78	Symptom onset age >24 months n=22	p-value
5HT rs1042173	GG	21	10	0.187	24	6	0.622
	GT	25	20		36	9	
	TT	14	10		18	7	
HTR3A rs1062613	CC	43	27	0.587	57	13	0.445
	CT	14	13		19	8	
	TT	3	0		2	1	
MLN rs2281820	CC	22	18	0.505	34	6	0.250
	CT	30	16		35	11	
	TT	8	6		9	5	
GHRL rs27647	CC	8	9	0.392	11	6	0.289
	CT	22	13		29	6	
	TT	30	18		38	9	
GHSR rs572169	GG	36	23	0.344	49	10	0.153
	GA	23	15		26	12	
	AA	1	2		3	0	

Genetic and environmental factors are also thought to play a role in constipation. In many studies, constipation has been questioned in the family. Edan and Yahya found the rate of history of constipation in the family was 21% in the constipated group and 0.7% in the control group[13]. Similar studies in the literature found the rate of family history of constipation to be between 41 and 70.8%[14]. In our study, a history of familial constipation was found in 40 (40%) patients, the rate similar to the literature.

Camilleri, who evaluated the effects of serotonin on the gastrointestinal tract, found that serotonin increased intestinal motility. In addition, he reported that serotonin polymorphism was high in patients with diarrhoea and low in patients with constipation[5]. Some studies showed association of serotonin levels and functions in the gastrointestinal tract and even in cases of increased intestinal peristalsis, others failed to show relationship of serotonin or its receptors with gastrointestinal diseases. For example, no difference was found in terms of polymorphism in the integral membrane protein SLC6A4 (5-HTT) involved in the presynaptic neuronal transfer of serotonin from the synaptic area in irritable bowel disease[15]. In our study, allele and genotype frequency states of *5-HTT* and *HTR3A* polymorphisms were examined in patients and control groups, and no significant difference was found between the groups. Similarly, when the patient group was evaluated within itself, no significant difference was found between the patient and control groups in terms of allele and genotype frequency states of *5-HTT* and *HTR3A* genes according to the constipation onset time, and positive family history and defecation frequency.

Recent studies in animals and humans related to ghrelin show that it also has a prokinetic activity in the lower gastrointestinal tract[16]. In another study, it has been shown that serum ghrelin levels are essential in response to lactulose treatment in patients with constipation[17]. In this study, patients' serum ghrelin levels were measured at the beginning of constipation treatment, and those with low serum ghrelin levels initially responded better to the lactulose treatment. In contrast, those who had high serum ghrelin levels did not respond adequately to the other group than lactulose treatment. The results of treatment suggest that the effect of ghrelin on intestinal motility may be more effective on functional constipation aetiology. There is no study in the literature investigating and evaluating ghrelin polymorphisms in patients with constipation. In our study, allele and genotype frequencies of GHRL and GHSR polymorphisms were examined in the patient and control groups, and no significant difference was found between the groups.

The most important task of the motilin hormone is to speed up bowel movements. Peak levels of motilin in plasma are correlated with peristaltic solid contractions[18]. Motilin binds to MTLRs on the cell surface to show its effect. In humans, MTLR density is highest in the gastroduodenal region and decreases gradually towards the colon[19]. In the literature, there are studies in which motilin levels are measured in patients with constipation, in which motilin and agonists are given to patients, and the efficacy is evaluated. In the study by Hirabayashi et al., dogs were given mitemcinal (GM-611), an agonist of motilin, and found that colon motility and the number of defecations increased[20]. Although motilin is a factor that may affect constipation, the motilin levels measured in constipated individuals are similar to those of healthy controls. For example, Penning et al. found that in adult patients with slow-pass constipation, fasting and toughness did not detect a change in plasma motilin level compared to the healthy control group, while Aydın et al. also found a significant reduction in the level of motilin compared to the control group in adult constipated patients[21,22]. A study on the relationship between polymorphism and serum motilin level found that there was no significant difference in *MLN* gene polymorphism between paediatric patients with constipation and those without[6]. In our study, allele and genotype frequencies of *MLN* gene polymorphisms were examined in the patient and control groups, and no significant difference was found between the groups.

LIMITATIONS

Studies involving larger patient groups are needed to support this result.

CONCLUSION

Our study results showed that gene polymorphisms of these three hormones may not be related to constipation in children. As in our study, the fact that the results of genetic studies in the literature do not provide a relationship with constipation in children reveals the need for epigenetic-based studies on this issue.

AUTHORS' CONTRIBUTIONS

BA: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **GD:** Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft. **SOB:** Resources, Formal Analysis, Data curation. **MC:** Resources, Formal Analysis, Data curation. **ME:** Project administration, Supervision, Visualization Investigation.

REFERENCES

1. Pession A, Leardini D, Muratore E, Masetti R. Chronic constipation in pediatrics: pathophysiology and diagnosis. In: Lima M, Ruggeri G, editors. Ano-rectal endosonography and manometry in paediatrics. Cham, Switzerland: Springer International Publishing; 2022. p. 113-8. https://doi.org/10.1007/978-3-030-97668-2_10
2. Vriesman MH, Koppen IJN, Camilleri M, Di Lorenzo C, Benninga MA. Management of functional constipation in children and adults. Nat Rev Gastroenterol Hepatol. 2020;17(1):21-39. https://doi.org/10.1038/s41575-019-0222-y
3. Sanger GJ, Furness JB. Ghrelin and motilin receptors as drug targets for gastrointestinal disorders. Nat Rev Gastroenterol Hepatol. 2016;13(1):38-48. https://doi.org/10.1038/nrgastro.2015.163
4. Camilleri M, Atieh J. New developments in prokinetic therapy for gastric motility disorders. Front Pharmacol. 2021;12:711500. https://doi.org/10.3389/fphar.2021.711500
5. Camilleri M. Serotonin in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes. 2009;16(1):53-9. https://doi.org/10.1097/med.0b013e32831e9c8e
6. Ulusoy E, Arslan N, Küme T, Ülgenalp A, Çirali C, Bozkaya Ö, et al. Serum motilin levels and motilin gene polymorphisms in children with functional constipation. Minerva Pediatr (Torino). 2016;73(5):420-5. https://doi.org/10.23736/S2724-5276.16.04369-X
7. Loening-Baucke V. Prevalence, symptoms and outcome of constipation in infants and toddlers. J Pediatr. 2005;146(3):359-63. https://doi.org/10.1016/j.jpeds.2004.10.046
8. Misra S, Lee A, Gensel K. Chronic constipation in overweight children. JPEN J Parenter Enteral Nutr. 2006;30(2):81-4. https://doi.org/10.1177/014860710603000281
9. Benninga MA, Büller HA, Heymans HSA, Tytgat GNJ, Taminiau JAJM. Is encopresis always the result of constipation? Arch Dis Child. 1994;71(3):186-93. https://doi.org/10.1136/adc.71.3.186
10. Plunkett A, Phillips CP, Beattie RM. Management of chronic functional constipation in childhood. Pediatr Drugs. 2007;9(1):33-46.
11. Kasirga E, Akil I, Yilmaz O, Polat M, Gözmen S, Egemen A. Evaluation of voiding dysfunctions in children with chronic functional constipation. Turk J Pediatr. 2006;48(4):340-3. PMID: 17290569
12. Zaffanello M, Banzato C, Piacentini G. Management of constipation in preventing urinary tract infections in children: a concise review. Eur Res J. 2019;5(2):236-43. https://doi.org/10.18621/eurj.412280
13. Edan OAA, Yahya FS. Socio-demographic characteristics and risk factors of functional constipation in children: a case-control study. Sri Lanka J Child Health. 2022;51(2):227-34. http://doi.org/10.4038/sljch.v51i2.10123
14. Yurdakul Ertürk E, Yalçin O. Patients with constipation and related factors. Middle Black Sea J Health Sci. 2019;5(1):1-5. https://doi.org/10.19127/mbsjohs.529445
15. Elamin E, Elshaikh N, Abdelghani S, Madani M, Waggiallah H, Bashier L, et al. Serotonin transporter gene polymorphism (5-HTTLPR and VNTR) among Sudanese patients with irritable bowel syndrome (IBS). Kuwait Med J. 2021;53(1):25-30.
16. Mohammadi EN, Pietra C, Giuliano C, Fugang L, Greenwood-Van Meerveld B. A comparison of the central versus peripheral gastrointestinal prokinetic activity of two novel ghrelin mimetics. J Pharmacol Exp Ther. 2019;368(1):116-24. https://doi.org/10.1124/jpet.118.250738
17. Gürkan OE, Dalgıç B, Bideci A. Relation between ghrelin level and treatment response in functional constipation. Turk J Gastroenterol. 2013;24(6):515-20. PMID: 24623291
18. Deloose E, Verbeure W, Depoortere I, Tack J. Motilin: from gastric motility stimulation to hunger signalling. Nat Rev Endocrinol. 2019;15(4):238-50. https://doi.org/10.1038/s41574-019-0155-0
19. Kato S, Takahashi A, Shindo M, Yoshida A, Kawamura T, Matsumoto K, et al. Characterization of the gastric motility response to human motilin and erythromycin in human motilin receptor-expressing transgenic mice. PLoS One. 2019;14(2):e0205939. https://doi.org/10.1371/journal.pone.0205939
20. Hirabayashi T, Morikawa Y, Matsufuji H, Hoshino K, Hagane K, Ozaki K. Stimulatory action of mitemcinal (GM-611), an acid-resistant non-peptide motilin receptor agonist, on colonic motor activity and defecation: spontaneous and mitemcinal-induced giant migrating contractions during defecation in dogs. Neurogastroenterol Motil. 2009;21(10):1085-e91. https://doi.org/10.1111/j.1365-2982.2009.01341.x
21. Penning C, Delemarre JB, Bemelman WA, Biemond I, Lamers CB, Masclee AA. Proximal and distal gut hormone secretion in slow transit constipation. Eur J Clin Invest. 2000;30(8):709-14. https://doi.org/10.1046/j.1365-2362.2000.00692.x
22. Aydin S, Donder E, Akin OK, Sahpaz F, Kendir Y, Alnema MM. Fat-free milk as a therapeutic approach for constipation and the effect on serum motilin and ghrelin levels. Nutrition. 2010;26(10):981-5. https://doi.org/10.1016/j.nut.2009.11.023

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220991

Homocysteine concentrations in overweight children and adolescents

Juliana Dias Gonçalves dos Santos[1], Fabíola Isabel Suano de Souza[1,2], João Carlos Pina Faria[1,3*], Luciana Satiko Sawamura[1,4], Anelise Del Vecchio Gessullo[1], Roseli Oselka Saccardo Sarni[1,2]

SUMMARY

OBJECTIVE: The aim of this study was to describe homocysteine concentrations in overweight and obese children and adolescents and relate them to blood pressure levels, renal function, and insulin resistance.

METHODS: This is a cross-sectional and observational study with 64 overweight children and adolescents (mean age: 11.6±3.5 years) in outpatient follow-up. The following parameters were evaluated: body mass index z-score, waist-to-height circumference ratio, pubertal stage, blood pressure, serum homocysteine, glycemia, insulin, lipid profile, renal function, high-sensitivity C-reactive protein, microalbuminuria, and creatinuria. Statistical analysis: analysis of variance and logistic regression (dependent variable: homocysteine) ($p<0.05$).

RESULTS: The mean body mass index z-score was 2.9±1.1. The mean homocysteine concentrations were 8.6±2.2 μmol/L (10th and 90th percentiles: 6.6 and 11.2 μmol/L, respectively), with no difference when compared with children with severe obesity and obesity/overweight ($p=0.431$). High values of waist-to-height ratio (93.8%), systolic blood pressure (18.8%), diastolic blood pressure (12.5%), glycemia (4.7%), low-density lipoprotein cholesterol (31.1%), triglycerides (35.9%), non-high-density lipoprotein cholesterol (34.4%), and microalbuminuria (21.9%) were obtained. The mean glomerular filtration rate was 122.9±24.6 mL/min/1.73 m^2. Homocysteine concentrations were not associated with any of the studied variables ($R^2=0.095$).

CONCLUSION: Homocysteine concentrations in overweight children and adolescents (mean 8.6±2.2 μmol/L) were not associated with body mass index z-score, blood pressure, renal function, and insulin resistance.

KEYWORDS: Homocysteine. Pediatric obesity. Biomarkers. Heart disease risk factors.

INTRODUCTION

Homocysteine (Hcy) is an amino acid containing sulfhydryl that forms during methionine metabolism. The interest in Hcy as a causal risk factor for cardiovascular diseases (CVDs) in childhood began with the observation that more than 50% of children with homocystinuria of genetic origin die prematurely from vascular diseases[1].

A meta-analysis evaluating the dose-response effect suggested that Hcy concentrations are linear and positively associated with all-cause mortality risk. This risk has increased by 33.6% for each 5 μmol/L increase in Hcy concentrations in adults (risk ratio: 1.336, 95%CI 1.254–1.422, $p<0.001$)[2].

Obesity seems to relate to high Hcy concentrations and represents a risk for the development of CVD, considering that overweight individuals experience events in earlier ages, live with events for longer periods, and have a shorter life expectancy compared to individuals with a normal body mass index (BMI)[3].

A recent meta-analysis involving 14 studies with adults found significantly higher Hcy concentrations in obese individuals compared to healthy controls, regarding their eating habits, insulin resistance (IR), and drug use[4].

In the pediatric age group, systematic review and meta-analysis, including studies published from 1999 to 2017, showed that these were predominantly cross-sectional and mainly evaluated adolescents. In the meta-analysis (n=6) and cross-sectional studies (n=3), the authors identified that high Hcy concentrations correlated weekly and directly with excess weight in children and adolescents (odds ratio [OR]: 1.08; 95%CI 1.04–1.11)[5].

Considering the participation of high Hcy concentrations and excess weight in the risk for the development of CVD and the lack of studies in the pediatric age group, especially in our country, this study aimed to describe the Hcy concentrations in overweight children and adolescents and to verify an association with blood pressure (BP), renal function, and IR.

[1]Centro Universitário Fundação Santo André, Faculdade de Medicina do ABC, Department of Pediatrics – Santo André (SP), Brazil.
[2]Universidade Federal de São Paulo, Department of Pediatrics – São Paulo (SP), Brazil.
[3]Universidade Nove de Julho, Department of Pediatrics – São Paulo (SP), Brazil.
[4]Universidade Municipal de São Caetano do Sul, Department of Pediatrics – São Caetano do Sul (SP), Brazil.
*Corresponding author: jocapf79@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on July 22, 2022. Accepted on September 03, 2022.

METHODS

Through a cross-sectional and observational study, 64 overweight and obese children and adolescents aged 5–19 years were followed up at the Nutrition Outpatient Clinic of the Department of Pediatrics of the Centro Universitário FMABC Santo André, Brazil.

Of the 80 children eligible for the study who regularly attended the outpatient clinic from August 2014 to May 2015, 64 (80%) were included.

Children and adolescents with obesity secondary to diseases of genetic or hormonal cause and carriers of other chronic diseases, birth weight of less than 2,500 g, and under medications that could interfere with renal function, lipid profile, and glucose tolerance were included.

A questionnaire containing questions related to obesity and associated morbidities, socioeconomic status, and morbid personal and family history of CVDs was applied.

Pubertal staging was classified according to the one proposed by Marshall and Tanner.

Anthropometric assessment was performed according to protocols standardized by the World Health Organization (WHO)[6]. Weight and height measurements were expressed as body mass index Z-score (zBMI), calculated using the WHO AnthroPlus software. For the anthropometric classification, the cutoff points recommended by the WHO were adopted[7]. The waist-to-height ratio (WHtR) was classified as altered when the value was equal to or greater than 0.5[8].

Systemic BP was measured at the time of the interview, according to the recommendation of the Task Force, 2004. BP values were classified according to sex, age, and height percentiles and were considered inadequate when above the 90th percentile[9]. BP measurements were performed by a pediatrician using calibrated equipment and periodically reviewed.

The examinations were performed at the Clinical Analysis Laboratory of the Centro Universitário FMABC. A sample of 10 mL of blood was obtained by peripheral venipuncture, after 12-h fast, to determine total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), non-HDL cholesterol (NHDL = TC - HDL), and triglycerides (TG) (colorimetric method); blood glucose (colorimetric method) and insulin (immunoenzymatic method), from which Homeostasis Model Assessment for insulin resistance (HOMA-IR) was calculated; urea and creatinine (colorimetric method); and high-sensitivity C-reactive protein (hs-CRP immunoenzymatic method). For lipid profile classification, the cutoff points recommended by the American Academy of Pediatrics were adopted[10].

Hcy analysis was performed by chemiluminescence method, with reference values between 5 and 15 µmol/L. The enzyme immunoassay was performed using automatic chemiluminescence equipment, model Immulite 2000. The chemiluminescence method showed results comparable to those obtained by high-performance liquid chromatography in school-age children[11].

Plasma creatinine was used to calculate the estimated creatinine clearance or estimated glomerular filtration rate (eGFR) according to Schwartz's equation: GFR (mL/min/1.73 m²) = 0.43 × height (cm)/plasma creatinine (mg/dL)[12].

Isolated urine sample (first in the morning, 20 mL) was collected for the calculation of albuminuria and creatinuria. Microalbuminuria (MA) was defined as the albumin/creatinine ratio with values between ≥30 and <300 mg/g[13].

For statistical analysis, the SPSS (IBM®) software version 24.0 was used. Categorical variables were presented as absolute and percentage values. The continuous ones were evaluated for their normality. The parametric variables were presented in the form of mean±standard deviation and the nonparametric variables in the form of median (minimum; maximum). The nonparametric variables (MA, hs-CRP, HOMA-IR, and insulin) underwent logarithmic transformation, and for the analyses, the analysis of variance test was used for comparison. Logistic regression was performed (dependent variable: Hcy). The significance level was set at 5%.

The research protocol was approved by the Ethics Committee of Centro Universitário FMABC (n 1,080,802).

RESULTS

Table 1 describes the general characteristics of overweight and obese children and adolescents included in the study. The mean age was 11.6±3.5 years. Most children and adolescents had obesity and severe obesity of 81.3% and the increased WHtR was observed in 93.8%. High systolic and diastolic BP (above the 90th percentile) occurred in 18.8 and 12.5%, respectively.

Table 2 describes the means and medians of BP values, anthropometric indicators, and laboratory variables. The mean zBMI was 2.9±1.1 with Hcy concentrations of 8.6±2.2 µmol/L (10th and 90th percentiles: 6.6 and 11.2 µmol/L, respectively).

The mean GFR was 122.9±24.7 mL/min/1.73 m² (minimum and maximum: 78.9 and 192.1 mL/min/1.73 m², respectively). Four (6.2%) patients had a GFR below 90 mL/min/1.73 m². Of these, three were adolescents, all obese, and one had an associated MA. There was no significant correlation between zBMI, insulin, and HOMA-IR with MA values or GFR.

There was no statistically significant difference when comparing the Hcy concentrations in overweight, obese, and severely obese children (Figure 1) (p=0.431). Table 3 shows that Hcy concentrations were also not associated with any of the studied variables (R^2=0.095) in this group of overweight children and adolescents.

DISCUSSION

This study showed mean concentrations of Hcy of 8.6±2.2 µmol/L in overweight children and adolescents. There was no association of the Hcy concentrations with BP, renal function, or IR.

One study described the distribution of total Hcy among a representative sample of American children and adolescents (n=2027, ages between 4 and 19 years) and tested the differences between sex, age, and race-ethnicity categories. The geometric mean concentrations of Hcy adjusted for age were 6.2 and 5.8 µmol/L in non-Hispanic Caucasian boys and girls, 6.4 and 6.1 µmol/L in non-Hispanic African-American boys and girls, and 6.4 and 5.5 µmol/L in Mexican-American boys and girls, respectively[14]. The values found in the American study were lower than those observed in our study.

High concentrations of Hcy have been associated with increased risk for the cardiovascular, cerebrovascular, and thromboembolic diseases in adults. Values of 10 µmol/L or smaller are probably safe for adult individuals, but values of 11 µmol/L or above may suggest the need for intervention. There are no indications of values related to negative outcomes for the pediatric age group[15].

The association between high concentrations of Hcy and excess weight seems related to the dysfunction of the adipose tissue with inhibition of lipolysis by activating the protein kinase influenced by adenosine monophosphate[16]. Evidences pointing to an association between high concentrations of Hcy and overweight in the pediatric age group are still insufficient, as described in a recently published meta-analysis. After the combination of studies in meta-analysis (n=6), there was a

Table 1. General characteristics of the studied sample of overweight children and adolescents.

Variable		N=64
Sex	Male	32 (50%)
Age	<10 years	24 (37.5%)
Pubertal stage	Prepubescent	29 (45.3%)
	Pubescent 2 and 3	24 (37.5%)
	Pubescent 4 and 5	11 (17.2%)
BMI Z-score	Severe obesity	24 (37.5%)
	Obesity	28 (43.8%)
	Overweight	12 (18.8%)
WHtR	≥0.5	60 (93.8%)
Systolic blood pressure	>90th percentile	12 (18.8%)
Diastolic blood pressure	>90th percentile	8 (12.5%)
Mean arterial pressure	>90th percentile	14 (21.9%)
Family history	Obesity	24 (37.5%)
	Systemic arterial hypertension	32 (50%)
	Diabetes	11 (17.2%)
	Dyslipidemia	11 (17.2%)
	Cardiovascular disease	25 (39.1%)
Fasting glucose	>100 mg/dL	3 (4.7%)
Total cholesterol	>200 mg/dL	18 (28.1%)
LDL-c	>130 mg/dL	20 (31.1%)
HDL-c	<45 mg/dL	17 (26.6%)
Triglycerides	>100 mg/dL	23 (35.9%)
Non-HDL-c	>145 mg/dL	22 (34.4%)
Microalbuminuria	>30 mg/g	14 (21.9%)

BMI Z-score: body mass index z-score; WHtR: waist-to-height circumference ratio; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.

Table 2. Characteristics of mean or median blood pressure values, anthropometric indicators, and laboratory variables evaluated in overweight children and adolescents (n=64).

Variable		Mean±SD or median (min-max)
Systolic blood pressure	mmHg	110.7±15.3
Diastolic blood pressure	mmHg	69.6±12.6
Body mass index	Z-score	2.9±1.1
Homocysteine	µmol/L	8.6±2.2
Microalbuminuria	mg/g	9.4 (0.7–300.7)
Creatinine clearance	mL/min/1.73 m²	122.9±24.6
hs-CRP	mg/dL	4.1 (0.3–38.3)
Total cholesterol	mg/dL	183.9±32.3
HDL-c	mg/dL	48.9±13.8
LDL-c	mg/dL	116.2±25.9
Triglycerides	mg/dL	100.7±56.3
Non-HDL-cholesterol	mg/dL	134.9±31.8
Glycemia	mg/dL	86.3±8.9
Insulin	µU/mL	8.7 (2.0–37.3)
HOMA-IR		1.8 (0.4–10.0)

hs-CRP: high-sensitivity C-reactive protein; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; Non-HDL-c: non-HDL cholesterol (total cholesterol - HDL-c); HOMA-IR: Homeostasis Model Assessment of insulin resistance.

weak and direct correlation between Hcy concentrations and BMI by age only in cross-sectional studies (n=3) and a direct but nonstatistically significant correlation in cohort studies (n=3). The authors emphasized that the majority of the studies were conducted with adolescents and indicates the necessity of developing future longitudinal studies to better identify the associations[5].

Moreover, it is a consensus that the concentrations may vary with age, sex, and pubertal staging in both healthy and obese children[16].

Brasileiro et al. did not find any differences between the total concentrations of Hcy of Brazilian adolescents with overweight/obesity and healthy adolescents[16]. The mean Hcy concentration in the study was 11.8 µmol/L, higher than what we observed (8.6 µmol/L), and folate deficiency was found in 68.6% of the sample. Some hypotheses can be suggested for this difference: the study was conducted exclusively with adolescents (mean age 16 years) and the present study with children and adolescents, and the data collection of the cited study was performed when the mandatory fortifications of corn and wheat flour with a minimum of 4.2 mg of iron and 150 µg of folic acid for every 100 g of flour were not yet put into force in Brazil, starting in 2004[17]. In countries such as the United States and Canada, it was found that the mandatory fortification of folic acid foods led to a decrease in total Hcy concentrations in all age groups, more expressive in individuals with higher pre-fortification values[18].

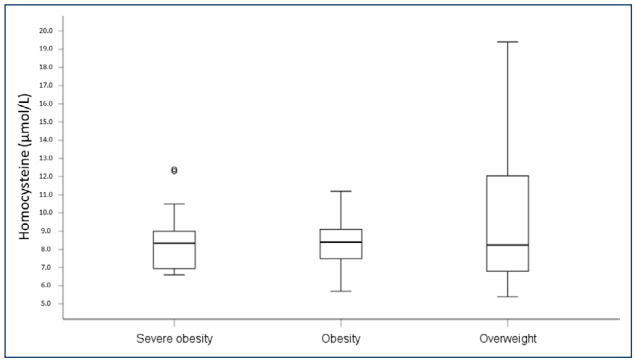

Figure 1. Homocysteine concentrations in overweight, obese, and severely obese children.

A cross-sectional and controlled study conducted in Turkey showed significantly higher concentrations of Hcy in obese children compared to controls without excess weight. Contrary to what was observed in our study, the authors have verified a significant correlation between the total concentrations of Hcy and age, BMI, TG, and HDL-c in the obese group. There was no association with the HOMA-IR[19]. The increased concentrations of insulin promote inhibition of hepatic cystathionine-B-synthase activity with consequent increase in circulating Hcy concentrations[20].

In this study, there was no association between Hcy concentrations and renal function evaluated based on MA and creatinine clearance. Current data suggest that a healthy kidney plays an important role in the clearance and metabolism of Hcy, as with other amino acids. Hcy concentrations increase as renal function declines and progresses to advanced renal disease, with the vast majority of dialysis patients presenting mild-to-moderate hyperhomocysteinemia. The values of GFR estimated from serum creatinine or calculated creatinine clearance are consistently and inversely correlated with plasma Hcy levels[21].

We have not found any association between Hcy concentrations and BP. Although higher plasma Hcy concentrations

Table 3. Association of studied variables with homocysteine concentrations in overweight children and adolescents (n=64).

Variable		B	95%CI		p-value
Age	Years	-0.005	-0.020	0.010	0.513
Systolic BP	mmHg	0.019	-0.043	0.082	0.532
Diastolic BP	mmHg	0.018	-0.054	0.090	0.625
WHtR	cm/cm	0.131	-7.505	7.767	0.973
hs-CRP (log)	mg/dL	-0.330	-1.277	0.616	0.487
HOMA-IR (log)		-0.389	-2.372	1.594	0.696
Microalbuminuria (log)	mg/g	-0.503	-1.536	0.530	0.333
Creatinine clearance	mL/min/1.73 m²	0.001	-0.023	0.025	0.937
BMI Z-score		-0.065	-0.623	0.493	0.815
Non-HDL-cholesterol	mg/dL	-0.005	-0.024	0.014	0.595

Logistic regression dependent variable: homocysteine (µmol/L). R^2=0.095.
BMI Z-score: body mass index z-score; BP: blood pressure; WHtR: waist-to-height circumference ratio; hs-CRP: high-sensitivity C-reactive protein; HOMA-IR: Homeostasis Model Assessment of insulin resistance.

have been associated with high BP in cross-sectional studies with adults[22,23], a cohort study entitled the Framingham Heart Study showed an association only in the unadjusted model; the multivariate analysis did not show a causal relationship[24].

This study presents some limitations such as the cross-sectional design that does not allow establishing a cause-effect relationship, the reduced sample size, and the absence of a healthy control group without excess weight.

CONCLUSION

The presence of complications/comorbidities was observed in about 30% of those with dyslipidemias, 20% with increased BP levels, and 22% with MA >30 mg/g. The mean total Hcy concentration was 8.6±2.2 µmol/L (10th and 90th percentiles: 6.6 and 11.2 µmol/L, respectively). There was no association between Hcy concentrations and zBMI, BP, renal function, or IR. In this study, the values that are higher than those described in other studies in the literature in countries that practice mandatory folic acid fortification of foods remind us the importance of monitoring Hcy concentrations in overweight individuals in the pediatric age group.

DECLARATION

All authors declare to be responsible for the content made available for publication.

ETHICAL ASPECTS

The study was approved by the Research Ethics Committee of the FMABC University Center, opinion number: 1080802. Families received information, risks, and benefits from the study. Literate children received a TALE and their guardians a TCLE.

AUTHORS' CONTRIBUTIONS

JDGS: Data curation, Formal Analysis, Writing – original draft. **FISS:** Conceptualization, Methodology, Validation, Writing – review & editing. **JCPF:** Data curation, Methodology, Project administration, Writing – original draft. **LSS:** Formal Analysis, Methodology. **ADVG:** Conceptualization, Methodology, Validation. **ROSS:** Conceptualization, Methodology, Visualization, Writing – review & editing.

REFERENCES

1. Koklesova L, Mazurakova A, Samec M, Biringer K, Samuel SM, Büsselberg D, et al. Homocysteine metabolism as the target for predictive medical approach, disease prevention, prognosis, and treatments tailored to the person. EPMA J. 2021;12(4):477-505. https://doi.org/10.1007/s13167-021-00263-0
2. Fan R, Zhang A, Zhong F. Association between homocysteine levels and all-cause mortality: a dose-response meta-analysis of prospective studies. Sci Rep. 2017;7(1):4769. https://doi.org/10.1038/s41598-017-05205-3
3. Werner N, Nickenig G, Sinning J-M. Complex PCI procedures: challenges for the interventional cardiologist. Clin Res Cardiol. 2018;107(Suppl 2):64-73. https://doi.org/10.1007/s00392-018-1316-1
4. Wang J, You D, Wang H, Yang Y, Zhang D, Lv J, et al. Association between homocysteine and obesity: a meta-analysis. J Evid Based Med. 2021;14(3):208-17. https://doi.org/10.1111/jebm.12412
5. Leite LO, Pitangueira JCD, Damascena NF, Costa PRF. Homocysteine levels and cardiovascular risk factors in children and adolescents: systematic review and meta-analysis. Nutr Rev. 2021;79(9):1067-78. https://doi.org/10.1093/nutrit/nuaa116
6. World Health Organization. The WHO child growth standards. Geneva: WHO; 2020. [cited on June, 2022]. Available from: https://www.who.int/growthref/en/
7. de Onis M. The use of anthropometry in the prevention of childhood overweight and obesity. Int J Obes Relat Metab Disord. 2004;28(Suppl 3):S81-5. https://doi.org/10.1038/sj.ijo.0802810
8. Haun DR, Pitanga FJG, Lessa I. Razão cintura/estatura comparado a outros indicadores antropométricos de obesidade como preditor de risco coronariano elevado [Waist-height ratio compared to other anthropometric indicators of obesity as predictors of high coronary risk] (in Portuguese). Rev Assoc Med Bras (1992). 2009;55(6):705-11. https://doi.org/10.1590/s0104-42302009000600015
9. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(Suppl 2 4th Report):555-76. https://doi.org/10.1542/peds.114.S2.555
10. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics. 2011;128(Suppl 5):S213-56. https://doi.org/10.1542/peds.2009-2107C
11. Gascón TM, Schindler F, Oliveira CGB, Souza FIS, Hix S, Sarni ROS, et al. Evaluation of chemiluminescence method for the analysis of plasma homocysteine and comparison with HPLC method in children samples (in English, Portuguese). Einstein (São Paulo). 2010;8(2):187-91. https://doi.org/10.1590/S1679-45082010AO1499
12. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20(3):629-37. https://doi.org/10.1681/ASN.2008030287

13. Jones CA, Francis ME, Eberhardt MS, Chavers B, Coresh J, Engelgau M, et al. Microalbuminuria in the US population: third National Health and Nutrition Examination Survey. Am J Kidney Dis. 2002;39(3):445-59. https://doi.org/10.1053/ajkd.2002.31388
14. Must A, Jacques PF, Rogers G, Rosenberg IH, Selhub J. Serum total homocysteine concentrations in children and adolescents: results from the third National Health and Nutrition Examination Survey (NHANES III). J Nutr. 2003;133(8):2643-9. https://doi.org/10.1093/jn/133.8.2643
15. Smith AD, Refsum H. Homocysteine - from disease biomarker to disease prevention. J Intern Med. 2021;290(4):826-54. https://doi.org/10.1111/joim.13279
16. Brasileiro RS, Escrivão MAMS, Taddei JAAC, D'Almeida V, Ancona-Lopez F, Carvalhaes JTA. Plasma total homocysteine in Brazilian overweight and non-overweight adolescents: a case-control study. Nutr Hosp. 2005;20(5):313-9. PMID: 16229398
17. Ministério da Saúde do Brasil. Agência nacional de vigilância sanitária. RESOLUÇÃO-RDC Nº 344, DE 13 DE DEZEMBRO DE 2002. [cited on June, 2022]. Available from: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2002/rdc0344_13_12_2002.html
18. Dai C, Fei Y, Li J, Shi Y, Yang X. A novel review of homocysteine and pregnancy complications. Biomed Res Int. 2021;2021:6652231. https://doi.org/10.1155/2021/6652231
19. Abaci A, Akelma AZ, Özdemir O, Hizli Ş, Razi CH, Akin, KO. Relation of total homocysteine level with metabolic and anthropometric variables in obese children and adolescents. Turk J Med Sci. 2012;42(1):69-76. https://doi.org/10.3906/sag-1011-1252
20. Badawy A, State O, El Gawad SSA, El Aziz OA. Plasma homocysteine and polycystic ovary syndrome: the missed link. Eur J Obstet Gynecol Reprod Biol. 2007;131(1):68-72. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0301211506005665 https://doi.org/10.1016/j.ejogrb.2006.10.015
21. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. J Am Soc Nephrol. 2001;12(10):2181-9. https://doi.org/10.1681/ASN.V12102181
22. Ganji V, Kafai MR, Third National Health and Nutrition Examination Survey. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2003;77(4):826-33. https://doi.org/10.1093/ajcn/77.4.826
23. Lim U, Cassano PA. Homocysteine and blood pressure in the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Epidemiol. 2002;156(12):1105-13. https://doi.org/10.1093/aje/kwf157
24. Sundström J, Sullivan L, D'Agostino RB, Jacques PF, Selhub J, Rosenberg IH, et al. Plasma homocysteine, hypertension incidence, and blood pressure tracking: the Framingham Heart Study. Hypertension. 2003;42(6):1100-5. https://doi.org/10.1161/01.HYP.0000101690.58391.13

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20221003

Diagnostic value of systemic immune inflammation index in acute appendicitis

Kemal Şener[1*], Adem Çakır[1], Hüseyin Kılavuz[2], Ertuğrul Altuğ[1], Ramazan Güven[1]

SUMMARY

OBJECTIVE: Clinical diagnosis of acute appendicitis is often difficult and involves a synthesis of clinical, laboratory, and radiological findings. The aim of this study was to investigate whether the systemic immune inflammation index can be used as an effective parameter in the diagnosis of acute appendicitis and its reliability in the differentiation of complicated vs. non-complicated appendicitis.

METHODS: The study was conducted retrospectively with patients admitted to the emergency department with abdominal pain and diagnosed with acute appendicitis. In total, 150 patients and 150 control cases were included in the study. Demographic data, medical history, white blood cell count, platelet count, neutrophil count, systemic immune inflammation index values, Alvarado score, adult appendicitis score, and pathology result of appendectomy material were retrieved from the hospital automation system and recorded in the data form.

RESULTS: Neutrophil-lymphocyte ratio and systemic immune inflammation index were significantly higher, and platelet-neutrophil ratio and lymphocyte-neutrophil ratio were significantly lower in the patient group compared to the control group ($p<0.001$). Receiver operating characteristic analysis revealed that the sensitivity and specificity of systemic immune inflammation index with a cutoff value of 840.13 was 82 and 66.7%, respectively, for the diagnosis of acute appendicitis. Correlation analysis revealed that systemic immune inflammation index, Alvarado score, and adult appendicitis score were positively correlated, and this correlation was statistically significant.

CONCLUSION: Systemic immune inflammation index may be used to promote the diagnosis of acute appendicitis and may reduce the need for radiation exposure and diagnostic imaging tests such as contrast-enhanced abdominal computed tomography. It can also be used to differentiate between complicated and non-complicated acute appendicitis cases.

KEYWORDS: Appendicitis. Inflammation mediators. Systemic immune-inflammation index.

INTRODUCTION

Acute appendicitis (AA) is one of the most common abdominal emergencies worldwide[1]. Lifetime risk is 8.6% in men and 6.7% in women[2]. In addition, AA is one of the most common causes of hospitalization in patients admitted to the emergency department with abdominal pain.

Clinical diagnosis of AA is often difficult and involves a synthesis of clinical, laboratory, and radiological findings. The diagnosis of AA can be made more accurately and reliably by using physical examination findings and inflammation markers. In addition, many scoring systems are used to estimate AA risk, including Alvarado score, acute appendicitis score, adult appendicitis score (AAS), Raja Isteri Pengiran Anak Saleha Appendicitis (RIPASA) score, appendicitis inflammatory response (AIR) score, and modified Alvarado score. Studies have shown that these scoring systems are helpful in the diagnosis and treatment of AA[3,4].

The systemic immune inflammation index (SIII) is a novel systemic inflammatory prognostic indicator associated with outcomes in patients with different tumors. Studies have shown an association between SIII and many chronic/acute inflammatory diseases[5]. Since SIII is easy to calculate, inexpensive, requires only complete blood count, and relies on no subjective findings, it will provide more accurate results in the diagnosis of AA.

Therefore, the aim of this study was to determine whether the diagnostic value SIII can be used as an effective parameter in the diagnosis of AA and its reliability in the distinction between complicated and non-complicated appendicitis.

METHODS

Study setting

This study began after obtaining the study approval from the ethics committee of our hospital (Ethics committee decision no.

[1]Başakşehir Çam ve Sakura Şehir Hastanesi, Department of Emergency Medicine – Istanbul, Turkey.
[2]Başakşehir Çam ve Sakura Şehir Hastanesi, Department of General Surgery – Istanbul, Turkey.
*Corresponding author: drkemalsener@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 21, 2022. Accepted on October 23, 2022.

2021.04.35; dated: April 28, 2021). The study was conducted retrospectively and in a single center. The study was conducted between May 1, 2021, and May 1, 2022, with patients who were admitted to the emergency department with abdominal pain, diagnosed with AA, and met the criteria for inclusion in the study. Demographic data, medical history, WBC, platelet count, neutrophil count, SIII values, Alvarado scores, AAS, and pathology results of appendectomy material were retrieved from the hospital automation system [Hospital Information Management System (HIMS)] and recorded in the case form. The Alvarado score is a diagnostic score that is based on the symptoms (migratory pain, anorexia, nausea, and/or vomiting), signs (tenderness, rebound tenderness, and elevated body temperature), and laboratory findings (leukocytosis and left shift). One point was given to the presence of each indicator, except 2 points for tenderness and leukocytosis, making a total score of 10. AA cases were divided into two groups as complicated appendicitis and non-complicated appendicitis based on the presence of complications (gangrenous, perforated, and abscess formation). The study included 150 confirmed cases of AA and 150 control cases who were admitted to the emergency department with abdominal pain and not diagnosed with AA.

Study group patients with confirmed AA diagnosis and control group patients who were not diagnosed with AA after being admitted to the emergency department with abdominal pain were included in the study. In all, 3 patients under the age of 18 years, 5 pregnant patients, and 12 patients with missing data were excluded from the study. Also, 10 patients whose outcome could not be followed and whose medical history was unknown were not included in the study. In addition, 12 patients with any medical history of malignancy, a history of hematological disease, bone marrow pathology, and those taking anti-inflammatory or immunosuppressive drugs were also excluded from the study. In addition, patients with non-appendicitis infection focus were excluded from the study.

Patient group

The control group was randomized from age- and gender-matched patients meeting the inclusion and exclusion criteria. As the control group, 150 patients who presented to the emergency department with the complaint of "abdominal pain" but were not diagnosed with AA were included. The group consisted of volunteers with a known medical history and no chronic disease history.

Data calculation

In the study, calculations were made from the obtained results of the cases. P, N, and L refer to peripheral platelet, neutrophil, and lymphocyte counts, respectively. Accordingly, NLR (N/L ratio), PLR (P/L ratio), PNR (P/N ratio), and LNR (L/N ratio) were calculated. SIII calculated as $[(P\times N)/L)]^5$. Mortality evaluation was based on the mortality rates of the patients during hospital stay. Due to the retrospective design of the study, mortality after discharge and its causes were not evaluated.

Statistical analyses

The data were analyzed with SPSS Package Program version 24.0. Number, percentage, mean, standard deviation, median, minimum, and maximum values were used in the presentation of descriptive data. The conformity of the data to normal distribution was evaluated by the Kolmogorov-Smirnov test. In the univariate analysis, continuous variables with normal distribution were expressed as mean±SD and compared using the t-test. Pearson's χ^2 test was used in the analysis of categorical variables. For categorical variables, Fisher's exact test was used in cases with less than five variables. Spearman's correlation test was used in correlation analysis of multiple variables. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis. The appropriate cutoff values were determined, and the sensitivity and specificity values were calculated for the parameters with an area below the curve (AUC) above 0.600. A $p<0.05$ was accepted as statistically significant in all analyses.

RESULTS

A total of 150 patients and 150 control cases were included in the study. The control group was randomly generated from age- and gender-matched patients. Mean age was 33.47±11.01 years in the patient group and 35.67±12.23 years in the control group. Mean pulse rate was significantly higher in the patient group compared to the control group. There was no significant difference between other vital parameters. WBC and neutrophil count were significantly higher and lymphocyte level was significantly lower in the patient group compared to the control group. There was no significant difference in platelet count between the two groups (Table 1).

Neutrophil-lymphocyte ratio (NLR) and SIII were significantly higher, and PNR and LNR were significantly lower in the patient group compared to the control group. There was no significant difference between the two groups with respect to mean PLR (Table 1).

The patient group was further subdivided as complicated and non-complicated cases based on the complication status. In all, 18 cases were evaluated as complicated appendicitis cases and 132 cases as non-complicated appendicitis cases. Both defense and rebound findings in physical examination

were significantly more common in complicated appendicitis cases. WBC and neutrophil count were significantly higher and lymphocyte count was significantly lower in complicated appendicitis cases compared with non-complicated cases. SIII, NLR, and PLR were significantly higher and PNR and LNR were significantly lower in complicated appendicitis cases.

Mean Alvarado score (14.5±2.83 and 11.40±1.98; p<0.001) and AAS (7.33±1.08 and 4.33±1.38; p<0.001) were significantly higher in complicated appendicitis cases. Peritonitis findings were significantly more pronounced in complicated appendicitis cases (p=0.003). There was no significant difference between the two groups in terms of mortality.

The ROC analysis revealed that the sensitivity and specificity of SIII with a cutoff value of 840.13 was 82 and 66.7%, respectively, for the diagnosis of AA. Furthermore, a cutoff value of 1782.94 for SIII had 88.9% sensitivity and 68.9% specificity for distinguishing between complicated vs. non-complicated cases (Figure 1 and Table 2).

Correlation analysis revealed that SIII, Alvarado score, and AAS were positively correlated, and this correlation was statistically significant.

DISCUSSION

Acute appendicitis is one of the leading abdominal emergencies worldwide. The diagnosis of AA is still not clearly established in emergency room conditions with tests that prolong the process, are cost-ineffective, and lead to radiation exposure. There is a search for new diagnostic tools in order to make the diagnosis of AA more accurate, more reliable, and cheaper. Recent studies show that SIII is both an accurate indicator of inflammation and a useful ratio that helps predict the diagnosis and prognosis of many diseases[6-8]. SIII is a newly defined, simple, and inexpensive index that reflects the balance between inflammatory and immune responses. Based on the results of this study, it was found that SIII is a reliable index that can be used both in the diagnosis of AA and in the differentiation of complicated and non-complicated AA cases.

Leukocyte count is one of the most commonly used diagnostic methods in the diagnosis of AA. However, the sensitivity and specificity of leukocyte count for the diagnosis of AA is limited[9]. In this study, leukocyte count was higher in the patient group diagnosed with AA. Likewise, NLR and PLR have been used as auxiliary parameters in the diagnosis and prognosis of many diseases[10-12]. In this study, NLR was significantly higher in patients diagnosed with AA, while no significant difference was found in PLR. In addition, based on the results of this study, PNR and LNR are also diagnostic ratios that can be used in the diagnosis of AA.

In patients with suspected AA, these ratios can optimize the use of risk classification with clinical scoring systems and diagnostic imaging, as well as guide decision-making to prevent negative exploratory surgeries. Due to these scoring systems, both unnecessary radiological examinations for AA diagnosis

Table 1. Comparison of demographic and clinical data of the patient and control groups.

Parameter	Subparameter	Patient group	Control group	p
Age		33.47±11.01	35.67±12.23	0.102*
Gender	Female	65 (48.5)	69 (51.5)	0.642*
	Male	85 (51.2)	81 (48.8)	
Vital parameters	Systolic BP (mmHg)	128.65±26.47	132.29±20.85	0.187**
	Diastolic BP (mmHg)	77.50±16.57	77.43±13.88	0.970**
	Pulse (pulse/min)	94.03±19.71	88.79±16.81	**0.014****
Laboratory values	WBC (×10^9/L)	13.42±4.57	8.72±2.89	**<0.001****
	Neutrophil (×10^9/L)	7.75±5.41	4.10±4.84	**<0.001****
	Lymphocytes (×10^9/L)	1.75±0.75	2.07±1.04	**0.004****
	Platelet (×10^9/L)	238.06±68.98	254.89±66.18	0.032**
Ratios	NLR	7.75±5.41	4.10±4.84	**<0.001****
	PLR	162.27±90.03	156.88±113.04	0.648**
	PNR	27.13±16.18	51.78±24.04	**<0.001****
	LNR	0.21±0.17	0.43±0.265	**<0.001****
	SIII (×10^9/L)	1759.62±1263.92	979.96±1032.33	**<0.001****

*Pearson's χ^2 test was used; **t-test was used. BP: blood pressure; NLR: neutrophil-lymphocyte ratio; PLR: platelet-lymphocyte ratio; PNR: platelet-neutrophil ratio; LNR: lymphocyte-neutrophil ratio; SIII: systemic immune inflammatory index; WBC: white blood cells. Bold indicates statistically significant p-values.

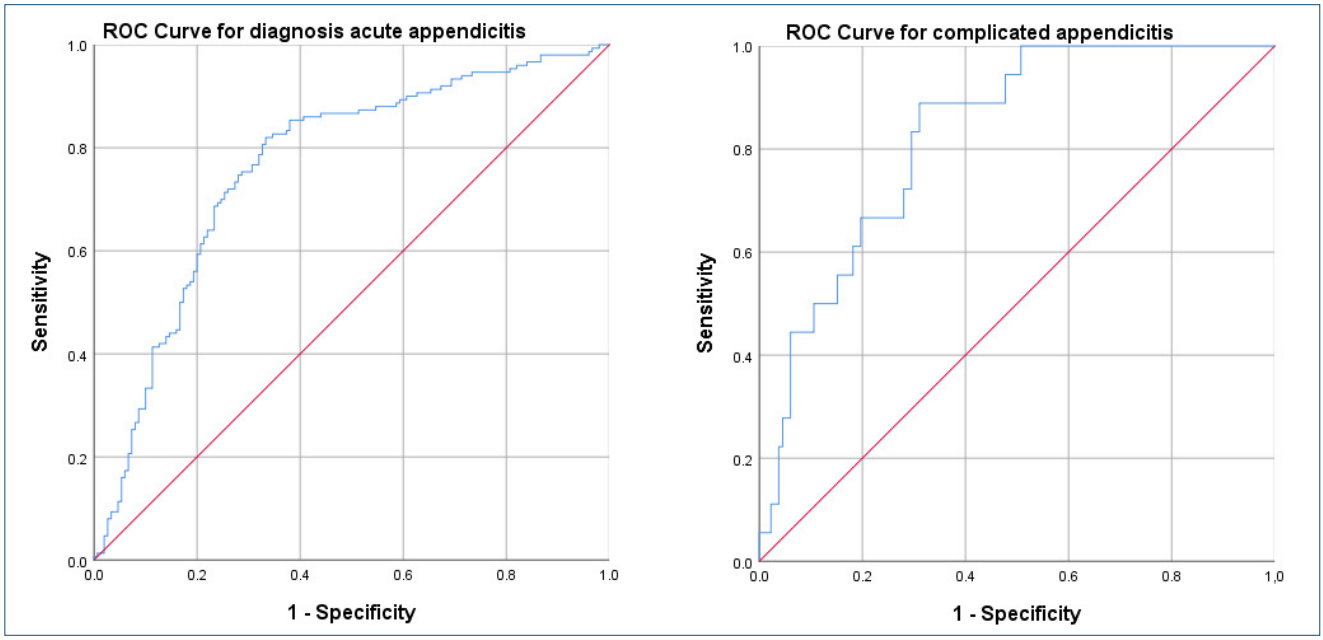

Figure 1. Systemic immune inflammation index receiver operating characteristic analysis for diagnosis of acute appendicitis and identifying complications.

Table 2. Systemic immune inflammation index receiver operating characteristic analysis results for the diagnosis of acute appendicitis and identifying complications.

Parameter	Cutoff value	Sensitivity	Specificity	Area under curve (AUC)	95%CI		p
					Lower bound	Upper bound	
Diagnostic value of SIII	840.13	82.0	66.7	0.764	0.709	0.819	**<0.001**
SIII for differentiating between complicated vs. non-complicated cases	1782.94	88.9	68.9	0.826	0.744	0.909	**<0.001**

ROC: receiver operating characteristic; SIII: systemic immune inflammatory index. Bold indicates statistically significant p-values.

and unnecessary surgeries are prevented. Many scoring systems such as Alvarado score, AAS, and AIR can be used in the diagnosis and risk classification of AA. In a meta-analysis, Kularatna et al. reported that the AIR scoring system was the most successful scoring system in terms of sensitivity and specificity[3,4,13,14]. Among the scoring systems evaluated in this study, Alvarado score and AAS were more successful in complicated AA cases compared to non-complicated cases. This is due to the fact that physical examination and peritoneal irritation findings and inflammatory markers are more prominent in complicated AA patients.

To the best of our knowledge, there are no studies in the literature investigating the diagnostic power of SIII for AA. Similarly, there are no studies investigating the efficacy of SIII in the distinction of complicated vs. non-complicated AA. In this study, the cutoff value of 840.13 ($\times 10^9$/L) for SIII had 82% sensitivity and 66.7% specificity. In addition, the cutoff value of 1,782.94 ($\times 10^9$/L) had 88.9% sensitivity and 88.9% specificity for differentiating between complicated and non-complicated cases. Compared to the study by Khairol et al., the sensitivity of SIII obtained in this study is higher than that of NLR, and its specificity is the same[15].

Dey et al. examined AA cases that were diagnosed histopathologically and found that there were misdiagnosed cases. For this reason, they investigated the correlation between histopathological diagnosis and Alvarado score and found a statistically significant positive correlation[16]. Canbak et al. investigated the correlation between Alvarado score and ultrasonography in the diagnosis of AA and found that their combined use reduced the rates of misdiagnosis and missed diagnosis[17]. In another study, Sousa-Rodrigues et al. found that the Alvarado score and the macroscopic appearance of the appendix were correlated for the diagnosis of AA; however,

this correlation only occurred in the advanced stage of AA[18]. In this study, both Alvarado score and AAS were positively correlated with SIII. This indicates that SIII can be successfully used in the diagnosis of AA. When evaluated together with Alvarado score and AAS, SIII will reduce the rates of misdiagnosis and missed diagnosis in AA cases.

CONCLUSION

Based on the results of this study, SIII may be used to promote the diagnosis of AA, and it can reduce the need for diagnostic imaging tests with radiation exposure, such as contrast-enhanced abdominal computed tomography. SIII is cost-effective and easy to calculate, and its use with Alvarado score and AAS will reduce both misdiagnosis and unnecessary operation rates.

Limitations

The limitations of our study are that it is a retrospective, single-center study. There is a need for multicenter, prospective studies with more patients.

ETHICAL APPROVAL

This study was approved by the Ministry of Healthy Başaksehir Çam and Sakura State Hospital Ethics Committee (decision no.: 2021.04.34). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

AVAILABILITY OF DATA AND MATERIALS

Ministry of Healthy Başaksehir Çam and Sakura State Hospital computer data system was used.

AUTHORS' CONTRIBUTIONS

KŞ: Conceptualization, Methodology, Writing – review & editing. **AÇ**: Conceptualization, Methodology, Writing – review & editing. **HK**: Conceptualization, Writing – review & editing. **EA**: Methodology, Writing – review & editing. **RG**: Conceptualization, Methodology.

REFERENCES

1. Bhangu A, Søreide K, Di Saverio S, Assarsson JH, Drake FT. Acute appendicitis: modern understanding of pathogenesis, diagnosis, and management. Lancet. 2015;386(10000):1278-87. https://doi.org/10.1016/S0140-6736(15)00275-5. Erratum in: Lancet. 2017;390(10104):1736. PMID: 26460662.
2. Snyder MJ, Guthrie M, Cagle S. Acute appendicitis: efficient diagnosis and management. Am Fam Physician. 2018;98(1):25-33. PMID: 30215950
3. Di Saverio S, Podda M, De Simone B, Ceresoli M, Augustin G, Gori A, et al. Diagnosis and treatment of acute appendicitis: 2020 update of the WSES Jerusalem guidelines. World J Emerg Surg. 2020;15(1):27. https://doi.org/10.1186/s13017-020-00306-3
4. Kularatna M, Lauti M, Haran C, MacFater W, Sheikh L, Huang Y, et al. Clinical prediction rules for appendicitis in adults: which is best? World J Surg. 2017;41(7):1769-81. https://doi.org/10.1007/s00268-017-3926-6
5. Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res. 2014;20(23):6212-22. https://doi.org/10.1158/1078-0432.CCR-14-0442
6. Liu X, Guan G, Cui X, Liu Y, Liu Y, Luo F. Systemic immune-inflammation index (SII) can be an early indicator for predicting the severity of acute pancreatitis: a retrospective study. Int J Gen Med. 2021;14:9483-9. https://doi.org/10.2147/IJGM.S343110
7. Chen JH, Zhai ET, Yuan YJ, Wu KM, Xu JB, Peng JJ, et al. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. World J Gastroenterol. 2017;23(34):6261-72. https://doi.org/10.3748/wjg.v23.i34.6261
8. Mazza MG, De Lorenzo R, Conte C, Poletti S, Vai B, Bollettini I, et al. Anxiety and depression in COVID-19 survivors: role of inflammatory and clinical predictors. Brain Behav Immun. 2020;89:594-600. https://doi.org/10.1016/j.bbi.2020.07.037
9. Agrawal CS, Adhikari S, Kumar M. Role of serum C-reactive protein and leukocyte count in the diagnosis of acute appendicitis in Nepalese population. Nepal Med Coll J. 2008;10(1):11-5. PMID: 18700623
10. Yao C, Liu X, Tang Z. Prognostic role of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio for hospital mortality in patients with AECOPD. Int J Chron Obstruct Pulmon Dis. 2017;12:2285-90. https://doi.org/10.2147/COPD.S141760
11. Wang D, Bai N, Hu X, Ou Yang XW, Yao L, Tao Y, et al. Preoperative inflammatory markers of NLR and PLR as indicators of poor prognosis in resectable HCC. PeerJ. 2019;7:e7132. https://doi.org/10.7717/peerj.7132
12. Paliogiannis P, Fois AG, Sotgia S, Mangoni AA, Zinellu E, Pirina P, et al. Neutrophil to lymphocyte ratio and clinical outcomes in COPD: recent evidence and future perspectives. Eur Respir Rev. 2018;27(147):170113. https://doi.org/10.1183/16000617.0113-2017
13. Andersson M, Kolodziej B, Andersson RE, STRAPPSCORE Study Group. Randomized clinical trial of appendicitis inflammatory response score-based management of patients with suspected appendicitis. Br J Surg. 2017;104:1451-61. https://doi.org/10.1002/bjs.10637
14. Coleman JJ, Carr BW, Rogers T, Field MS, Zarzaur BL, Savage SA, et al. The Alvarado score should be used to reduce emergency department length of stay and radiation exposure in select patients with abdominal pain. J Traum Acute Care Surg. 2018;84(6):946-50. https://doi.org/10.1097/TA.0000000000001885

15. Ahmad KA, Ideris N, Aziz SHSA. A cross-sectional study of neutrophil-to-lymphocyte ratio in diagnosing acute appendicitis in hospital melaka. Malays J Med Sci. 2019;26(6):55-66. https://doi.org/10.21315/mjms2019.26.6.6

16. Dey S, Mohanta PK, Baruah AK, Kharga B, Bhutia KL, Singh VK. Alvarado scoring in acute appendicitis-a clinicopathological correlation. Indian J Surg. 2010;72(4):290-3. https://doi.org/10.1007/s12262-010-0190-5

17. Canbak T, Acar A. Does alvarado score reduce the need for ultrasonography in the diagnosis of acute appendicitis? Niger J Clin Pract. 2020;23(6):764-7. https://doi.org/10.4103/njcp.njcp_328_19

18. Sousa-Rodrigues CF, Rocha AC, Rodrigues AK, Barbosa FT, Ramos FW, Valões SH. Correlation between the Alvarado Scale and the macroscopic aspect of the appendix in patients with appendicitis. Rev Col Bras Cir. 2014;41(5):336-9. https://doi.org/10.1590/0100-69912014005007

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20221005

Modelling anaerobic peak power assessed by the force-velocity test among late adolescents

Diogo Vicente Martinho[1,2], Rafael Baptista[1], Anderson Santiago Teixeira[3], Tomás Oliveira[2], João Valente-dos-Santos[2,4], Manuel João Coelho-e-Silva[1,2*], Amândio Cupido-dos-Santos[1,2]

SUMMARY

OBJECTIVES: The aim of this study was to examine the concurrent contributions of body size, estimates of whole-body composition, and appendicular volume in addition to participation in competitive basketball to explain inter-individual variance in anaerobic peak power output during late adolescence. The study also tested non-participation versus participation in basketball as an independent predictor of peak power output.

METHODS: The sample of this cross-sectional study was composed of 63 male participants (basketball: n=32, 17.0±0.9 years; school: n=31, 17.4±1.0 years). Anthropometry included stature, body mass, circumferences, lengths, and skinfolds. Fat-free mass was estimated from skinfolds and lower limbs volume predicted from circumferences and lengths. Participants completed the force-velocity test using a cycle ergometer to determine peak power output.

RESULTS: For the total sample, optimal peak power was correlated to body size (body mass: r=0.634; fat-free mass: r=0.719, lower limbs volume: r=0.577). The best model was given by fat-free mass and explained 51% of the inter-individual variance in force-velocity test. The preceding was independent of participating in sports (i.e., the dummy variable basketball vs. school did not add significant explained variance).

CONCLUSION: Adolescent basketball players were taller and heavier than school boys. The groups also differed in fat-free mass (school: 53.8±4.8 kg; basketball: 60.4±6.7 kg), which was the most prominent predictor of inter-individual variance in peak power output. Briefly, compared to school boys, participation in basketball was not associated with optimal differential braking force. Higher values in peak power output for basketball players were explained by a larger amount of fat-free mass.

KEYWORDS: Adolescent. Sport. Physiology. Anaerobic.

INTRODUCTION

Basketball is an intermittent sport involving repeated transitions between offence and defence phases. Periods of high-intensity activity were interspersed with low- to moderate-intensity activities[1]. The preceding emphasizes the need for basketball players to perform extensive sprinting and high-intensity shuffling activities during matchplay. The maximal efforts are predominantly supported by the anaerobic re-synthesis of adenosine triphosphate from phophocreatine and glycolysis[2]. The Wingate test (WAnT) is perhaps the most popular protocol to assess anaerobic fitness[3]. It requires a 30-s maximal effort in the cycle ergometer, adopting a standardized braking force (Fb) calculated as 7.5% of body mass (BM) as recommended by the original authors[4]. Nevertheless, a recent study adopted an Fb of 10% of BM to assess 32 trained male athletes from different sports (track and field, tennis, basketball, and football) in the WAnT[5].

Youth basketball players tend to plot above the median of the US reference data for boys[6,7]. An interesting research question emerges regarding whether Fb follows a constant proportionality in relation to BM as assumed by the WAnT protocol. Alternatively, the force-velocity test (FVT) has been used to assess peak power output in both school boys[8] and youth basketball players[7]. The FVT protocol requires participants to execute 3–5 maximal intensity efforts lasting 10 s or less to allow the estimation of the optimal braking force (Fb_{opt}) and associated optimal peak power (PP_{opt}). The calculation is obtained from a parabolic function that represents the relationship between peak power and Fb[7]. In fact, among youth basketball players aged 8.4–12.3 years, PP_{opt} assessed by the FVT was determined by adopting an Fb corresponding to 0.089 kg per unit of BM[9].

Considering that youth basketball players tend to be characterized by a larger body size compared to the normal population,

[1]Universidade de Coimbra, Faculty of Sport Sciences and Physical Education – Coimbra, Portugal.
[2]Universidade de Coimbra, Research Center for Physical Activity – Coimbra, Portugal.
[3]Universidade Federal de Santa Catarina, Research Group for Development of Football and Futsal, Physical Effort Laboratory, Sports Center – Florianópolis (SC), Brazil.
[4]Universidade Lusófona de Humanidades e Tecnologias, Centro de Investigação em Desporto, Educação Física e Exercício e Saúde – Lisboa, Portugal.
*Corresponding author: mjcesilva@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 30, 2022. Accepted on November 14, 2022.

the aim of this study was to examine the independent and combined effects of sports participation status, body size, and estimates of body composition to predict inter-individual variance in PP_{opt} assessed by the FVT among late adolescents. It was hypothesized that although the traditional standardized Fb would be questionable, the principle of geometric similarity applies to both non-athlete late adolescents and athletes of the same chronological age and fat-free mass (FFM) would be confirmed as the best predictor.

METHODS

Procedures

This cross-sectional study was approved by the Ethical Committee of the University of Coimbra [CE/FCDEF-UC/00122014] and followed the recommendations by the World Medical Association for research with humans[10]. Parents or legal guardians signed an informed consent. All measurements were completed by the same observers at the same hours of the day, that is, during the mornings of non-school days, under the same conditions as previously reported elsewhere[7]. Participants were advised to avoid eating food at least 3 h before the functional protocol and not to drink caffeine-containing beverages for at least 8 h before the laboratory assessment. All tests occurred at the Coimbra University Stadium.

Participants

The sample included 31 non-athlete adolescent boys (aged 17.4±1.0 years) recruited in secondary schools with which the University of Coimbra had agreements to carry out research projects. Inclusion criteria were as follows: (1) not participating in organized sports and (2) any physical limitations to perform maximal tests such as asthma. In parallel, 32 male adolescent basketball players (aged 17.0±0.9 years) were assessed as an independent group. The basketball players were recruited in four clubs registered in Portuguese Basketball Federations who already completed at least two seasons at the time of the observations. They regularly train three to four sessions per week of 90–120 min each under the supervision of a certified coach and in official basketball competitions (usually on weekend days).

Body size and body composition

Anthropometry was completed by a single observer following standardized procedures[11]. Stature was measured to the nearest 0.1 cm using a stadiometer (Harpenden model 98.603, Holtain LTD, Crosswell, UK). A portable balance (SECA model 770, Hanover, MD, USA) was used to measure BM to the nearest 0.1 kg. Skinfold thickness was measured at two sites (triceps and subscapular) to the nearest 1 mm using a Lange calliper (Beta Technology Incorporate Cambridge, MD, USA). A non-invasive equation recommended for male adolescents[12] was used to determine body fat expressed as a percentage of BM (%FM). Subsequently, fat mass (FM) and FFM in kg were derived.

Lower limbs volume

Estimates of lower limbs volume (LLV) were determined as previously detailed[7]. The lower limb was fractionated using geometric truncated cones. It requires circumferences and partial lengths between consecutive transverse plans. Lengths and circumferences were measured to the nearest 0.1 cm. The protocol partitioned the lower limb into truncated cones. The circumferences were measured as follows: at the most proximal gluteal furrow; at the level of the largest mid-thigh circumference; at the minimum circumference above the knee; at the maximum circumference around the knee, that is, at the patella level; at the minimum circumference below the knee; at the maximum calf circumference; and at the minimum ankle circumference. The lengths between consecutive transverse plans corresponding to each circumference (from the gluteal furrow to the minimum ankle circumference) were measured. To calculate the volume of a truncated cone, the following equation was used: $V = [A_1 + A_2 + (A_1 \times A_2)^{0.5}] \times h \div 3$, where A_1 (e.g., area at the proximal circumference level) and A_2 (e.g., area at the distal circumference level) are the areas at the sections that define the truncated cone, and h is the length between the two transverse plans. The areas (A_1, A_2) were derived from leg circumferences (C) as follows: $A = C^2/4\pi$. LLV (in L) was calculated as the sum of the volumes of the truncated cones.

Force-velocity test

Participants completed the FVT on a cycle ergometer interfaced to a computer (Monark 824E; Monark AB, Vargerg, Sweden). The standardized warm-up consisted of pedalling for 5 min at 60 revolutions per minute (rpm) against the basket (resistance: 1 kg) interspersed with a 3-s "all-out" sprint at the second, third, and fourth minutes. The FVT involved a set of three to six "all-out" sprints against random breaking forces. The initial resistance was set at 0.74 N kg^{-1} with subsequent Fbs randomly above and below the initial load. Flywheel velocity was measured using an optical sensor (Opto Sensor 2000; Sports Medicine Industries Inc., St. Cloud, MN, USA). The test was automatically interrupted when the optical sensor detected that rpm declined for three consecutive revolutions. Each sprint was interspaced by a 5-min active recovery (pedalling at 60 rpm with minimal resistance, i.e., the 1 kg the basket of the ergometer). PP_{opt} and Fb_{opt} were individually calculated[3,7,13].

Analyses

Descriptive statistics were calculated for school-aged adolescents and basketball players. The mean differences between school-aged adolescents and basketball participants were examined with the t-test for independent samples. The magnitude of differences was interpreted as follows[14]: <0.20 (trivial), 0.20–0.59 (small), 0.60–1.19 (moderate), 1.20–1.99 (large), 2.0–3.9 (very large), and ≥4.0 (nearly perfect). The linear relationship among body size descriptors and PP_{opt} was examined using Pearson product-moment correlation, and the magnitude of correlations was interpreted as follows[14]: trivial ($r<0.1$), small ($0.1\leq r<0.3$), moderate ($0.3\leq r<0.5$), large ($0.5\leq r<0.7$), very large ($0.7\leq r<0.9$), and nearly perfect ($r\geq 0.9$).

An initial model[15] was obtained using multiple linear regression analysis and the log-transformed values of BM, FFM, and LLV. In addition, sports participation status was encoded as a dummy variable (school=0; basketball=1). From an initial model including all predictors, it was tested whether it was possible to extract a more economical solution of predictors without a significant decline in explained variance (backward method of multiple regression analysis). For the final model, it was summarized as follows: coefficient R (multiple regression coefficient), standard error of estimate (SEE), squared R (explained variance), and significance value. For each predictor, an unstandardized coefficient was presented. The significance level was set at 5%. Statistical analyses were performed using the IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 5.03 software (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Table 1 summarizes descriptive statistics separately for school-aged adolescents and basketball players, who were, on average, +8.5 cm taller and +8.2 kg heavier compared to non-athletes. Basketball players produced +123 W in the cycle ergometer test protocol compared to their school peers. The Fb_{opt} was 11.7% of BM (specific values were 11.3 and 12.2% of BM, respectively, for non-athlete adolescents and basketball players). For the total sample, the gradient of the correlation coefficients between PP_{opt} and each body size descriptor were as follows: LLV ($r_{PPopt,\ LLV}=0.577$; 95%CI 0.384–0.722; large), BM ($r_{PPopt,\ BM}=0.634$; 95%CI 0.458–0.762; large), and FFM ($r_{PPopt,\ FFM}=0.719$; 95%CI 0.573–0.821; very large). Table 2 summarizes the initial solution that considered log-transformed values for the three anthropometric variables in addition to sports participation as a dummy variable (school boys vs. basketball players). It explained 52.3% of the variance. Nevertheless, another significant model was obtained after excluding the dummy variables, suggesting that the sports status was not essential to explain the performance variable. In fact, the explained variance was reduced to 51.9%. Afterwards, it was also possible to exclude LLV with a minimal impact on explained variance ($R^2=0.509$). Finally, by excluding BM from the set of predictors, a model exclusively including FFM as a predictor was significant ($R=0.712$, $SEE=0.130$, $p<0.001$; 50.6% explained variance). The obtained equation is presented in Figure 1. The anti-log function corresponds to $PP_{opt}=2.106+FFM^{1.150}$. It is generalized to both non-athletes and basketball participants.

Table 1. Descriptive statistics by sports status (non-athletes vs. basketball players) for chronological age, body size, body composition, and force-velocity test outputs among male post-pubertal adolescents.

Variable	Unit	Comparisons between non-sports participants and basketball players					
		Non-athletes (n=31)	Basketball players (n=32)	t	p	Magnitude effect	
						d	(Qualitative)
Chronological age	years	17.4±1.0	17.0±0.9	1.461	0.149	0.43	(Small)
Stature	cm	171.8±4.5	180.3±7.8	5.225	<0.001	1.35	(Large)
Body mass	kg	64.9±8.6	73.1±10.3	3.398	0.001	0.88	(Moderate)
Fat mass	%	16.4±6.9	16.9±4.7	0.309	0.758	0.09	(Trivial)
	kg	11.1±6.0	12.7±5.0	1.120	0.267	0.29	(Small)
Fat-free mass	kg	53.8±4.8	60.4±6.7	4.484	<0.001	1.15	(Moderate)
Lower limbs volume	L	12.9±2.1	15.5±3.1	3.904	<0.001	1.08	(Moderate)
Optimal braking force	kg	7.3±1.4	8.8±1.8	3.562	0.001	0.94	(Moderate)
	$kg.kg^{-1}$	0.113±0.016	0.120±0.028	1.484	0.143	0.40	(Small)
	$N.kg^{-1}$	1.11±0.16	1.18±0.28	1.477	0.145	0.40	(Small)
PP_{opt}	W	806±140	929±157	3.267	0.002	0.84	(Moderate)

PP_{opt}, optimal peak power; d, Cohen's d-value.

Table 2. Modelling of peak power output among male adolescents (n=63).

Step	Variables in the model	Excluded in the model	Model summary						Coefficients				
			R	SEE	R^2	R^2 adjusted	F	p	Scaling			Constant	
									k	(95%CI)	p	a	p
1[a]	ln BM												
	ln FFM												
	ln LLV												
	School vs BB		0.723	0.131	0.523	0.490	15.887	<0.001					
2[b]	ln BM												
	ln FFM												
	ln LLV	School vs. BB	0.720	0.131	0.519	0.494	21.195	<0.001					
3[c]	ln BM												
	ln FFM	ln LLV	0.713	0.131	0.509	0.493	31.105	<0.001					
4[d]	ln FFM	ln BM	0.712	0.130	0.506	0.498	62.547	<0.001	1.150	(0.859–1.440)	<0.001	2.106	<0.001

ln BM, log-transformed body mass; ln FFM, log-transformed fat-free mass; ln LLV, log-transformed lower limbs volume; school vs. BB, dummy variable: school=0 and basketball=1; R, multiple correlation coefficient; R^2, explained variance; SEE, standard error of estimation; 95%CI, 95% confidence interval. [a]Model 1: $\ln(PP_{opt})=k_1*\ln(BM)+k_2*\ln(FFM)+k_3*\ln(LLV)+a+b*$dummy variable$+\ln(\varepsilon)$. [b]Model 2: $\ln(PP_{opt})=k_1*\ln(BM)+k_2*\ln(FFM)+k_3*\ln(LLV)+a+\ln(\varepsilon)$. [c]Model 3: $\ln(PP_{opt})=k_1*\ln(BM)+k_2*\ln(FFM)+a+\ln(\varepsilon)$. [d]Model 4: $\ln(PP_{opt})=k_1*\ln(FFM)+a+\ln(\varepsilon)$.

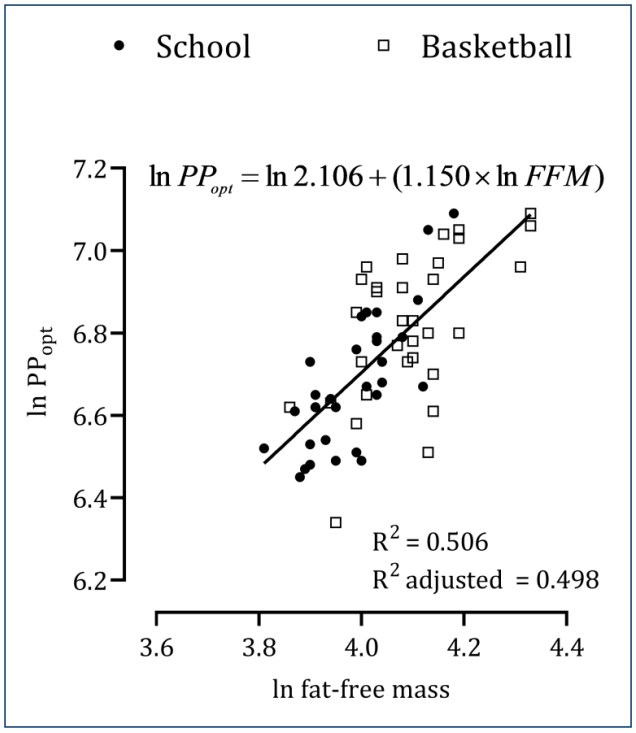

Figure 1. Linear regression of the ln transformed optimal peak output with the ln transformed fat-free mass.

DISCUSSION

This study examined the contribution of concurrent size descriptors to explain inter-individual variation on PP_{opt} obtained from the FVT protocol among a sample of late adolescent males combining non-athletes and basketball players. Compared to school boys, current basketball players presented larger amounts of LLV and FFM. The differences between sports participants and non-athletes do not necessarily follow the principles of geometric similarity. The final solution to explain PP_{opt} suggested that, among post-pubertal males, FFM was the best single predictor. The previous studies highlighted the importance of metabolically active tissues and appendicular volume to interpret inter-individual performance in the anaerobic performance output under discussion.

Few studies have examined anaerobic power among male adolescent basketball players combining WAnT and FVT. The preceding protocols were used in youth and adult basketball[16], and peak power derived from WAnT were 864, 700, and 1,039 W, respectively, for under-15 (n=35), under-18 (n=35), and elite adult players (n=31). The corresponding mean values obtained from the FVT protocol were 868, 1,086, and 1,255 W. The authors found that both WAnT and FVT protocols consistently detected variation of the mean performance values by playing position. Guards and forwards scored better than centers, more pronounced at senior level[16].

In this study, Fb_{opt} was 11.3% of BM (1.11 $N.kg^{-1}$) among school-aged adolescents and 12.0% (1.18 $N.kg^{-1}$) for basketball players. The above values were different from 7.5% of BM (0.74 $N.kg^{-1}$) as recommended in the WAnT protocol. Finally, pre-pubertal basketball players aged 10.8 years assessed using the FVT showed an estimated Fb_{opt} of

8.9% of BM. These results confirm that PP_{opt} is not associated to a standardized Fb as proposed by WAnT and, additionally, the size descriptor having largest shared variance to anaerobic performance is FFM. Consequently, body composition should be part of batteries aimed at assessing basketball players. In fact, body composition is a discriminant characteristic between non-athletes and basketball players. Finally, strength training designed to gain muscle mass may be a valid goal to increase anaerobic performance.

Despite the limitations of using the ratio standard[17,18], maximal short-term power output derived from FVT and WAnT protocols is often expressed per unit of BM (watt/kg). Previous study[6] suggested allometric scaling as the recommended option to obtain a size-free understanding of inter-individual variance which is believed to be relevant in sports such as basketball characterized by selection based on body size. Briefly, the simple ratio tends to penalize heavier individuals and rarely represents an appropriated approach to examine variability among participants[17,19,20]. The current study illustrated a linear relationship among FFM and anaerobic peak power derived from the FVT; nevertheless, it should be recognized that future studies need to use a better methodology in the assessment of body composition.

CONCLUSION

This study suggested that Fb_{opt} to assess anaerobic peak power should not be standardized at 0.075 kg per unit of BM. It has also been demonstrated that inter-individual variability in PP_{opt} in post-pubertal male school boys and adolescent basketball players is largely related to differences in the amount of FFM. Regardless of participation in basketball, among post-pubertal adolescents, FFM was confirmed as the most relevant body size descriptor to explain maximal intensity short-term output given by FVT.

AUTHORS' CONTRIBUTIONS

RB: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation. **MJCS:** Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **ACS:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **DVM:** Formal Analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. **JVS:** Investigation, Software, Validation. **TO:** Formal Analysis, Resources, Software, Validation. **AST:** Software, Validation.

REFERENCES

1. Stojanović E, Stojiljković N, Scanlan AT, Dalbo VJ, Berkelmans DM, Milanović Z. The activity demands and physiological responses encountered during basketball match-play: a systematic review. Sports Med. 2018;48(1):111-35. https://doi.org/10.1007/s40279-017-0794-z
2. Armstrong N, Barker AR, McManus AM. Muscle metabolism during exercise. In: Armstrong N, Van Mechelen W, editors. Oxford textbook of children's exercise science and medicine. Oxford: Oxford University Press; 2017. p. 69-87.
3. Doré E, Martin R, Ratel S, Duché P, Bedu M, Van Praagh E. Gender differences in peak muscle performance during growth. Int J Sports Med. 2005;26(4):274-280. https://doi.org/10.1055/s-2004-821001
4. Inbar O, Bar-Or O, Skinner JS. The wingate anaerobic test. Champaign (IL): Human Kinetics; 1996.
5. Ozkaya O, Balci GA, As H, Vardarli E. The test-retest reliability of new generation power indices of Wingate all-out test. Sports (Basel). 2018;6(2):31. https://doi.org/10.3390/sports6020031
6. Coelho e Silva MJ, Figueiredo AJ, Carvalho HM, Malina RM. Functional capacities and sport-specific skills of 14-to 15-year-old male basketball players: size and maturity effects. Eur J Sport Sci. 2008;8(5):277-85. https://doi.org/10.1080/17461390802117177
7. Martinho DV, Baptista R, Teixeira AS, Duarte JP, Valente-Dos-Santos J, Coelho-E-Silva MJ, et al. Allometric scaling of force-velocity test output among pre-pubertal basketball players. Int J Sports Med. 2021;42(11):994-1003. https://doi.org/10.1055/a-1327-2727
8. Santos AMC, Armstrong N, Croix MBADS, Sharpe P, Welsman JR. Optimal peak power in relation to age, body size, gender, and thigh muscle volume. Pediatr Exerc Sci. 2003;15(4):406-18. https://doi.org/10.1123/pes.15.4.406
9. Williams CA, Ratel S. Maximal-intensity exercise. In: Armstrong N, Van Mechelen W, editors. Oxford textbook of children's sport and exercise medicine. Oxford: Oxford University Press; 2017. p. 105–17.
10. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4. https://doi.org/10.1001/jama.2013.281053
11. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign (IL): Human Kinetics; 1988.
12. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol. 1988;60(5):709-23. PMID: 3224965
13. Coelho-E-Silva MJ, Rebelo-Gonçalves R, Martinho D, Ahmed A, Luz LGO, Duarte JP, et al. Reproducibility of estimated optimal peak output using a force-velocity test on a cycle ergometer. PLoS One. 2018;13(2):e0193234. https://doi.org/10.1371/journal.pone.0193234
14. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc. 2009;41(1):3-13. https://doi.org/10.1249/MSS.0b013e31818cb278
15. Nevill AM, Holder RL. Modelling maximum oxygen uptake-a case-study in non-linear regression model formulation and comparison. J R Stat Soc Ser C Appl Stat. 1994;43(4):653-66. https://doi.org/10.2307/2986263
16. Nikolaidis P, Calleja-González J, Padulo J. The effect of age on positional differences in anthropometry, body composition, physique and anaerobic power of elite basketball players. Sport Sci Health. 2014;10:225-33. https://doi.org/10.1007/s11332-014-0198-5

17. Tanner JM. Fallacy of per-weight and per-surface area standards, and their relation to spurious correlation. J Appl Physiol. 1949;2(1):1-15. https://doi.org/10.1152/jappl.1949.2.1.1

18. Nevill AM, Ramsbottom R, Williams C. Scaling physiological measurements for individuals of different body size. Eur J Appl Physiol Occup Physiol. 1992;65(2):110-17. https://doi.org/10.1007/BF00705066

19. Armstrong N, Welsman JO. Sex-specific longitudinal modeling of short-term power in 11- to 18-year-olds. Med Sci Sports Exerc. 2019;51(5):1055-63. https://doi.org/10.1249/MSS.0000000000001864

20. Welsman J, Armstrong N. Interpreting aerobic fitness in youth: the fallacy of ratio scaling. Pediatr Exerc Sci. 2019;31(2):184-90. https://doi.org/10.1123/pes.2018-0141

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20221012

Finding the best method for screening for gestational diabetes mellitus: fetal thymic-thoracic ratio or fetal thymus transverse diameter

Koray Gök[1*], Selçuk Özden[1]

SUMMARY

OBJECTIVE: The aim of this study was to compare the efficiency of fetal thymic-thoracic ratio and fetal thymus transverse diameter measurements in gestational diabetes mellitus.

METHODS: Fetal thymic-thoracic ratio and fetal thymus transverse diameter were assessed in 360 pregnant women. Patients were examined in two groups: 180 gestational diabetes mellitus (study group) and 180 healthy pregnant women (control group).

RESULTS: There were no statistically significant differences between the cases with gestational diabetes mellitus and the control group in terms of fetal thymus transverse diameter; however, the fetal thymic-thoracic ratio was found to be significantly lower in cases with gestational diabetes mellitus compared to that in the control group ($p<0.001$).

CONCLUSION: The fetal thymic-thoracic ratio is superior to the fetal thymus transverse diameter in evaluating the fetal thymus size.

KEYWORDS: Fetus. Diabetes, gestational. Thymus gland. Ultrasonography, prenatal.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as the glucose tolerance that occurs or is determined for the first time during pregnancy in an individual with no preexisting diabetes[1]. It has been estimated to affect 5–20% of all pregnancies[2]. Although the pathophysiology of GDM has not yet been fully elucidated, it has been suggested that it causes low-grade systemic inflammation that exacerbates maternal immune responses[3]. As it leads to serious maternal and fetal complications if not monitored, it is of great importance that it should be screened during pregnancy and, if detected, should be appropriately followed up and treated[2].

The thymus is a lymphoepithelial organ originating from the third brachial cleft at the 9th gestational week and descending to the anterior/superior mediastinum at the 12th gestational week[4]. The fetal thymus is detected ultrasonographically at three-vessel levels, in front of the pulmonary artery, aorta, and superior vena cava, behind the sternum, and between both lungs[5-7]. Various methods have been reported for the ultrasonographic measurement of fetal thymus size including transverse diameter, anterior-posterior diameter, circumference, volume, and thymic-thoracic ratio[8-11].

There are numerous studies evaluating the fetal thymus size. It has been reported that the fetal thymus size increases in proportion to the gestational week in healthy pregnant women while the fetal thymic-thoracic ratio remains constant[8]. However, it has been reported that this ratio decreased in some complicated pregnancy cases, including diabetic pregnancies[12-16].

To the best of our knowledge, there are studies investigating the fetal thymic-thoracic ratio in diabetic pregnant women[14-16]; however, there are no studies evaluating the fetal thymus transverse diameter in GDM. Being the first study on this subject, the aim was to compare fetal thymic-thoracic ratio and fetal thymus transverse diameter in the evaluation of fetal thymus size in GDM.

METHODS

This study was approved by the Sakarya University Ethics Committee (decision no.: 40019-376, approval date: June 30, 2021). It included pregnant women diagnosed with gestational diabetes (n=180), who were admitted to the Sakarya University Education and Research Hospital, Gynecology and Obstetrics Clinic Perinatology Department between November 1, 2018, and June 15, 2021, and delivered in the same hospital. GDM was defined as a single abnormal result from a 2-h 75-g oral glucose tolerance test or two abnormal results from a 3-h 100-g oral glucose tolerance test[17]. These pregnant women were classified into two groups: diet-controlled gestational diabetes (GDd, n=106) and insulin-dependent gestational diabetes

[1]Sakarya Üniversitesi, Faculty of Medicine, Department of Obstetrics and Gynecology – Sakarya, Turkey.
*Corresponding author: drkorayctf@hotmail.com

Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 26, 2022. Accepted on November 04, 2022.

(GDi, n=74). In the control group, completely healthy pregnant women (n=180) at a similar gestational week without any pregnancy complications were included in the study. The data of both groups were obtained from the medical records of the hospital. The fetal thymic-thoracic ratio data were measured in the third trimester in both groups. The values of fetal thymus transverse diameter and fetal thymic-thoracic ratio measured in the third trimester were obtained from medical records. Preeclamptic pregnant women, pregnant women diagnosed with pre-GDM, pregnant women with any known medical disease (chronic hypertension, chronic liver disease, chronic kidney disease, and rheumatological diseases), pregnant women with HIV or other accompanying chronic inflammatory diseases, amniotic fluid disorders (polyhydramnios and oligohydramnios), preterm labor, pregnant women diagnosed with premature rupture of membranes, pregnant women with suspected fetal macrosomia in ultrasonographic measurements, cases with fetal structural or chromosomal disorders, cases with a history of corticosteroid use during pregnancy, cases with evidence of placental insufficiency in Doppler parameters, and pregnant women with sonographic estimated fetal weight <10% were not included in the study.

All ultrasound examinations were performed by a single sonographer (Koray Gök) using a Voluson 730 and a Voluson E6 (GE Medical Systems, Milwaukee, WI, USA) ultrasound machine. Thus, the measurements were standardized and the bias was limited. All measurements were carried out in the absence of fetal movements. Fetal thymus transverse diameter measurements were performed as previously described by Zalel et al.[7] The thymus was identified in the three vessels as a homogeneous structure in the anterior mediastinum. The transverse diameter of the fetus was measured by placing the ultrasound calipers perpendicular to the junction between the sternum and the spine. Fetal thymic-thoracic ratio measurement was performed as previously described by Chaoui et al.[8] Thymus was detected in the three vessels and trachea (3VT) views as a hypoechogenic structure with echogenic dots filling the space between the vessels posteriorly and the anterior chest wall (sternum and ribs) anteriorly. The anteroposterior diameter of the thymus was determined in addition to the midline between the transverse aortic arch border posteriorly and the posterior chest wall anteriorly. Also, the mediastinal sagittal diameter was determined in addition to the line traced to measure the thymic diameter, as the distance between the anterior edge of the thoracic vertebral body at the level of the transverse arch posteriorly and the internal edge of the sternum anteriorly.

The statistical evaluations were carried out using the SPSS 24.0 software (SPSS Inc. and Lead Tech. Inc., Chicago, USA). The Kolmogorov-Smirnov test was used to examine the normality of the distribution of the data. Nonparametric data were reported as the median and interquartile range . Nonparametric data were compared using the Mann-Whitney U test. Multiple groups were compared using the Kruskal-Wallis test and the Bonferroni post-hoc correction. Receiver operating characteristic (ROC) analysis was used to evaluate the predictive performance of the fetal thymic-thoracic ratio for GDM. An alpha <0.05 for Bonferroni correction and a p-value <0.05 for other tests were considered to be statistically significant.

RESULTS

The characteristics of the GDM cases and the control group and the comparison of fetal thymus transverse diameter and fetal thymic-thoracic ratio in both groups are presented in Table 1. No statistically significant differences were found between the gestational diabetes cases and the control group in terms of

Table 1. Comparison of characteristics, fetal thymic-thoracic ratio, and fetal thymus transverse diameter between gestational diabetes mellitus cases and control group.

	Gestational diabetes group (n=180)	Control group (n=180)	p-value
Maternal age (years)	32 (20–44)	32 (20–43)	0.901
Gravidity	3 (1–8)	3 (2–7)	**0.000**
Parity	1 (0–5)	2 (0–4)	**0.000**
Body mass index (BMI) (kg/m^2)	26.8 (23.7–29.6)	26.4 (22.8–29.4)	0.081
Gestational age at the time of the study (weeks)	31.42 (29.7–33.84)	31.56 (29.56–33.42)	0.226
Gestational age at birth (weeks)	38.84 (36–40.14)	39 (37–39.56)	**0.043**
Birth weight (g)	3405 (2580–4400)	3387 (2640–3900)	0.068
Transverse diameter (mm)	32.8 (27.8–36.6)	33.1 (27.9–35.6)	0.070
Thymic-thoracic ratio	0.324 (0.292–0.408)	0.43 (0.392–0.462)	**0.000**

Data are expressed as median (minimum–maximum). p<0.05 indicates a significant difference (denoted in bold).

age and body mass index (BMI). There were no statistically significant differences between the cases with GDM and the control group in terms of fetal thymus transverse diameter; however, the fetal thymic-thoracic ratio was found to be significantly lower in cases with GDM compared to that in the control group (p<0.001).

The fetal thymic-thoracic ratio was evaluated using the Kruskal-Wallis test between the diet-controlled gestational diabetes and insulin-dependent gestational diabetes group and the control group, and statistically significant differences were determined between the groups (p<0.001) (Table 2). By evaluating the fetal thymic-thoracic ratios within the groups using the Mann-Whitney U test, a statistically significant difference was found among all three groups (p<0.001).

A cutoff value was determined for the fetal thymic-thoracic ratio using the ROC curve, and its success in predicting GDM was analyzed. By setting the fetal thymic-thoracic ratio cutoff value as 0.407 for the prediction of GDM, the sensitivity was found to be 92.8% and the specificity to be 86.1% (p<0.001) (Figure 1).

DISCUSSION

The following results were obtained in the present study:

1. The fetal thymic-thoracic ratio was found to be lower in the GDM than that in the control group.
2. Evaluating the patients with GDM among themselves, it was found that the fetal thymic-thoracic ratio was lower in the insulin-dependent gestational diabetes group.
3. Contrary to the fetal thymic-thoracic ratio, there were no differences between the groups in terms of fetal thymus transverse diameter measurements.

The first study on the fetal thymic-thoracic ratio in diabetic pregnant women was carried out by Dörnemann et al. The researchers included healthy pregnant women, gestational diabetic pregnant women, and pregestational diabetic pregnant women at gestational weeks similar to those adopted in the present study. The fetal thymic-thoracic ratio was found to be lower in diabetic pregnant women compared to that in healthy pregnant women; however, no statistically significant differences were found when examining the diabetic pregnant women among themselves. Accepting the fetal thymic-thoracic ratio cutoff value as 0.332, it has been reported that the sensitivity of this value in predicting GDM was 87.6%, the specificity was 76.2%, and the AUC value was 0.895. The researchers have stated that the fetal thymic-thoracic ratio could be used in the management of diabetic pregnancies[14]. In a study by Ghalandarpoor-Attar et al. with a fewer number of patients, patient groups similar to those of Dörnemann et al. were evaluated and found that the fetal thymic-thoracic ratio decreased in diabetic pregnant women compared to that in the healthy pregnant women. The researchers also found that the decrease in the fetal thymic-thoracic ratio in diabetic pregnant women was more evident in the pregestational group; however, they did not determine a cutoff value. The researchers, though, recommended its use to predict diabetes during pregnancy[15]. In the present study, it was found that the thymic-thoracic ratio decreased in pregnant women with GDM compared to that in healthy pregnant women. By setting the cutoff value

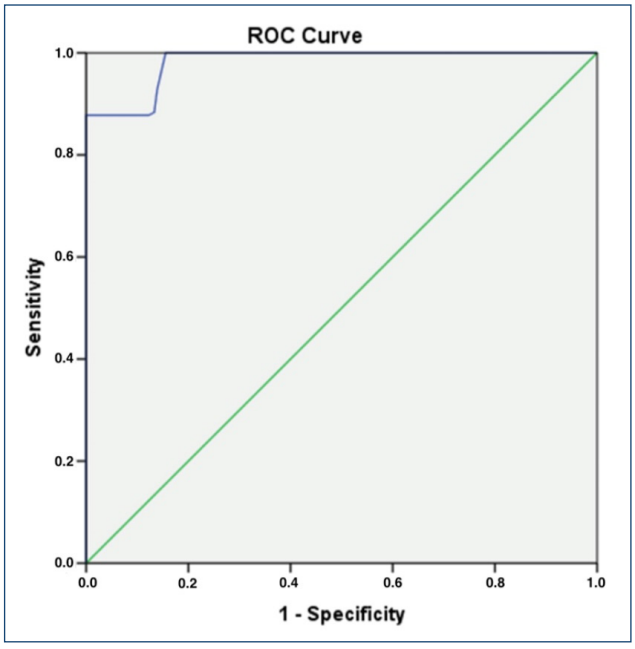

Figure 1. Receiver operating characteristic curve of fetal thymic-thoracic ratio to predict gestational diabetes. Diagonal segments are produced by ties.

Table 2. Comparison of fetal thymic-thoracic ratio between diet-controlled gestational diabetes and insulin-dependent gestational diabetes group and control group using Kruskal-Wallis test.

	Control group (n=180)	Diet-controlled gestational diabetes (n=106)	Insulin-dependent gestational diabetes (n=74)	p-value
Thymic-thoracic ratio	0.43 (0.392–0.462)	0.326 (0.296–0.408)	0.317 (0.292–0.348)	**0.000**

Data are expressed as median (minimum–maximum). p<0.05 indicates a significant difference (denoted in bold).

of the thymic-thoracic ratio in determining gestational diabetes as 0.407, the sensitivity was calculated as 92.8% and the specificity as 86.1%. The reason for the higher sensitivity and specificity of the cutoff value that was set in the present study than that of Dörnemann et al. can be the better standardization of the present study. Unlike the others, the present study included only gestational diabetic pregnant women. In addition, the number of patients in the study was higher, BMI values known to be associated with insulin resistance were similar, and all the pregnant women comprised only patients in the third trimester. Furthermore, it was determined that the thymic-thoracic ratio decreased more significantly in the insulin-dependent gestational diabetes group in parallel with the severity of GDM. Ghalandarpoor-Attar et al. reported that the decrease in the thymic-thoracic ratio was more pronounced in the pre-GDM, which is a more severe form of diabetes[15]; however, Dörnemann et al. did not report such a case[14]. In both studies, a more significant decrease in this ratio was not determined in insulin-dependent GDM, which is considered to be a more serious case[14,15]. In another recent study, researchers found that the anteroposterior diameter of the fetal thymus and the fetal thymic-thoracic ratio decreased in GDM. However, in this study, it is seen that the researchers did not divide diabetic pregnant women into groups according to the severity of the disease, as in our study[16]. In the present study, it is possible to discuss about a relationship between the severity of diabetes and the decrease in the thymic-thoracic ratio in gestational diabetic pregnant women, whereas in other studies, it is not possible to discuss about such a relationship between the severity of diabetes and the decrease in the thymic-thoracic ratio.

In the present study, although the fetal thymic-thoracic ratio has been argued to be the best ultrasonographic method for evaluating fetal thymus size in the literature, fetal thymus transverse diameter was also evaluated. However, no differences were found between diabetic pregnant women and healthy pregnant women. This may be due to the fact that fetal thymus transverse diameter measurement is a simpler method to evaluate fetal thymus size compared to fetal thymic-thoracic ratio measurement. However, there are studies showing that fetal thymus transverse diameter may be valuable in some complicated pregnancies[18,19].

For healthy fetal development, it is necessary to have various maternal anatomical and physiological adaptations. Sex hormones play an important role in the coordination of these adaptations. Sex hormones use mediators such as receptor activators of nuclear factor kappa-B (RANK) to perform these functions[20]. During pregnancy, RANK has been shown to regulate thymus functions in addition to bone metabolism through sex hormones. Regulatory T cells (Treg) in the thymus are expected to increase via RANK for a healthy pregnancy. Thymic depletion of RANK causes Treg cells to accumulate in adipose tissue while reducing their level in the placenta. Its effect on adipose tissue leads to an increase in the size of adipocyte cells, tissue inflammation, increased glucose intolerance, and the development of gestational diabetes. The decrease in these Treg cells in the placenta results in fetal losses. In addition to fetal losses, the decrease in Treg cells in the placenta in gestational diabetic pregnancies indicates the presence of abnormal placentation[20]. The hypoxic and metabolic stress environment caused by this abnormal placentation may have led to a decrease in fetal thymus size in pregnant women with GDM, as determined in the present study.

Although this study is retrospective, it has strong strengths including a large sample with similar BMI values and gestational weeks among the groups, and the measurements were made by a single specialist.

CONCLUSION

It was determined that the fetal thymic-thoracic ratio decreased in gestational diabetic pregnant women, which was more pronounced in the insulin-dependent gestational diabetes group, indicating more severe form of GDM. It was shown that the fetal thymic-thoracic ratio is superior to the fetal thymus transverse diameter in evaluating the fetal thymus size. The measurement of the fetal thymic-thoracic ratio was seen to be beneficial in determining the severity of the disease in gestational diabetic pregnant women.

ETHICAL APPROVAL

This study was approved by the clinical research ethics committee of the Sakarya University Clinical Research Ethics Board (decision no.: 40019-376, approval date: June 30, 2021).

AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44(Suppl 1):S15-33. https://doi.org/10.2337/dc21-S002
2. Vladu IM, Clenciu D, Mitrea A, Amzolini A, Micu SE, Crisan AE, et al. Maternal and fetal metabolites in gestational diabetes mellitus: a narrative review. Metabolites. 2022;12(5):383. https://doi.org/10.3390/metabo12050383
3. Sharma S, Banerjee S, Krueger PM, Blois SM. Immunobiology of gestational diabetes mellitus in Post-Medawar Era. Front Immunol. 2022;12:758267. https://doi.org/10.3389/fimmu.2021.758267
4. Tangshewinsirikul C, Panburana P. Sonographic measurement of fetal thymus size in uncomplicated singleton pregnancies. J Clin Ultrasound. 2017;45(3):150-9. https://doi.org/10.1002/jcu.22419
5. Cho JY, Min JY, Lee Y-H, McCrindle B, Hornberger LK, Yoo S-J. Diameter of the normal fetal thymus on ultrasound. Ultrasound Obstet Gynecol. 2007;29(6):634-8. https://doi.org/10.1002/uog.3979
6. Gamez F, De Leon-Luis J, Pintado P, Perez R, Robinson JN, Antolin E, et al. Fetal thymus size in uncomplicated twin and singleton pregnancies. Ultrasound Obstet Gynecol. 2010;36(3):302-7. https://doi.org/10.1002/uog.7578
7. Zalel Y, Gamzu R, Mashiach S, Achiron R. The development of the fetal thymus: an in utero sonographic evaluation. Prenat Diagn. 2002;22(2):114-7. https://doi.org/10.1002/pd.257
8. Chaoui R, Heling K-S, López AS, Thiel G, Karl K. The thymic-thoracic ratio in fetal heart defects: a simple way to identify fetuses at high risk for microdeletion 22q11. Ultrasound Obstet Gynecol. 2011;37(4):397-403. https://doi.org/10.1002/uog.8952
9. Li L, Bahtiyar MO, Buhimschi CS, Zou L, Zhou Q-C, Copel JA. Assessment of the fetal thymus by two- and three-dimensional ultrasound during normal human gestation and in fetuses with congenital heart defects. Ultrasound Obstet Gynecol. 2011;37(4):404-9. https://doi.org/10.1002/uog.8853
10. De Leon-Luis J, Ruiz Y, Gamez F, Pintado P, Oyelese Y, Pereda A, et al. Comparison of measurements of the transverse diameter and perimeter of the fetal thymus obtained by magnetic resonance and ultrasound imaging. J Magn Reson Imaging. 2011;33(5):1100-5. https://doi.org/10.1002/jmri.22547
11. Tonni G, Rosignoli L, Cariati E, Martins WP, Miyague AH, Bruns RF, et al. Fetal thymus: visualization rate and volume by integrating 2D- and 3D-ultrasound during 2nd trimester echocardiography. J Matern Fetal Neonatal Med. 2016;29(14):2223-8. https://doi.org/10.3109/14767058.2015.1081892
12. Nau TG, de Murcia KO, Möllers M, Braun J, Abhari RE, Steinhard J, et al. Foetal thymus size in pregnancies after assisted reproductive technologies. Arch Gynecol Obstet. 2018;298(2):329-36. https://doi.org/10.1007/s00404-018-4795-4
13. Monteiro M, Aires T, Pimentel K, Pedrosa K, Lima S, Aquino M, et al. Ultrasonographic evaluation of the fetal thymic-thoracic ratio and its association with conotruncal heart defects. J Gynecol Obstet Hum Reprod. 2022;51(2):102281. https://doi.org/10.1016/j.jogoh.2021.102281
14. Dörnemann R, Koch R, Möllmann U, Falkenberg MK, Möllers M, Klockenbusch W, et al. Fetal thymus size in pregnant women with diabetic diseases. J Perinat Med. 2017;45(5):595-601. https://doi.org/10.1515/jpm-2016-0400
15. Ghalandarpoor-Attar SN, Borna S, Ghalandarpoor-Attar SM, Hantoushzadeh S, Khezerdoost S, Ghotbizadeh F. Measuring fetal thymus size: a new method for diabetes screening in pregnancy. J Matern Fetal Neonatal Med. 2020;33(7):1157-61. https://doi.org/10.1080/14767058.2018.1517309
16. Karaşin SS, Akselim B, Tosun Ö, Karaşin ZT. Decreased fetal thymus size at 24 weeks gestation by ultrasound measurement in gestational diabetes mellitus fetal thymus examination for diabetes. J Obstet Gynaecol Res. 2022;48(6):1348-54. https://doi.org/10.1111/jog.15229
17. Tsakiridis I, Giouleka S, Mamopoulos A, Kourtis A, Athanasiadis A, Filopoulou D, et al. Diagnosis and management of gestational diabetes mellitus: an overview of national and international guidelines. Obstet Gynecol Surv. 2021;76(6):367-81. https://doi.org/10.1097/OGX.0000000000000899
18. Musilova I, Hornychova H, Kostal M, Jacobsson B, Kacerovsky M. Ultrasound measurement of the transverse diameter of the fetal thymus in pregnancies complicated by the preterm prelabor rupture of membranes. J Clin Ultrasound. 2013;41(5):283-9. https://doi.org/10.1002/jcu.22027
19. Ekin A, Gezer C, Taner CE, Solmaz U, Gezer NS, Ozeren M. Prognostic value of fetal thymus size in intrauterine growth restriction. J Ultrasound Med. 2016;35(3):511-7. https://doi.org/10.7863/ultra.15.05039
20. Paolino M, Koglgruber R, Cronin SJF, Uribesalgo I, Rauscher E, Harreiter J, et al. RANK links thymic regulatory T cells to fetal loss and gestational diabetes in pregnancy. Nature. 2021;589(7842):442-7. https://doi.org/10.1038/s41586-020-03071-0

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20221093

Evaluation of functionality and socioeconomic status of patients with chronic pain

Michelle dos Santos Severino Costa[1*], Renato Santiago Gomez[1], Gustavo Rodrigues Costa Lages[2], Ariel de Freitas Quintão Américo[2], Joao Marcelo Guimarães de Abreu[3], Fernanda Ribeiro Faria[3], Letícia Maia Azevedo[3]

SUMMARY

OBJECTIVE: This study aimed to evaluate the influence of chronic pain on functionality and its consequences on work and patient income.

METHODS: A total of 103 patients from the Multidisciplinary Pain Center of the Clinics Hospital of Universidade Federal de Minas Gerais were interviewed between January 2020 and June 2021, applying questionnaires on mobile devices. Socioeconomic data, multidimensional characterization of pain, and instruments for assessing pain functionality and intensity were analyzed. Pain intensity was categorized as mild, moderate, or intense for comparative analysis. Ordinal logistic regression was used to identify risk factors and variables that jointly influence the outcome of pain intensity.

RESULTS: The patients had a median age of 55 years, were predominantly female, married or in a stable relationship, white race, and completed high school. The median family income was R$2,200. Most patients were retired due to disability and pain-related causes. Functionality analysis showed severe disability directly associated with pain intensity. The financial impacts observed were correlated with the pain intensity of the patients. Age was a risk factor for pain intensity, while sex, family income, and duration of pain served as protective factors.

CONCLUSION: Chronic pain was associated with severe disability, decreased productivity, and exit from the labor market, with a negative impact on financial condition. Age, sex, family income, and duration of pain were directly associated with pain intensity.

KEYWORDS: Chronic pain. Income. Disability evaluation. Retirement. Sick leave.

INTRODUCTION

By definition, according to the International Association for the Study of Pain, pain is "an unpleasant sensory and emotional experience associated, or similar to that associated, with a real or potential tissue injury"[1]. It is recognized as chronic when it lasts or recurs for a period longer than 3 months, causing impairment in functionality, social and psychological well-being, and the financial life of patients.

Global data indicate that the prevalence of chronic pain is, on average, 28%[2]. In Brazil, there is great regional variability in this rate: in Londrina, the prevalence was 51.44%; in Salvador, this rate was 41.4%[3]; in São Paulo, it was 28.7%[4]. Despite these findings, less is known about the epidemiological aspects of patients with chronic pain in Brazil.

According to the World Health Organization (WHO), the definition of the term disability refers to the scope of the various manifestations of a disease, resulting from the interaction between the organic or structural dysfunction presented by the individual, the limitation of their activities, and the restriction in social participation, determining impairments in the functions of the body and difficulties in performing the tasks of daily living. When work becomes a burden for patients, there is greater use of the social benefits of sick leave and early retirement[5], especially in developing countries, such as Brazil.

Socioeconomic status is a determinant of health that is directly associated with the experience of pain[6,7]. Among the indicators of this status that showed a relationship with chronic pain are education, employment status, financial difficulties, and income[7-9]. Given the complexity of pain, studies capable of evaluating its impact are important for understanding the epidemiological scenario of pain and allowing a broad view of the current scenario, providing support for the planning and direction of strategic preventive actions of health services. Therefore, the objective of this study was to examine the relationship between chronic pain and functionality and to evaluate the labor situation, income, and financial changes resulting from pain.

[1]Universidade Federal de Minas Gerais, Department of Sciences Applied to Surgery – Belo Horizonte (MG), Brazil.
[2]Universidade Federal de Minas Gerais, Hospital das Clínicas – Belo Horizonte (MG), Brazil.
[3]Faculdade de Minas – Belo Horizonte (MG), Brazil.
*Corresponding author: santosseverinomichelle@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on August 19, 2022. Accepted on October 26, 2022.

METHODS

This is an observational, cross-sectional, noncontrolled, and descriptive study conducted at the Pain Center of the Clinics Hospital of the Universidade Federal de Minas Gerais. The study followed the ethical standards of Resolution no. 196/96 of the National Health Council and was approved by the Research Ethics Committee (no. 21291119.0.0000.5149). To participate in this study, participants signed an informed consent form.

Data were collected during an interview from 2020 to 2021. Instruments were used to evaluate pain intensity (*visual numerical scale* – VNS) and functionality (*The Pain Disability Questionnaire* – PDQ). For the categorization of pain intensity (VNS), the patients who answered 0, 1, 2, or 3 were classified as mild pain; those who answered 4, 5, 6, or 7 were classified as moderate pain; and those who answered 8, 9, or 10 were classified as severe pain. The sample was defined by convenience and totaled 103 patients.

A total of 103 patients were interviewed. Patients of both sexes, older than 18 years, and with cognitive deficits were excluded from the study.

For the statistical analysis, an exploratory analysis was initially performed using the *Shapiro-Wilk* test. The comparative analyses were performed using the *Kruskal-Wallis* test, followed by the Dunn post-test. The correlation analyses were performed using the *Spearman's* test. Variables with a level of statistical significance ($p \leq 0.05$) remained in the final multivariate logistic model. The strength of the association was determined by the *odds ratio* (OR), with a 95% confidence interval (CI). The likelihood ratio test was used to define and fit the final model.

Statistical analyses were performed using *GraphPad Prism*® software (GraphPad Software, version 8.0, La Jolla, CA, USA, www.graphpad.com) for Windows and *Stata*® software (version 14.0, Stata Corp., College Station, TX, USA).

RESULTS

The sociodemographic characterization is evaluated in Table 1.

Regarding the work situation, most patients (34.95%) were retired due to disability. In 63.89% of these patients, disability was related to pain. The second most cited labor situation by patients was sick leave (22.33%), followed by unemployed (13.59%). Together, these three categories accounted for 70.87% of the sample. There was no difference in relation to the labor situation when the different categories of pain were considered ($p>0.05$).

In the general population (n=103), the median pain time was 60 months. Considering the intensity of pain, patients in the intense pain group had a median time of pain higher than that presented by the patients in the moderate pain group. In the general population, most patients (29.13%) reported having this pain for 10 years or more. In patients with severe pain, this condition was observed in 41.30% of the sample. According to the EVN pain scale, the median score obtained by the general population was six. Of the total number of patients analyzed, 44.66% (n=46) reported severe pain. There were no significant differences between the groups in this study regarding frequency, location, and cause of pain ($p>0.05$).

The classification of the functionality of the patients, according to the PDQ instrument, indicated a severe degree of disability in the general study population, with a mean total score of 96 points. In the group with moderate pain, the mean total score on the PDQ was 87 points (severe disability), and in patients with mild pain, it was 50 points (moderate disability). By stratifying patients according to pain intensity, it was possible to observe that the group of patients with mild pain showed better results in all items of this instrument ($p<0.05$) (Table 2).

Most participants (74.76%) reported that they suffered some financial impact due to pain. Regarding the type of financial difficulty, the patients reported increased spending on health (37.40%), retirement or sick leave (35.77%), unemployment (13.01%), and decreased income (13.82%). Again, there was no significant difference between the groups categorized by pain intensity ($p>0.05$). According to the results obtained, patients in the mild pain group showed a moderate negative correlation between the presence of financial difficulties and the location of pain ($p=0.042$ and $r=-0.592$). In the intense pain group, the financial changes were directly correlated with the cause of pain ($p=0.028$ and $r=0.323$). The other variables used to characterize the pain were not significantly correlated with the presence of financial changes ($p>0.05$).

According to the final result of multivariate analysis, the variable age (age groups 18–40 years and 61–80 years) was 2.5 times more likely to be associated with pain intensity than patients aged 41–60 years. Conversely, males were 0.3 times less likely to have high levels of pain than females. Patients with family income above two minimum wages were 0.5 times less likely to have intense levels of pain compared to patients receiving up to one minimum wage. Time was also associated with pain intensity: patients with shorter pain duration were 0.3 times less likely to have intense levels of pain compared to patients with long periods of pain (Table 3).

DISCUSSION

Pain is considered a serious public health problem worldwide[10]. Considering that prolonged pain compromises the health

Table 1. Sociodemographic characteristics of the study population, considering the general sample and pain intensity (mild, moderate, and severe).

Variable	Overall (n=103)		Mild pain (n=12)		Moderate pain (n=45)		Severe pain (n=46)		p-value
	n	%	n	%	n	%	n	%	
Age (years)									
Median (P25–P75)	55 (48–63)		56 (39.5-61)		56 (51–64)		54 (47–61)		0.224[KW]
Mín–Max	22–79		22–67		35–79		26–77		
18–30	4	3.88	2	16.67	-	-	2	4.35	0.064[KW]
31–40	5	4.85	1	8.33	2	4.44	2	4.35	
41–50	24	23.30	2	16.67	8	17.78	14	30.43	
51–60	35	33.98	4	33.33	15	33.33	16	34.78	
61–70	25	24.27	2	16.67	14	31.11	9	19.57	
71–80	10	9.71	1	8.33	6	13.33	3	6.52	
Sex (n=103)									
Female	75	72.82	8	66.67	31	68.89	36	78.26	0.534[KW]
Male	28	27.18	4	33.33	14	31.11	10	21.74	
Marital status (n=103)									
Married or stable relationship	59	57.28	4	33.33	32	71.11	23	50.00	**0.041[a KW]**
Single	24	23.30	5	41.67	6	13.33	13	28.26	
Separate or divorced	13	12.62	2	16.67	4	8.89	7	15.22	
Widowed	7	6.80	1	8.33	3	6.67	3	6.52	
Race (n=103)									
White	41	39.81	2	16.66	17	37.78	22	47.83	0.411 [KW]
Brown	38	36.89	5	41.67	17	37.78	16	34.78	
Black	23	22.33	5	41.67	10	22.22	8	17.39	
Asian	1	0.97	-	-	1	2.22	-	-	
Schooling (n=103)									
Illiterate	3	2.91	-	-	2	4.44	1	2.17	0.958[KW]
Complete elementary education	21	20.39	3	25.00	10	22.22	8	17.39	
Incomplete elementary education	29	28.16	3	25.00	10	22.22	16	34.78	
Complete high school	35	33.98	5	41.67	17	37.78	13	28.26	
Incomplete high school	5	4.85	-	-	3	6.67	2	4.35	
Technical training	3	2.91	-	-	1	2.22	2	4.35	
Complete Higher Education	6	5.83	1	8.33	2	4.44	3	6.52	
Incomplete Higher Education	1	0.97	-	-	-	-	1	2.17	
Income (R$)									
Median (P25–P75)	R$ 2.200 (R$ 1.100–2.200)		R$ 1.650 (1.100–2.200)		R$ 2.200 (1.100–3.300)		R$ 1.100 (1.100–2.200)		0.065[KW]
Mín–Max	R$ 275–16.500		R$ 275–4.400		R$ 275–16.500		R$ 275–5.500		
Up to 1 minimum wage	46	44.66	6	50.00	16	35.56	24	52.17	0.196[KW]
1–2 minimum wages	32	31.07	4	33.34	13	28.89	15	32.61	
2–3 minimum wages	17	16.50	1	8. .34	11	24.44	5	10.87	
>4 minimum wages	8	7.77	1	8.34	5	11.11	2	4.35	

[a]Significant p-values. Differences were observed between mild and moderate pain. [KW]Kruskal-Wallis test.

condition and functional capacity, the present study evaluated this relationship, as well as the work situation, income, and financial changes resulting from the pain.

The analysis of sociodemographic characteristics revealed that most participants had a profile of severe or moderate pain, with a median age of 55 years, and were predominantly female. Most patients were white and had completed high school. Previous studies described that populations affected by chronic pain had characteristics similar to those observed in the present study[11].

The number of female and male patients was not homogeneous in the present study, precluding a direct correlation analysis between the determining factors for pain and sex. However, the results obtained in the multivariate logistic regression analysis identified the male sex as a protective factor for severe pain compared to the female sex.

The results obtained in the multivariate analysis also indicated that the age groups 18–40 and 61–80 years were more likely to be associated with pain intensity. This result can be explained by the fact that in older patients, chronic pain seems to be associated with diseases and conditions typical of older age, while in younger patients, the demands at work could determine more intense pain. Previous studies are inconclusive on the influence of age on pain intensity[12].

Among the work situations observed in the present study, retirement due to disability, sick leave, and unemployment were the classes most presented by the studied sample. The significant number of patients receiving some type of social benefit presented in this study demonstrates the direct implications of pain for the economy. The unemployment rates presented in this study are higher than the unemployment rates of the Brazilian population according to the Continuous National Household Sample Survey in 2019[13]. This relationship between chronic pain and unemployment is complex. There may also be underemployment, reduced working hours, loss of productivity, and frequent changes in employment. In women, unemployment raises concerns about family stability, exacerbating pain while men show the opposite data[9]. It is important to note that, according to the Institute of Applied Economic Research (IPEA), in 2019, women commanded 45% of Brazilian households, being the main contributor to family income. In this study, the unemployed and retired population by contribution or age had a lower association with pain intensity. This could be explained by the demands of the professional function, with work activity leading to more severe pain. In this context, retirement or lack of employment would represent protective factors, as they ensure lower physical demand.

In the multivariate analysis, income above two minimum wages served as a protective factor for severe pain. This finding indicates that the higher the family income is, the less intense the patient's pain tends to be. Several authors state that chronic pain is more common in less privileged segments of the population and that low income is consistently related to increased pain[9,14]. Andersson et al. (1993) correlated chronic pain with low income; however, according to Gerdle et al. (2004), annual income should be seen as a consequence and not only as a predictor of pain[6]. Although the educational level in the present study was not significant, this parameter is often related to chronic pain.

Socioeconomic indicators assess the status of the patient to obtain resources but fail to evaluate the financial needs, responsibilities, and obligations. The presence of economic difficulties

Table 3. Ordinal logistic regression (factors associated with pain intensity–visual numerical scale): final model.

Ordinal logistics regression (variable answer=VNS) final model**			
Explanatory variables	**Odds ratio**	**95%CI**	**p-value**
Age (categories)	2.5	1.0–6.2	**0.053***
Sex	0.3	0.1–0.9	**0.033***
Race/color	0.6	0.3–1.1	0.097
Family income	0.5	0.2–0.8	**0.010***
Financial changes	1.4	0.9–2.3	0.153
Work status	0.6	0.3–1.0	0.069
Time of pain (categories)	0.3	0.1–1.0	**0.046***
PDQ_total index	1.0	1.0–1.1	**0.005***

*Significant p-values ($p<0.05$). **Likelihood log=-60.177/Observation number=90/Pseudo-R^2=0.327.

Table 2. Characterization of disability, according to Pain Disability Questionnaire instruments.

Instrument	Overall population (n=103)	Mild pain (n=12)	Moderate pain (n=45)	Severe pain (n=46)	p-value
	Median	Median	Median	Median	
PDQ					
Psychosocial component	40.0[c]	23.0[a,b,c]	36.0[b]	40.0[a]	0.000*
Functional status	58.0[c]	27.0[a,b,c]	49.0[b]	58.0[a]	<0.0001*
Total index	96.0[c]	50.0[a,b,c]	87.0[b]	96[a]	<0.0001*

*Significant p-values. [a]Significant differences were observed between the mild pain and severe pain groups. [b]Significant differences were observed between the mild pain and moderate pain groups. [c]Significant differences were observed between the mild and general pain groups.

is closely linked to socioeconomic status, representing a personal perception, and an important determinant of disability and psychological distress. In this study, most participants reported the occurrence of some negative impact on their financial life. Low income, therefore, behaves as a risk factor for the development of pain and, at the same time, can be negatively influenced by pain, which is a bidirectional variable[6,9,14].

When analyzing income and the variables that characterize pain, in patients with mild pain, the financial changes were negatively correlated with the location of pain, suggesting that the greatest financial impacts occurred in patients with a particular location of pain. This fact can be explained by a relationship between the location of pain and the demands of professional practice. Thus, even though pain is considered mild, its occupational function could lead to significant impacts. In the severe pain group, the financial changes were directly correlated with the cause of pain, suggesting that some etiologies have a greater impact on the patients' functionality for work. The analysis of the duration of pain, in turn, revealed a median period of 60 months, and this result was also observed in previous studies[15]. In the multivariate analysis, pain time served as a protective variable for pain intensity because patients with shorter pain time had lower chances of severe pain. This finding corroborates the comparative analysis, where the intense pain group exhibited significantly longer pain time, suggesting an increase in intensity as the duration of pain increases.

The results showed a severe change in functionality. According to the data obtained in the comparative analysis between the groups, the degree of disability was related to the intensity of pain, suggesting that the higher the intensity, the greater the interference in the functionality of patients. Dorner et al. (2011) reported that the level of disability is a good way to measure the severity of pain.

CONCLUSION

Chronic pain was associated with changes in the functionality of patients, characterized by a severe degree of physical and psychosocial disability. A large portion of the patients analyzed was outside the labor market and received social security benefits due to early retirement or sick leave. Chronic pain had a strong negative impact on the financial condition of patients, and among the variables analyzed, age, sex, family income, and duration of pain were directly associated with pain intensity.

Limitations

The study is cross sectional, and the sample was limited to a single study center, defined by convenience, and was not homogeneous in relation to gender.

AUTHORS' CONTRIBUTIONS

MSSC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Project administration, Writing – original draft. **GRCL:** Investigation, Formal Analysis, Methodology, Visualization. **AFQA:** Investigation, Formal Analysis, Methodology, Visualization. **JMGA:** Data curation, Formal Analysis, Validation, Visualization. **FRF:** Data curation, Formal Analysis, Validation, Visualization. **LMA:** Data curation, Formal Analysis, Validation, Visualization. **RSG:** Methodology, Supervision, Validation, Writing – review & editing.

REFERENCES

1. DeSantana JM, Perissinotti DMN, Oliveira Junior JO, Correia LMF, Oliveira CM, Fonseca PRB. Definition of pain revised after four decades. Braz J Pain. 2020;3(3):197-8. https://doi.org/10.5935/2595-0118.20200191
2. Stauder J. Unemployment, unemployment duration, and health: selection or causation? Eur J Health Econ. 2019;20(1):59-73. https://doi.org/10.1007/s10198-018-0982-2
3. Sá KN, Baptista AF, Matos MA, Lessa Í. Chronic pain and gender in Salvador population, Brazil. Pain. 2008;139(3):498-506. https://doi.org/10.1016/j.pain.2008.06.008
4. Cabral DMC, Bracher ESB, Depintor JDP, Eluf-Neto J. Chronic pain prevalence and associated factors in a segment of the population of São Paulo City. J Pain. 2014;15(11):1081-91. https://doi.org/10.1016/j.jpain.2014.07.001
5. Cáceres-Matos R, Gil-García E, Barrientos-Trigo S, Porcel-Gálvez AM, Cabrera-León A. Consequences of chronic non-cancer pain in adulthood. Scoping review. Rev Saude Publica. 2020;54:1-13. https://doi.org/10.11606/s1518-8787.2020054001675
6. Gerdle B, Björk J, Henriksson C, Bengtsson A. Prevalence of current and chronic pain and their influences upon work and healthcare-seeking: a population study. J Rheumatol. 2004;31(7):1399-406. PMID: 15229963
7. Dorner TE, Muckenhuber J, Stronegger WJ, Ràsky É, Gustorff B, Freidl W. The impact of socio-economic status on pain and the perception of disability due to pain. Eur J Pain. 2011;15(1):103-9. https://doi.org/10.1016/j.ejpain.2010.05.013
8. Malhotra C, Harding R, Teo I, Ozdemir S, Koh GCH, Neo P, et al. Financial difficulties are associated with greater total pain and suffering among patients with advanced cancer: results from the COMPASS study. Support Care Cancer. 2020;28(8):3781-9. https://doi.org/10.1007/s00520-019-05208-y

9. Evans MC, Bazargan M, Cobb S, Assari S. Mental and physical health correlates of financial difficulties among African-American older adults in low-income areas of Los Angeles. Front Public Health. 2020;8:21.https://doi.org/10.3389/fpubh.2020.00021

10. Rice ASC, Smith BH, Blyth FM. Pain and the global burden of disease. Pain. 2016;157(4):791-6.https://doi.org/10.1097/j.pain.0000000000000454

11. Elliott AM, Smith BH, Penny KI, Smith WC, Chambers WA. Epidemiology of chronic pain. Handbook of Pain Syndromes. 2020;354:12-31.

12. Zajacova A, Rogers RG, Grodsky E, Grol-Prokopczyk H. The relationship between education and pain among adults aged 30–49 in the United States. J Pain. 2020;21(11-12):1270-80. https://doi.org/10.1016/j.jpain.2020.03.005

13. Instituto Brasileiro de Geografia e Estatística (IBGE). Pesquisa Nacional por Amostra de Domicílios: Nota Técnica. 2020. p. 132.

14. Fliesser M, Witt Huberts J, Wippert P-M. Education, job position, income or multidimensional indices? Associations between different socioeconomic status indicators and chronic low back pain in a German sample: a longitudinal field study. BMJ Open. 2018;8(4):1-7. http://dx.doi.org/10.1136/bmjopen-2017-020207

15. Sanson N, Hach S, Moran R, Mason J. Behavioural activation and inhibition systems in relation to pain intensity and duration in a sample of people experiencing chronic musculoskeletal pain. Musculoskelet Sci Pract. 2020;47:102129. https://doi.org/10.1016/j.msksp.2020.102129

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20221116

Predicting mortality in neonates with gastroschisis in a Southeastern state of Brazil

Virginia Maria Muniz[1,2], Antônio Lima Netto[2], Katia Souza Carvalho[3], Claudia Saleme do Valle[4], Cleodice Alves Martins[5], Luciane Bresciani Salaroli[1,5*], Eliana Zandonade[1]

SUMMARY

OBJECTIVE: This study aimed to verify risk factors associated with gastroschisis mortality in three neonatal intensive care units located in the state of Espírito Santo, Brazil.

METHODS: A retrospective cohort study of neonates with gastroschisis was performed between 2000 and 2018. Prenatal, perinatal, and postsurgical variables of survival or nonsurvival groups were compared using chi-square statistical test, t-test, Mann-Whitney U test, and logistic regression. Tests with $p<0.05$ were considered statistically determined.

RESULTS: A total of 142 newborns were investigated. Mean maternal age, gestational age, and birth weight were lower in the group of nonsurvival ($p<0.05$). Poor clinical conditions during admission, complex gastroschisis, closure with silo placement, the use of blood products, surgical complications, and short bowel syndrome were more frequent in the nonsurvival group ($p<0.05$). Complex gastroschisis [adjusted odds ratio (OR) 3.74, 95% confidence interval (95%CI) 1.274–11.019] and short bowel syndrome (adjusted OR 7.55, 95%CI 2.177–26.225) increased the risk of death. Higher birth weight inversely reduced the risk for mortality (adjusted OR 0.99, 95%CI 0.997–1.000).

CONCLUSION: Complex gastroschisis and short bowel syndrome increased the risk of death, with greater birth weight being inversely correlated with the risk of mortality. The findings of this research can contribute to the formulation of protocols to improve the quality and safety of care in order to reduce neonatal mortality associated with gastroschisis.

KEYWORDS: Infant, newborn. Gastroschisis. Infant mortality. Congenital abnormalities. Risk factors.

INTRODUCTION

The advances in neonatal intensive care, improvement of parenteral nutrition solutions, and evolution of pediatric surgical strategies have contributed to reduce the mortality of gastroschisis rates to less than 10% in high-income countries (HICs). However, postsurgical complications and length of stay remain high. In these countries, efforts to improve outcomes in gastroschisis are centered on reducing morbidity and the burden on hospitals and healthcare systems[1]. Contrariwise, in low-middle income countries (LMICs), morbidity and mortality rates related to this congenital anomaly are still unacceptably high, conceivably due to limited financial resources and the fragility of the healthcare systems[2,3]. Among the risk factors associated with mortality in newborns with gastroschisis are low birth weight[4], prematurity[4,5], complex gastroschisis (CG)[5], sepsis[4,6], no antenatal diagnosis, outborn babies, and poor clinical conditions at admission[7].

Assuredly, the proper identification of risk factors for gastroschisis mortality may have a pivotal role in the definition of strategies by public health authorities and hospital managers to improve the survival rates of neonates with this birth defect. The aim of this study was to identify risk factors associated with gastroschisis mortality in three neonatal intensive care units located in the Metropolitan Region of Great Vitoria of Espírito Santo (GVMR-ES), Brazil. We present the following article in accordance with the STROBE reporting checklist[8].

METHODS

Study population

A retrospective cohort study was conducted with all newborns admitted to three neonatal intensive care units (NICU) at

[1]Universidade Federal do Espírito Santo, Postgraduate Program in Collective Health – Vitória (ES), Brazil.
[2]Hospital Estadual Infantil Nossa Senhora da Glória – Vitória (ES), Brazil.
[3]Hospital Estadual Infantil e Maternidade Alzir Bernardino Alves – Vila Velha (ES), Brazil.
[4]Hospital Estadual Dr. Jayme Santos Neves – Serra (ES), Brazil.
[5]Universidade Federal do Espírito Santo, Postgraduate Program in Nutrition and Health – Vitória (ES), Brazil.
*Corresponding author: lucianebresciani@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 05, 2022. Accepted on November 14, 2022.

GVMR-ES between January 2000 and December 2018, with the diagnosis of isolated gastroschisis, confirmed by a pediatric surgeon. The data collection period varied according to the availability of medical records from each hospital: NICU A from January 1, 2000, to December 31, 2018; NICU B from February 23, 2013, to December 31, 2018; and NICU C from November 11, 2010, to December 31, 2018. Exclusion criteria were genetic syndromes or other major congenital malformations and newborns who were transferred to other hospitals.

Patients were treated by healthcare teams from each study site. NICU A is located in a pediatric hospital and only admits outborn babies. NICU B is attached to a maternity hospital and only admits inborn babies. NICU C is also attached to a maternity hospital; however, it admits both inborn and outborn babies.

Patients were divided into two groups: survival and nonsurvival. To determine the possible association of probable causes of mortality after birth, the study included data from prenatal, perinatal, and postsurgical care until the outcome of discharge or death.

Study variables

Variables of prenatal and perinatal periods were maternal age, prenatal diagnosis by ultrasound, number of prenatal consultations, route of delivery, 1-min APGAR score and 5-min APGAR score[9] bulletin 1 and 2, gestational age by somatic Capurro method, birth weight, gender, birthplace (inborn or outborn), time between birth and first repair surgery, and clinical conditions at admission indicated by clinical pediatrician's notes as poor conditions (dysthermia, hypoactivity, hydroelectrolytic or metabolic disorders, and infection) or good conditions, since none of the three services have used a standardized neonatal death prediction score. Variables of postoperative care were the type of gastroschisis according to the surgeon's report by complex (atresias, strictures, volvulus, necrosis, and large gastroschisis) or simple[10], time on mechanical ventilation (MV), total parenteral nutrition time (TPN), use of vasoactive substances, use of antimicrobials, type of venous access (peripherally inserted central catheter [PICC], central intravenous access catheter by puncture or dissection, and peripheral intravenous access), use of blood products, clinical neonatal sepsis diagnosed by attending physician or confirmed by blood culture, surgical complications (surgical reinterventions, compartment syndrome, and necrotizing enterocolitis [NEC] after closure), short bowel syndrome (SBS) defined as the need for TPN greater than 60 days after intestinal resection or intestinal length less than 25% of expected for age[11], and length of stay (LOS). Furthermore, only variables with complete data in the medical record were included in this study.

Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 22.0 for Windows. Data were presented with absolute and relative frequencies for categorical variables. The chi-square test was used for categorical variables. For quantitative variables, normality tests were performed. In the case of normality, Student's t-test was performed, and results were presented with means and standard deviations. In the case of non-normality, Mann-Whitney nonparametric test was utilized, and results were presented with median and interquartile range (IQR). The level of significance adopted was 5% ($p<0.05$).

Some variables were included in the logistic regression model when the p-value is less than 0.20 in bivariate analysis. For logistic regression, the entry of variables in blocks by the Enter method was used. Crude odds ratios (OR) and adjusted OR (aOR) were calculated with 95% confidence intervals (95%CI). The level of significance adopted was 5% ($p<0.05$).

Ethical and legal aspects of research

This project was approved by Research Ethics Committee – Opinion nº 2671249/CEP – CIAS/Unimed-Vitória (CAAE 87878918.1.0000.5061). Terms of adherence to this research by hospitals were obtained through the Secretary of State for Health – ES.

RESULTS

The data from 144 newborns with gastroschisis were assessed, and the data from 2 newborns were excluded since they were transferred to another NICU not a participant in the current study, after being operated on.

NICU admissions by birthplace were: NICU A–74 (100% outborn), NICU B–29 (100% inborn), and NICU C–39 (87% inborn/13% outborn). The total mortality rate was 33% (NICU A: 21/28%, NICU B: 10/35%, and NICU C: 17/43%, p=0.266). Sepsis was the most common cause of death (58%), followed by NEC (19%) and compartment syndrome (8%). Moreover, complications that led to SBS were multiple surgeries with bowel resection (13/42%), NEC (9/29%), dependency on parenteral nutrition over 60 days after surgery to repair intestinal stenosis or atresias (8/26%), and compartment syndrome (1/3%). The total rate of CG was 44% (63/142). The SBS rate in patients with CG was 74% (23/31, p=0.000).

Table 1 shows the bivariate analysis regarding the characteristics of the prenatal, perinatal, and postsurgical cares for neonates, according to groups of survival or nonsurvival.

Table 1. Bivariate analysis of sample characterization variables referring to prenatal, perinatal, and postsurgical care of patients with gastroschisis admitted to three neonatal care units at the Greater Vitória Metropolitan Region between 2000 and 2018, according to groups of survival or nonsurvival.

Variables	Category	Survival N=94	Nonsurvival N=48	Total N=142	P-value
		N (%)	N (%)	Total	
Admission hospital	NICU A	53 (56.4)	21 (43.8)	74 (52.1)	0.266†
	NICU B	19 (20.2)	10 (20.8)	29 (20.4)	
	NICU C	22 (23.4)	17 (35.4)	39 (27.5)	
Maternal age (years) mean/SD		20 (±3.7)	18.5 (±4.0)	19.5 (±3.8)	0.040‡*
Antenatal consultations	≥6	45 (47.9)	22 (45.8)	67 (47.2)	0.818†
Antenatal diagnosis	Yes	50 (53.2)	32 (66.7)	82 (57.7)	0.124†
Route of birth	Cesarean section	57 (60.6)	33 (68.7)	90 (63.4)	0.343†
Gender	Male	47 (50)	31 (64.6)	78 (54.9)	0.099†
Gestational age (weeks) mean±SD		37.2 (±1.8)	35.8 (±2.4)	36.7 (±2.1)	0.001‡**
Birth weight (g) mean±SD		2.504 (±457)	2.188 (±467)	2.397 (±483)	0.000‡**
Apgar 1 mean±SD		7.4 (±1.4)	6.9 (±1.8)	7.2 (±1.5)	0.057‡
Apgar 2 mean±SD		8.5 (±0.9)	8.5 (±1.1)	8.5 (±0.9)	0.833‡
Birth-surgery time (h) median (IQR		3.0 (1–7)	2 (1–7)	3 (1–7)	0.455§
Birthplace	Inborn	39 (41.5)	24 (50)	63 (44.4)	0.334†
Clinical conditions	Poor	20 (21.3)	21 (43.8)	41 (28.8)	0.005 †**
Gastroschisis	Complex	28 (29.8)	35 (72.9)	63 (44.3)	0.001†**
Wall closure	Silo	17 (18.1)	23 (47.8)	40 (28.1)	0.001†**
Venous access	PICC	48 (51)	19 (39.6)	67 (47.2)	0.378†
Vasoactive substances	Yes	60 (63.8)	38 (79.2)	98 (69)	0.062†
Antibiotics	≥2 courses	71 (75.5)	36 (75)	107 (75.4)	0.945†
Time on MV median (IQR)		6 (4–11)	8 (3–15)	6 (4–13)	0.456§
Time on TPN median (IQR)		22 (16–30)	16 (0.5–35.5)	21 (13–32)	0.007§**
Sepsis	Yes	61 (64.9)	37 (77.1)	98 (69)	0.137†
Blood products	Yes	66 (70.2)	42 (87.5)	108 (76.1)	0.022†*
Surgical complications	Yes	18 (19.1)	25 (52.1)	43 (30.2)	0.001†**
Short bowel syndrome	Yes	7 (7.4)	24 (50)	31 (21.8)	0.001†**
LOS median (IQR)		28.5 (19–44)	24 (2–52)	28 (18–44)	0.002§**

†Chi-square test; ‡Student's t-test; §Mann-Whitney nonparametric test; *P-value <0.05; **P-value <0.01; SD: standard deviation; IQR: interquartile range; GS: gastroschisis; MV: mechanical ventilation, TPN: total parenteral nutrition; PICC: peripherally inserted central catheter; LOS: length of stay; poor clinical conditions: if the patient had one or more of following signs and symptoms: dysthermia, hypoactivity, hydroelectrolytic or metabolic disorders, and infection; good conditions: included cases that did not meet criteria for poor conditions; complex gastroschisis: according to surgeon's report by gastrointestinal tract or abdominal wall complications: atresias, strictures, volvulus, necrosis, and large gastroschisis.

Mean maternal age, gestational age at birth, and birth weight were lower in the nonsurviving group (p<0.05). Percentages of newborns with poor clinical conditions at the time of admission, CG, staged closure of abdominal wall with the placement of silo, shorter time of TPN, use of blood products, and surgical complications were higher in the group of nonsurvival (p<0.05). Among postsurgical complications, reinterventions were the most frequent ones (survival 13.8%/ nonsurvival 22.9%). SBS was more frequent in neonates who died (p<0.05). There were no statistical differences between the two groups, regarding NICU (A, B, and C) admission, number of prenatal consultations, diagnosis of gastroschisis during pregnancy, route of birth, gender, Apgar 1 and 2, birthplace, birth-surgery time, type of venous access, use of vasoactive drugs, use of antibiotics, time on MV, and sepsis. Table 2 shows the results of logistic regression. CG (aOR 3.74, 95%CI 1.274–11.019) and SBS (aOR 7.55, 95%CI 2.177–26.225) increased the risk of death. An increase in birth weight reduced the risk for mortality (aOR 0.99, 95%CI 0.997–1.000).

DISCUSSION

Very few studies on gastroschisis have been carried out in LMICs, where the mortality rates of this birth defect are the highest. Possibly, these unfavorable figures are related to the paucity of protocols for improving the quality of care to patients with gastroschisis in those countries[12]. Implementation of such protocols has been shown to improve outcomes and reduce deaths in gastroschisis cohorts from LMICs[13].

There were no statistically significant differences among mortality rates in the three NICUs. A previous study evaluated the influence of birthplace on outcomes of this cohort and concluded that this finding may reflect the fact that, once admitted to tertiary referral centers, the outborn patients can benefit over time from high technology used in neonatal care, which would reduce the differences faced in prehospital period[14].

The mortality rate of 33% of this cohort was higher than the ones reported in other studies performed in the southeastern region of Brazil, which found a variation between 4 and 29%[15], and lower than those reported by studies carried out in the country's north and northeast regions, where mortality

Table 2. Logistic regression with some variables of prenatal, newborns, and postsurgical care of patients with gastroschisis admitted to three neonatal care units at the Greater Vitória Metropolitan Region – Espírito Santo between 2000 and 2018, according to groups of survival or nonsurvival, for prediction of mortality as a proposed theoretical model.

Variable	Category	Crude OR (95%CI)	P-value	Adjusted odds ratio (aOR)† (95%CI)	P-value
Maternal age (years)		**0.901 (0.815–0.997)**	**0.043***	0.924 (0.811–1.054)	0.240
Antenatal diagnosis	Yes No	1.760 (0.853–3.631) 1	0.126	1.197 (0.423–3.387)	0.734
Birth weight (g)		**0.998 (0.998–0.999)**	**0.001****	**0.998 (0.997–1.000)**	**0.027****
Gestational age (weeks)		**0.746 (0.624–0.891)**	**0.001****	1.023 (0.745–1.330)	0.993
Gastroschisis	Complex Simple	**6.346 (2.924–13.775)** **1**	**0.001****	**3.747 (1.274–11.019)**	**0.016***
Wall closure	Silo Primary	**4.341 (1.997–9.436)** **1**	**0.001****	2.656 (0.888–7.945)	0.080
Surgical complications	Yes No	**4.589 (2.136–9.859)** **1**	**0.001****	2.489 (0.822–7.536)	0.107
Vasoactive substances	Yes No	2.153 (0.954–4.859) 1	0.065	0.990 (0.299–3.281)	0.987
Sepsis	Yes No	1.820 (0.822–4.031) 1	0.140	0.421 (0.111–1.604)	0.205
Blood products	Yes No	**2.970 (1.134–7.778)** **1**	**0.027****	3.031 (0.578–15.875)	0.189
Short bowel syndrome	Yes No	**12.429 (4.780–32.315)** **1**	**0.001****	**7.556 (2.177–26.225)**	**0.001****
Clinical conditions	Poor Good	**2.878 (1.353–6.119)** **1**	**0.006****	2.052 (0.727–5.791)	0.174

*P<0.05; **P<0.01. OR: odds ratio; CI: confidence interval; aOR was obtained through logistic regression and adjusted by the input of block variables using the Enter method; poor clinical conditions: if the patient had one or more of the following signs and symptoms: dysthermia, hypoactivity, hydroelectrolytic or metabolic disorders, and infection; good clinical conditions (included cases that did not meet criteria for poor conditions); complex gastroschisis: according to surgeon's report by gastrointestinal tract or abdominal wall complications: atresias, strictures, volvulus, necrosis, and large gastroschisis.

rates were 51.2 and 51.6%, respectively[4,7]. In comparison to other countries, the mortality rate in our study was close to the 39% rate reported in China[7]. Although sepsis was not a risk factor associated with mortality in this cohort, it was the main cause of death. Sepsis is a common cause of mortality in studies performed in LMICs[4,7].

CG increased the risk of death in our study. Another study also reported this association[16]. However, a large population study was carried out in an HIC, and CG was not a risk factor for death. This fact was attributed to the progress of neonatal surgical techniques and the manipulation of safer parenteral feeding solutions[17].

In this cohort, SBS was a risk factor associated with mortality, and its main cause was multiple surgeries with bowel resection. A study carried out in northern Brazil reported SBS in 25% of patients with gastroschisis. Most of those patients were outborn who had severe intestinal injuries on admission due to conditions that included inadequate neonatal transport[4].

A significant number of neonates with gastroschisis are low birth weight[18]. In this study, the higher the birth weight, the lower the risk of death from gastroschisis. In Brazil, a retrospective cohort conducted in the northern region showed that patients with birth weight less than 2,500 g had 2.4 times increased risk of death[4].

Young maternal age is an important risk factor associated with gastroschisis, and its prevalence among adolescent mothers is more than 7 times higher than those aged 25 years or older[19]. In this present study, the mean age of mothers in the group of nonsurvival was statistically lower than that of survival.

The mean gestational age at birth was lower in the group of individuals with gastroschisis who died. This fact may be related to low birth weight, which was one of the risk factors associated with mortality in this study. Prematurity may complicate the postoperative period of patients with gastroschisis due to its comorbidities[20]. Two studies performed in an HIC found different results. In the first one, prematurity was associated with worse outcomes in gastroschisis, including mortality. In the second one, although prematurity is associated with greater morbidity, it was not a risk factor associated with gastroschisis mortality[21].

Patients with poor clinical conditions on admission to the NICU were more frequent in the nonsurvival group. A study also carried out in Brazil observed that poor clinical conditions on admission to NICU increased the risk of death, which was higher among outborn patients[7]. Another study carried out in LICs found an increased risk of mortality in patients with CG and hypovolemia at admission, due to poor neonatal transport conditions. Furthermore, the use of blood products was more frequent in patients who died, and this fact may be related to coagulation disorders caused by sepsis[3].

TPN time was shorter in the nonsurvivors group. This finding may be explained by the fact that these patients presented clinical instability, a condition in which the use of TPN is not allowed.

Some limitations must be considered in this study. Since it is a retrospective and hospital-based study, it is subjected to information and selection bias. Moreover, the study period was long and uneven among the studied NICU. However, there was completeness of data, and the accuracy of gastroschisis diagnosis is ensured, as it was performed by pediatric surgeons, in contrast to what was found in studies using population databases, which have flaws in filling in some variables and underreporting. Finally, future studies with prospective designs are expected to be carried out to better understand the effects of assistance on gastroschisis morbidity and mortality. To that end, a group of Brazilian surgeons, called Paedsurg Brazil, has been recently created to investigate the most prevalent congenital surgical anomalies in Brazil, including gastroschisis[22].

CONCLUSION

In this gastroschisis cohort, the CG and short bowel syndrome increased the risk of death, with greater birth weight being inversely correlated with the risk of mortality. We hope that our findings could be used as a tool for professionals who routinely assist patients with gastroschisis in the elaboration of protocols for the improvement of quality of care to reduce mortality from birth defects preventable by surgical treatment, such as gastroschisis.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Marcelo Ramos Muniz for the text review.

AUTHORS' CONTRIBUTIONS

VMM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ALN:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, vaLidation, Visualization, Writing – original draft, Writing – review & editing. **KSC:** Conceptualization, Data curation, Formal Analysis, Funding

acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CSV:** Formal Analysis, Software. **CAM:** Visualization, Writing – original draft, Writing – review & editing. **LBS:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **EZ:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

REFERENCES

1. Wissanji H, Puligandla PS. Risk stratification and outcome determinants in gastroschisis. Semin Pediatr Surg. 2018;27(5):300-03. https://doi.org/10.1053/j.sempedsurg.2018.08.007
2. Global PaedSurg Research Collaboration. Mortality from gastrointestinal congenital anomalies at 264 hospitals in 74 low-income, middle-income, and high-income countries: a multicentre, international, prospective cohort study. Lancet. 2021;398(10297):325-39. https://doi.org/10.1016/S0140-6736(21)00767-4
3. PaedSurg Africa Research Collaboration. Paediatric surgical outcomes in sub-Saharan Africa: a multicentre, international, prospective cohort study. BMJ Glob Health. 2021;6(9):e004406. https://doi.org/10.1136/ bmjgh-2020-004406
4. Bilibio JP, Beltrão AM, Vargens AC, Gama TB, Lorenzzoni PL. Gastroschisis during gestation: prognostic factors of neonatal mortality from prenatal care to postsurgery. Eur J Obstet Gynecol Reprod Biol. 2019;237:79-84. https://doi.org/10.1016/j.ejogrb.2019.04.015
5. Marshall Niles SG, Mitchell-Fearon K, Gill MI, DeSouza CJ, Fearon IC, Abel CA, et al. Mortality-related factors in gastroschisis – a Jamaican perspective. J Pediatr Surg. 2017;52(4):530-03. https://doi.org/10.1016/j.jpedsurg.2016.10.045
6. Du L, Pan WH, Cai W, Wang J, Wu YM, Shi CR. Delivery room surgery: an applicable therapeutic strategy for gastroschisis in developing countries. World J Pediatr. 2014;10(1):69-73. https://doi.org/10.1007/s12519-014-0455-3
7. Vilela PC, Ramos De Amorim MM, Falbo GH, Santos LC. Risk factors for adverse outcome of newborns with gastroschisis in a Brazilian hospital. J Pediatr Surg. 2001;36(4):559-64. https://doi.org/10.1053/jpsu.2001.22282
8. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007;4(10):e297. https://doi.org/10.1371/journal.pmed.0040297.
9. American Academy of Pediatrics, Committee on Fetus and Newborn; American College of Obstetricians and Gynecologists and Committee on Obstetric Practice. The apgar score. Pediatrics. 2006;117(4):1444-47. https://doi.org/10.1542/peds.2006-0325
10. Bergholz R, Boettcher M, Reinshagen K, Wenke K. Complex gastroschisis is a different entity to simple gastroschisis affecting morbidity and mortality—a systematic review and meta-analysis. J Pediatr Surg. 2014;49:1527-32. https://doi.org/10.1016/j.jpedsurg.2014.08.001
11. Laje P, Fraga MV, Peranteau WH, Hedrick HL, Khalek N, Gebb JS, et al. Complex gastroschisis: clinical spectrum and neonatal outcomes at a referral center. J Pediatr Surg. 2018;53(10):1904-07. https://doi.org/10.1016/j.jpedsurg.2018.03.011
12. Chandra R, Kesavan A. Current treatment paradigms in pediatric short bowel syndrome. Clin J Gastroenterol. 2018;11(2):103-12. https://doi.org/10.1007/s12328-017-0811-7
13. Wright N, Abantanga F, Amoah M, Appeadu-Mensah W, Bokhary Z, Bvulani B, et al. Developing and implementing an interventional bundle to reduce mortality from gastroschisis in low-resource settings. Wellcome Open Res. 2019;4:46. https://doi.org/10.12688/wellcomeopenres.15113.1
14. Wesonga A, Situma M, Lakhoo K. Reducing gastroschisis mortality: a quality improvement initiative at a Ugandan pediatric surgery unit. World J Surg. 2020;44(5):1395-9. https://doi.org/10.1007/s00268-020-05373-w
15. Muniz VM, Lima Netto A, Carvalho KS, Valle CSD, Salaroli LB, Zandonade E. Influence of birthplace on gastroschisis outcomes in a state in the southeastern region of Brazil. J Pediatr (Rio J). 2021;97(6):670-5. https://doi.org/10.1016/j.jped.2021.02.004
16. Osmundo Junior GS, Mohamed SHM, Nishie EN, Tannuri ACA, Gibelli MABC, Francisco RPV, et al. Association of spontaneous labor onset with neonatal outcomes in pregnancies with fetal gastroschisis: a retrospective cohort analysis. Acta Obstet Gynecol Scand. 2019;98(2):154-61. https://doi.org/10.1111/aogs.13473
17. Cárdenas-RuizVelasco JJ, Pérez-Molina JJ, Corona-Rivera JR, Flores-García BG. Intraoperative findings associated to inpatient mortality from patients with gastroschisis in western Mexico. J Surg Res. 2020;254:58-63. https://doi.org/10.1016/j.jss.2020.03.065
18. Dekonenko C, Fraser JD, Deans KJ, Fallat ME, Helmrath M, Kabre R, et al. Outcomes in gastroschisis: expectations in the postnatal period for simple vs complex gastroschisis. J Perinatol. 2021;41(7):1755-9. https://doi.org/10.1038/s41372-021-01093-8
19. O'Connell RV, Dotters-Katz SK, Kuller JA, Strauss RA. Gastroschisis: a review of management and outcomes. Obstet Gynecol Surv. 2016;71(9):537-44. https://doi.org/10.1097/OGX.0000000000000344
20. Overcash RT, DeUgarte DA, Stephenson ML, Gutkin RM, Norton ME, Parmar S, et al. Factors associated with gastroschisis outcomes. Obstet Gynecol. 2014;124(3):551-7. https://doi.org/10.1097/AOG.0000000000000425
21. Utria AF, Wong M, Faino A, Jacobson E, Javid PJ. The role of feeding advancement strategy on length of stay and hospital costs in newborns with gastroschisis. J Pediatr Surg. 2022;57(3):356-9. https://doi.org/10.1016/j.jpedsurg.2021.04.011
22. Paedsurg Brazil [Internet]. Global PaedSurg [cited on Nov. 2021, 11] Available from: http://globalpaedsurg.com/introducing-paedsurg-brazil/

ORIGINAL ARTICLE

Em marcação
XML para AOP

https://doi.org/10.1590/1806-9282.20221125

Investigation of the effectiveness of the Quick Sequential Organ Failure Assessment-Troponin scores in non- ST-elevation myocardial infarction

Başar Cander[1], Bahadır Taşlıdere[1*], Ertan Sönmez[1]

SUMMARY

OBJECTIVE: A reliable predictor is needed for non-ST-elevation myocardial infarction patients with high mortality risk. The aim of this study was to assess the effectiveness of the Global Registry of Acute Coronary Events and Quick Sequential Organ Failure Assessment-Troponin (qSOFA-T) scores on in-hospital mortality rate in non-ST-elevation myocardial infarction patients.

METHODS: This is an observational and retrospective study. Patients admitted to the emergency department with acute coronary syndrome were evaluated consecutively. A total of 914 patients with non-ST-elevation myocardial infarction who met inclusion criteria were included in the study. The Global Registry of Acute Coronary Events and qSOFA scores were calculated and investigated its contribution to prognostic accuracy by adding cardiac troponin I (cTnI) concentration to the qSOFA score. The threshold value of the investigated prognostic markers was calculated by receiver operating characteristic curve analysis.

RESULTS: We found the in-hospital mortality rate to be 3.4%. The area under the receiver operating characteristic curve for Global Registry of Acute Coronary Events and qSOFA-T is 0.840 and 0.826, respectively.

CONCLUSION: The qSOFA-T score, which can be calculated easily, quickly, and inexpensively and obtained by adding the cTnI level, had excellent discriminatory power for predicting in-hospital mortality. Difficulty in calculating the Global Registry of Acute Coronary Events score, which requires a computer, can be considered a limitation of this method. Thus, patients with a high qSOFA-T score are at an increased risk of short-term mortality.

KEYWORDS: Acute coronary syndrome. Troponin I. Mortality. Non-ST elevated myocardial infarction.

INTRODUCTION

Chest pain constitutes a significant portion of all emergency department (ED) admissions[1]. Approximately 5–20% of patients who enter the ED with chest pain (typical or atypical) are diagnosed with acute coronary syndrome (ACS)[2]. This syndrome is one of the leading causes of death[3]. Even in ACS patients with timely medical intervention, 1-year mortality is 5%, and in-hospital mortality is 7.5%[4]. Approximately 70% of all ACS present as non-STEMI (ST-elevation myocardial infarction)[5]. The international cardiac guidelines recommend that patients presenting to the ED with chest pain should be evaluated using a risk score[6]. A frequently used and high-performing tool for this purpose is the Global Registry of Acute Coronary Events (GRACE)[7]. The GRACE identifies risk factors that help independently predict in-hospital and 6-month mortality rates. The score is calculated based on clinical parameters such as creatinine, troponin value, Killip class, and vital signs. The Quick Sequential Organ Failure Assessment (qSOFA) tool was developed to predict the prognosis and need for intensive care in sepsis patients. The qSOFA measurement is a simple score composed of three parameters, i.e., respiratory rate, Glasgow Coma Scale, and blood pressure[8]. Many studies have shown that the qSOFA score can be used to predict the need for intensive care and the probability of mortality[9]. Serum cardiac troponins are used to verify a diagnosis of ACS and predict its prognosis[10]. But a more reliable predictor is needed for ACS patients with high mortality risk. Therefore, we investigated the prognostic accuracy of the qSOFA score by adding cTnI concentration (as a fourth parameter). The aim of this study was to assess the effectiveness of the GRACE and qSOFA-T scores on in-hospital mortality rate in non-STEMI patients.

METHODS

This study was conducted retrospectively between January 1, 2016, and December 31, 2018, on patients over the age of 18 years who were admitted to the ED. All patients who presented to the ED with ACS were evaluated consecutively (symptoms

[1]Bezmiâlem Vakıf Üniversitesi, Faculty of Medicine, Department of Emergency Medicine – İstanbul, Turkey.
*Corresponding author: drbahadir@yahoo.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on August 24, 2022. Accepted on October 26, 2022.

determined by the American Heart Association). Patients diagnosed with non-STEMI were included in the study. Informed consent was waived (a retrospective study). This is an observational study, in accordance with the Declaration of Helsinki. The Ethics Committee's approval was obtained. Patient information was obtained using the International Classification of Diseases (*ICD*)-10. The study excluded patients with trauma, pregnant women, patients with missing data, patients whose scores could not be calculated, patients with STEMI, patients with unstable angina pectoris (USAP), and patients with a Charlson Comorbidity Index (CCI) score of 3 or higher. The CCI is a useful measure of comorbidity to standardize the evaluation of patients. Infectious pathologies affecting the qSOFA score were excluded. A total of 1,996 patients were evaluated. Of these, 126 were discharged from the ED. There were 641 patients with a CCI score of 3 or higher, 126 had chest pain due to other causes, 146 patients were hospitalized in non-cardiology departments, and 43 refused treatments.

Definitions and variables

All patients were managed as per the institutional protocol. The blood sample and scores were calculated in the first 6 h of observation in the ED. Documentation that was previously produced was used to record the collected variables, including demographic data, comorbidities, laboratory test results, vital parameters, and physical examination findings. The GRACE and qSOFA scores were calculated. We selected the GRACE score, which is recommended for long-term prognosis and mortality prediction, as the appropriate score for comparison. GRACE score parameters include age, heart rate, blood pressure, Killip class, ST segment, creatinine, and troponin level. By definition, the qSOFA score consists of three parameters, namely, blood pressure, respiratory rate, and Glasgow Coma Scale[9]. We defined the qSOFA-T score by combining these characteristics with the troponin value. Anyone with a cTnI value greater than 40 ng/L received 1 point (the value where the corporate reference value is positive). The precision of the qSOFA-T score obtained by adding troponin to the qSOFA score has never been investigated in any previous study. Two emergency medicine physicians worked independently during the acquisition of the data. The serum high-sensitive cTnI levels were based on patients' baseline values at admission (the normal reference range in our biochemistry laboratory was 0–40 ng/L; Abbott Lab., Chicago, IL, USA). Receiver operating characteristic (ROC) curve analysis was used to determine the threshold value for the prognostic markers (GRACE score and qSOFA-T score) that were investigated in the study. All patients with ACS who required cardiac follow-up constituted the study sample (medical or CABG in their follow-up). In a sample of size n=869, we had 80% power at an α value of 0.05 to find a difference of 1%.

Statistical analysis

Centralization and measures of variance, such as mean±standard deviation (SD), were used to express quantitative variables. Fisher's exact test and the chi-square test were used to identify differences in the ratios and relationships between categorical variables. To determine the behavioral differences in the group averages, the Mann-Whitney U test was used when the assumptions of normality and equivalence were not met. The ROC analysis was used to determine the threshold values of the numerical parameters that were used to predict disease status and to evaluate the indicators' accuracy. The statistical significance level was set at p=0.05 and below. For this purpose, the IBM SPSS Statistics for Windows software package (Armonk, NY) was used. Distribution statistics for the categorical demographic variables are shown as n (%), and distribution statistics for the numerical variables are shown as mean±SD// median (min-max).

RESULTS

Of the 914 patients, 628 (68.7%) were male and 286 (31.3%) were female (p=0.478). The mean age was 52.95±13.73 years (p=0.003). The number of in-hospital deaths was 31 (3.4%). The most common chronic diseases in deceased patients were coronary artery disease, hypertension, and diabetes mellitus, and in living patients, they were hypertension, diabetes mellitus, and coronary artery disease. There was no statistically significant difference between the hemogram and routine biochemistry tests between the two groups. The mean GRACE score in the in-hospital deceased group was 149.77±29.31. In the survivor group, it was found to be 103.3±34.58 (p<0.001) (Table 1). The mean qSOFA-T score for the in-hospital deaths group was 2.03±1.09. It was calculated as 1.09±0.34 in the survivor group (p<0.001) (Table 1). The GRACE score has already been a proven prognostic scoring system. In our study, the area under the ROC curve was 0.840 (95% confidence interval (CI): 0.782–0.899) with a cutoff value of 139.5. The ROC analysis was also performed to determine whether the qSOFA-T

Table 1. Risk scores.

	Deceased patients	Living patients	p
GRACE score	149.7±29.3	103.3±34.5	<0.001
qSOFA-T	2.03±1.09	1.09±0.34	<0.001

score had a diagnostic value for in-hospital mortality. The area under the ROC curve was 0.826 (95%CI 0.743–0.91) with a cutoff value of 1.5 (Table 2; Figure 1). Considering the area covered by the ROC curve, the GRACE score was 139.5 and the qSOFA-T score was 1.5.

DISCUSSION

When compared to participants in similar previous studies, our patients were younger, more often male, and had similar prevalence rates of diabetes and hypertension[11]. This study examined the predictive value of GRACE score, and qSOFA-T score in a large sample of patients who were diagnosed with non-STEMI. qSOFA is widely used to predict mortality in many diseases. For example, it has been demonstrated to have a significant correlation with mortality from conditions such as acute decompensated heart failure and sepsis[12]. These successful results were due to the precise selection of the reviewed parameters. Systolic blood pressure is a combination of cardiac output and systemic peripheral resistance. A normal-to-high measurement may indicate better-preserved cardiac function. Another parameter, respiratory rate, was found to be a predictor of mortality in patients with ACS in selected studies[13], owing to changes in respiratory control due to cardiac dysfunction manifested themselves as an increase in respiratory rate. This suggests that respiratory rate should be included in risk assessment strategies for patients with ACS[14]. Many studies[15] have shown that the third parameter, the Glasgow Coma Scale, has a predictive value for survival after hospital discharge. Despite these features, the effectiveness of the qSOFA score in determining prognosis in ACS (especially non-STEMI) patients at high risk of adverse events has not been adequately studied. To increase the logistic regression power of qSOFA, we added the cTnI level as a fourth parameter in the score. In doing so, we found that the AUC of the qSOFA-T score reached 0.826 (an excellent discriminatory power). As a result, the qSOFA-T score is appropriate for use in EDs since it is easy, quick, inexpensive, and effective. Studies have shown that the GRACE score has the highest predictive accuracy for mortality in patients with ACS[16]. In addition, the European Society of Cardiology guideline accepts GRACE as a method of risk scoring. GRACE score has been thoroughly validated for assessing prognosis in non-STEMI, based on registries and large trials[16]. Our study found that the AUC of the GRACE score was 0.840 (the qSOFA-T score was found to be 0.826). When the results obtained are found to be 0.8≤AUC<0.9, it indicates excellent discriminating power[17]. In a similar study, GRACE score (AUC=0.80) for non-STEMI patients was found to have excellent discriminatory power[18]. The qSOFA-T score has never been studied before for predicting mortality in ACS patients. According to the study, both scores can be used to identify patients at high risk of coronary events in the context of non-STEMI. In a study, it was shown that a GRACE score >133 is significant in terms of acute conditions[19]. In our study, the mean GRACE score in the in-hospital mortality group was 149.77 with a cutoff value of 139.5. According to GRACE's guidelines, in-hospital mortality is above 3% when the score is above 140 points[20]. In our study, this rate was similarly found to be 3.4%. The mean value for qSOFA-T was 2.03 with a cutoff value of 1.5. For this reason, it is necessary to ensure that the qSOFA-T score is positive for at least two of the four parameters.

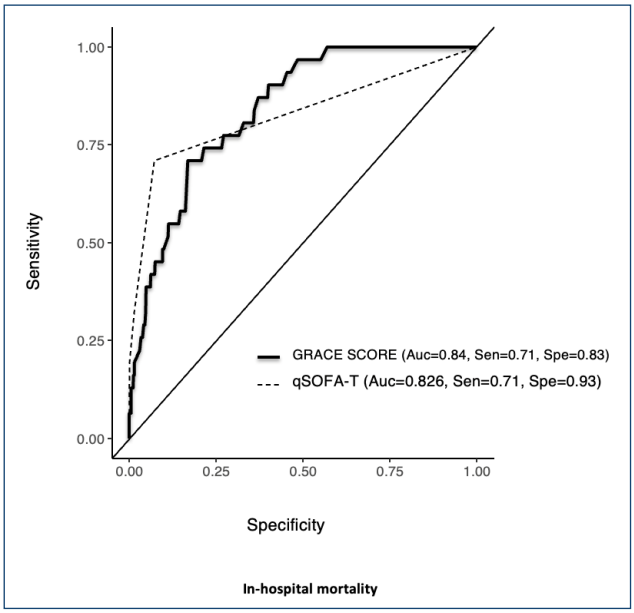

Figure 1. Comparison of in-hospital mortality using Global Registry of Acute Coronary Events and qSOFA-T.

Table 2. Comparison of scores by receiver operating characteristic analysis.

Variable	AUC	95%CI	Sensitivity	Specificity	Threshold
GRACE score	0.840	0.782–0.899	0.710	0.831	139.5
qSOFA-T score	0.826	0.743–0.91	0.710	0.928	1.5

Limitations

One limitation is that this is a single-center and retrospective design study. Second, anyone with a cTnI value above 40 received 1 point. This could be a bias point.

CONCLUSION

A reliable predictor is needed for non-STEMI patients with mortality risk. The GRACE score is a previously known score with proven effectiveness. Difficulty in calculating the GRACE score, which requires a computer, can be considered a limitation of this method. The qSOFA-T score, obtained by adding cTnI level to the qSOFA score, has excellent discriminatory power for predicting in-hospital mortality. The AUC of the GRACE score was 0.840, and the AUC of the qSOFA-T score was 0.826; both scores had excellent discriminatory power for predicting in-hospital mortality ($0.8 \leq AUC < 0.9$, an excellent discriminating power). In estimating the qSOFA-T score as a predictor of in-hospital mortality, the cutoff value was 1.5, and the mean value was 2.03. Care should be taken if the calculated qSOFA-T score is 2 or higher. According to the results of this study, patients who have a high qSOFA-T score, which can be calculated easily, quickly, and inexpensively, are at a higher risk of short-term mortality.

ETHICAL APPROVAL

The study was approved by the ethics committee of the Bezmialem Vakif University (approval number 2022-45; dated August 2, 2022).

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients to participate in the study.

AUTHORS' CONTRIBUTIONS

BT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **ES:** Formal Analysis, Project administration, Resources, Writing – original draft, Writing – review & editing. **BC:** Data curation, Formal Analysis, Supervision.

REFERENCES

1. Shah P, Patel K, Vasudev R, Patel H, Thakkar S, Adalja D, et al. Gender differences in the revascularization rates and in-hospital outcomes in hospitalizations with ST segment elevation myocardial infarction. Ir J Med Sci. 2020;189(3):873-84. https://doi.org/10.1007/s11845-019-02147-9
2. Penumetsa SC, Mallidi J, Friderici JL, Hiser W, Rothberg MB. Outcomes of patients admitted for observation of chest pain. Arch Intern Med. 2012;172(11):873-7. https://doi.org/10.1001/archinternmed.2012.940
3. Neves VB, Roman RM, Vendruscolo T, Heineck G, Mattos CAS, Mattos EI, et al. Validation of the Grace risk score to predict in-hospital and 6-month post-discharge mortality in patients with acute coronary syndrome. Int J Cardiovasc Sci. 2022;35(2):174-80. https://doi.org/10.36660/ijcs.20210019
4. Parco C, Brockmeyer M, Kosejian L, Quade J, Tröstler J, Bader S, et al. Modern NCDR and ACTION risk models outperform the GRACE model for prediction of in-hospital mortality in acute coronary syndrome in a German cohort. Int J Cardiol. 2021;329:28-35. https://doi.org/10.1016/j.ijcard.2020.12.085
5. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction. J Am Coll Cardiol. 2018;72(18):2231-64. https://doi.org/10.1016/j.jacc.2018.08.1038
6. McEvoy JW, Chen Y, Ndumele CE, Solomon SD, Nambi V, Ballantyne CM. et al. Six-year change in high-sensitivity cardiac troponin T and risk of subsequent coronary heart disease, heart failure, and death. JAMA Cardiol. 2016;1(5):519-28. https://doi.org/10.1001/jamacardio.2016.0765
7. Eggers KM, Baron T, Hjort M, Nordenskjöld AM, Tornvall P, Lindahl B. GRACE 2.0 score for risk prediction in myocardial infarction with nonobstructive coronary arteries. J Am Heart Assoc. 2021;10(17):e021374. https://doi.org/10.1161/JAHA.121.021374
8. Koch C, Edinger F, Fischer T, Brenck F, Hecker A, Katzer C, et al. Comparison of qSOFA score, SOFA score, and SIRS criteria for the prediction of infection and mortality among surgical intermediate and intensive care patients. World J Emerg Surg. 2020;25;15(1):63. https://doi.org/10.1186/s13017-020-00343-y
9. Goulden R, Hoyle MC, Monis J, Railton D, Riley V, Martin P, et al. qSOFA, SIRS and NEWS for predicting inhospital mortality and ICU admission in emergency admissions treated as Sepsis. Emerg Med J. 2018;35(6):345-9. https://doi.org/10.1136/emermed-2017-207120
10. Tarquinio N, Viticchi G, Zaccone V, Martino M, Fioranelli A, Morciano P, et al. The value of admission Troponin I to predict outcomes in suspected infections in elderly patients admitted in Internal Medicine: results from the SOFA-T collaboration, a multicenter study. Intern Emerg Med. 2021;16(4):981-8. https://doi.org/10.1007/s11739-020-02610-x
11. Poldervaart JM, Langedijk M, Backus BE, Dekker IMC, Six AJ, Doevendans PA, et al. Comparison of the GRACE, HEART and TIMI score to predict major adverse cardiac events in chest pain patients at the emergency department. Int J Cardiol. 2017;227:656-61. https://doi.org/10.1016/j.ijcard.2016.10.080
12. Jiang J, Yang J, Mei J, Jin Y, Lu Y. Head-to-head comparison of qSOFA and SIRS criteria in predicting the mortality of infected patients in the emergency department: a meta-analysis. Scand J Trauma Resusc Emerg Med. 2018;26(1):56. https://doi.org/10.1186/s13049-018-0527-9

13. Shlomai G, Kopel E, Goldenberg I, Grossman E. The association between elevated admission systolic blood pressure in patients with acute coronary syndrome and favorable early and late outcomes. J Am Soc Hypertens. 2015;9(2):97-103. https://doi.org/10.1016/j.jash.2014.11.005

14. Barthel P, Wensel R, Bauer A, Müller A, Wolf P, Ulm K, et al. Respiratory rate predicts outcome after acute myocardial infarction: a prospective cohort study. Eur Heart J. 2013;34(22):1644-50. https://doi.org/10.1093/eurheartj/ehs420

15. Kurowski A, Szarpak L, Frass M, Samarin S, Czyzewski L. Glasgow coma scale used as a prognostic factor in unconscious patients following cardiac arrest in prehospital situations: preliminary data. Am J Emerg Med. 2016;34(6):1178-9. https://doi.org/10.1016/j.ajem.2016.03.055

16. Akyuz S, Yazici S, Bozbeyoglu E, Onuk T, Yildirimturk O, Karacimen D, et al. Validity of the updated GRACE risk predictor (version 2.0) in patients with the non-ST-elevation acute coronary syndrome. Rev Port Cardiol. 2016;35(1):25-31. https://doi.org/10.1016/j.repc.2015.07.010

17. Zheng W, Wang G, Ma J, Wu S, Zhang H, Zheng J, et al. Evaluation and comparison of six GRACE models for the stratification of undifferentiated chest pain in the emergency department. BMC Cardiovasc Disord. 2020;20(1):199. https://doi.org/10.1186/s12872-020-01476-3

18. Shaikh MK, Hanif B, Shaikh K, Khan W, Parkash J. Validation of Grace Risk Score in predicting in-hospital mortality in patients with non-ST-elevation myocardial infarction and unstable angina. J Pak Med Assoc. 2014,64(7):807-11. PMID: 25255591

19. Mahmood M, Achakzai AS, Akhtar P, Zaman KS. Comparison of the TIMI and the GRACE risk scores with the extent of coronary artery disease in patients with the non-ST-elevation acute coronary syndrome. J Pak Med Assoc. 2013,63(6):691-5. PMID: 23901666

20. Tscherny K, Kienbacher C, Fuhrmann V, van Tulder R, Schreiber W, Herkner H, et al. Risk stratification in acute coronary syndrome: evaluation of the GRACE and CRUSADE scores in the setting of a tertiary care centre. Int J Clin Pract. 2020;74(2):e13444. https://doi.org/10.1111/ijcp.13444

ORIGINAL ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20221252

Selvester score and myocardial performance index in acute anterior myocardial infarction

Mustafa Kaplangoray[1], Cihan Aydın[2*], Kenan Toprak[3], Yusuf Cekici[4]

SUMMARY

BACKGROUND: The simplified Selvester QRS score is a parameter for estimating myocardial damage in ST-elevation myocardial infarction. ST-elevation myocardial infarction leads to varying degrees of impairment in left ventricular systolic and diastolic function. Myocardial performance index is a single parameter that can predict combined left ventricular systolic and diastolic performance.

OBJECTIVE: We investigated the relationship between Selvester score and myocardial performance index in patients undergoing primary percutaneous coronary intervention for acute anterior myocardial infarction.

METHODS: The study included 58 patients who underwent primary percutaneous coronary intervention for acute anterior myocardial infarction. Selvester score of all patients was also calculated at 72 h. Patients were categorized into two groups according to the Selvester score. Those with a score <6 (low score) were considered group 1 and those with a score ≥6 (high score) were considered group 2.

RESULTS: When compared with group 1, patients in group 2 were older (p=0.01) and had lower left ventricular ejection fractions (50.3±4 vs. 35.6±6.9, p=0.001), and conventional myocardial performance index (0.52±0.06 vs. 0.69±0.08, p=0.001), lateral tissue Doppler-derived myocardial performance index (0.57±0.08 vs. 0.72±0.08, p=0.001), and septal tissue Doppler-derived myocardial performance index (0.62±0.07 vs. 0.76±0.08, p=0.001) were higher. There was a high correlation between lateral tissue Doppler-derived myocardial performance index and conventional myocardial performance index and Selvester score (r=0.80, p<0.001; r=0.86, p<0.001, respectively) and a moderate correlation between septal tissue Doppler-derived myocardial performance index and Selvester score (r=0.67, p<0.001).

CONCLUSIONS: The post-procedural Selvester score can predict lateral tissue Doppler-derived myocardial performance index and conventional myocardial performance index with high sensitivity and acceptable specificity in patients undergoing primary percutaneous coronary intervention for acute anterior myocardial infarction.

KEYWORDS: Echocardiography. Infarction. Percutaneous coronary intervention.

INTRODUCTION

The severity of myocardial damage in survivors of ST-elevation myocardial infarction (STEMI) has great prognostic importance[1,2]. In 12-lead electrocardiography (ECG), the Selvester QRS scoring system, developed by Selvester et al., calculates the infarct area (IS) based on QRS waveforms[3]. This system is easy to use, accessible, and inexpensive and provides important information about prognosis after acute MI. Many studies have been performed by comparing Selvester QRS scoring with radionuclide ventriculography, creatinine kinase peak level, and myocardial perfusion imaging by single-photon emission computed tomography (SPECT) in determining IS and showed that Selvester QRS scoring provides comparable information with these methods[4]. Delayed enhancement magnetic resonance imaging (DE-MRI) is an important imaging tool that can provide accurate and direct measurement of IS[5]. Recent studies have also shown a good correlation between the Selvester QRS scoring system and DE-MRI in determining IS[6].

Myocardial performance index (MPI) provides important information in the evaluation of left heart systolic and diastolic functions. The prognostic value of MPI in various cardiac diseases such as MI has been proven in many studies. In the classical approach, MPI is obtained using a pulsed-wave Doppler. In recent years, tissue Doppler-derived MPI (tMPI) has been used instead of conventional MPI (cMPI). This is due to the fact that tMPI is not affected by preload and heart rate variability[7]. To the best of our knowledge, there are no studies investigating the relationship between Selvester QRS score and MPI in patients undergoing pPCI for acute anterior MI. Our aim was to examine the relationship between Selvester QRS score and both cMPI and tMPI.

[1]Sağlık Bilimleri Üniversitesi, Mehmet Akif İnan Research and Training Hospital, Department of Cardiology – Şanlıurfa, Turkey.
[2]Tekirdağ Namık Kemal Üniversitesi, Department of Cardiology – Tekirdağ, Turkey.
[3]Republic of Turkey Ministry of Health, Siverek State Hospital, Department of Cardiology – Şanlıurfa, Turkey.
[4]Sağlık Bilimleri Üniversitesi, Adana Health Practice and Research Center, Department of Cardiology – Adana, Turkey.
*Corresponding author: drcihanaydin@hotmail.com
Conflicts of interest: the authors declare there is no conflict of interest. Funding: none.
Received on September 22, 2022. Accepted on November 15, 2022.

METHODS

Study population

Between January 2021 and May 2022, 58 patients with anterior STEMI who were admitted to our hospital within the first 12 h of MI were included in the study. STEMI was diagnosed according to the European Society of Cardiology Guidelines[7]. Patients with left bundle branch block on ECG, >50% stenosis in vessels other than the vessel responsible for the lesion, pacemaker rhythm, left fascicular block, evidence of left ventricular (LV) hypertrophy on ECG, atrial fibrillation, and patients with severe heart failure and cardiogenic shock were excluded. The study protocol was approved by the local ethics committee and informed written consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki. The definition of risk factors was explained in previous studies.

Percutaneous coronary intervention procedure

Coronary angiography was performed using the Judkins technique via femoral or right radial artery access. Guideline-directed medical and interventional therapies were performed on all our patients.

Echocardiographic evaluation

Patients were evaluated in the lateral decubitus position with a Philips Envisor C echocardiograph (Philips Medical Systems, Andover, MA, USA) using a 3.5-MHz transducer, and ECG recording was performed simultaneously 72 h after the onset of MI. The interval between the end and beginning of the mitral inflow velocity was determined as "a." Pulsed Doppler analysis of LV outflow was performed by placing the sample volume just below the aortic valve in all five cavity windows and the interval between the beginning and end of LV outflow was determined as "b." The mean values of ''a" and ''b" were calculated as the average of the values obtained from three consecutive cardiac cycles, and the conventional MPI was calculated as (a–b)/b. Peak early (Em) and late (Am) diastolic velocities and peak systolic (Sm) annular velocity were recorded from these sites. MPI, based on TDI (tMPI), was calculated as follows: (IVCT + IVRT) / ET.

Electrocardiography interpretation and Selvester QRS score calculation

All patients underwent ECG on admission, after primary PCI, 90 min after PCI, and daily thereafter during hospitalization. The height of ST elevation was measured 20 ms after the J point. Total ST elevation was measured as the sum (mm) of ST elevation in leads D1, aVL, and V1 to V6. Total ST elevation was determined as STE1 at admission and as STE2 at 90 min. Modified Selvester QRS scoring was used in the study[8].

Statistical analysis

The SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data conforming to normal distribution were expressed as mean±standard deviation (SD) and the data not conforming to normal distribution were expressed as median (minimum-maximum). Categorical variables were expressed as percentages and compared using χ^2 or Fischer's exact test. Continuous data with normal distribution were compared with Student's t-test. ROC analysis was performed to determine the optimum threshold value of septal tMPI, lateral tMPI, and cMPI for the prediction of patients with high Selvester score. Univariate and multivariate logistic regression analyses were performed to identify independent predictors of high Selvester score. The Spearman's correlation test was performed to determine the relationship between high Selvester score and septal tMPI, lateral tMPI, and cMPI. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 64 patients with acute anterior MI were included in the study. Six patients were excluded from the study due to poor echocardiographic appearance. The mean age of the patients was 58.13±11.3 years and 20.7% of patients were female. Baseline demographic and laboratory characteristics are shown in Table 1. The patients were divided into two groups according to Selvester scores: group 1 with score <6 (low score) and group 2 with score ≥6 (high score).

Group 2 was older (56±12.6 vs. 60±9.8; p=0.01) and LVEFs were lower (50.3±4 vs. 35.6±6.9, p=0.001), and cMPI (0.52±0.06 vs. 0.69±0.08; p=0.001), lateral tMPI (0.57±0.08 vs. 0.72±0.08, p=0.001), and septal tMPI (0.62±0.07 vs. 0.76±0.08, p=0.001) were higher. Notably, 72 h QRS time and STE2 were lower in group 1 (Table 2). According to logistic regression analysis, septal tMPI, lateral tMPI, and cMPI were independent risk factors for high Selvester score (p<0.001) (Table 3). Correlation analysis was also performed to reveal the relationship between Selvester score and septal/lateral tMPI, and cMPI. The Spearman's correlation analysis showed a high correlation (r=0.80, p<0.001; r=0.86, p<0.001, respectively) between lateral tMPI and cMPI and Selvester score.

In our ROC curve analysis to identify patients with high Selvester scores, the findings were as follows: septal tMPI [(AUC=0.84, 95%CI 0.72–0.96, p<0.001)], lateral tMPI (AUC=0.90, 95%CI 0.82–0.98, p<0.001), and cMPI (AUC=0.88, 95%CI 0.78–0.97, p<0.001).

Table 1. Demographic and clinical characteristics.

	Patients (n=58)
Gender (M/F), n (%)	46/12 (79.3%/20.7%)
Age (years), mean±SD	58.13±11.3
Weight (kg), mean±SD	80.2±9.8
Height (cm), mean±SD	173.13±6.6
BMI (kg/m^2), mean±SD	26.7±3.5
Diabetes, n (%)	24 (41.4%)
Hypertension, n (%)	24 (41.4%)
Hyperlipidemia, n (%)	32 (55.2%)
Smoking, n (%)	28 (48.3%)
Family history of coronary artery disease (%)	14 (24.1%)
Echocardiographic measurements	
LVEF (%)	42.76±9.3
Mitral E velocity (cm/s)	68.7±18.5
Mitral A velocity (cm/s)	76.4±17.6
Mitral E/A ratio	0.94±0.38
IVRT (ms)	95.6±11.4
IVCT (ms)	68.1±16.5
ET (ms)	266.6±15.2
cMPI	0.61±0.11
Lateral IVRT (ms)	92.8±11.4
Lateral IVCT (ms)	76.1±18.7
Lateral ET (ms)	257.6±15.5
Lateral tMPI	0.65±0.11
Septal IVRT (ms)	100.2±11.2
Septal IVCT (ms)	77.3±17.5
Septal ET (ms)	254.3±13.6
Septal tMPI	0.70±0.1
Electrocardiographic measurements	
STE1 (mV)	11.5±4.9
STE2 (mV)	5.2±3.34
72 h Selvester score	6.1±2.4
Laboratory results	
Glucose (mg/dl)	198.72±105.9
Creatinine (mg/dl)	1.08±0.42
Sodium (mmol/L)	140±8.5
Hemoglobin	16.3±5
Platelet count (×10^3/μl)	294.6±71.8
High-density lipoprotein (mg/dl)	36.5±5.6
Low density lipoprotein (mg/dl)	133.3±28.8
Total cholesterol (mg/dl)	216.2±39.9
Triglycerides (mg/dl)	178.3±22.5
Medication	
Acetylsalicylic acid	30 (51.7%)
ACE-I /AT-II blocker	26 (44.8%)
Beta-blocker	14 (24.1%)
Statin	12 (20.7%)

BMI: body mass index; LVEF: left ventricular ejection fraction; IVRT: isovolumetric relaxation time; IVCT: isovolumetric contraction time; ET: ejection time; cMPI: conventional myocardial performance index; tMPI: tissue Doppler-derived myocardial performance index; Sm: mitral annular peak systolic; Em: mitral annular early diastolic velocity; Am: mitral annular late diastolic velocity; STE1: the sum of ST segment elevations at baseline; STE2: the sum of ST segment elevations at 90 min; ACE-I: angiotensin-converting enzyme inhibitors; AT-II: angiotensin-II.

DISCUSSION

Myocardial performance index (MPI) provides important information about both systolic and diastolic functions of the heart as a single parameter and is used in many cardiac diseases including MI leading to myocardial dysfunction. Conventional MPI is the sum of IVCT and IVRT divided by the ejection time (ET). The intervals here are not intervals of the same cardiac cycle but are derived from consecutive cycle intervals. Therefore, many factors, especially heart rate variability, reduce the reliability of cMPI. However, tMPI can be obtained by the ratio of the relaxation and contraction intervals to the ET of the same cardiac cycle. Therefore, it also provides reliable measurements in cases of heart rate fluctuation. To the best of our knowledge, there are no studies investigating the relationship between Selvester QRS score and LV MPI. In addition, previous studies have shown that the efficacy of the Selvester QRS score is more valuable in patients with anterior MI. Some studies have shown that there are differences between cMPI and tMPI, especially in patients with previous MI. In this study, both cMPI and tMPI values measured from the lateral and septal regions of all patients were higher than normal. When we categorized the patients into high Selvester score and low Selvester score, it was also revealed that both cMPI and septal and lateral tMPI were more impaired in patients with high Selvester score. The correlation analysis between MPI and Selvester score showed that there was a strong correlation between Selvester score and cMPI and lateral tMPI. This was interpreted that both cMPI and tMPI were globally affected by systolic and diastolic functions of the heart. In patients with MI, changes in TDI-based intervals occur due to the intraventricular conduction system, asynchrony, and the effects of relaxation and contraction times, resulting in an increase in MPI. Rojo et al.[9], on a control group consisting of healthy individuals and patients who had a previous MI, revealed the incompatibility between tMPI and cMPI. They interpreted this difference as longer systolic intervals and shorter diastolic intervals in TDI-based measurements. In our study, it was revealed that cMPI and septal/lateral tMPI values were numerically different. This discordance in MPI values is also present in the measurements of healthy individuals, but this difference is even more prominent in MI survivors[8]. Therefore, this should be taken into account when using TDI-based MPI.

Kurisu et al.[10] showed that there is a good correlation between total perfusion defect measured by SPECT and Selvester score in patients who underwent pPCI for anterior MI. Therefore, the Selvester score can be used in the prediction of IS in clinics like ours where cardiovascular magnetic resonance (CMR) is not common and we used it in our study.

Table 2. Comparison of clinical characteristics of patients with 72 h Selvester score values of <6 and ≥6.

Variables	Group 1 (72 h Selvester score <6) (n=28)	Group 2 (72 h Selvester score ≥6) (n=30)	p
Age	56±12.6	60±9.8	0.01
LVEF (%)	50.3±4	35.6±6.9	<0.001
cMPI	0.52±0.06	0.69±0.08	<0.001
Lateral tMPI	0.57±0.08	0.72±0.08	<0.001
Septal tMPI	0.62±0.07	0.76±0.08	<0.001
72 h QRS duration (ms)	83±10.7	94±10.3	<0.001
STE1	10.4±5.2	12.3±4.4	0.141
STE2	3.2±1.7	6.8±3.5	<0.001

Table 3. Univariate and multivariate logistic regression analyses of the independent indicators of high Selvester score.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p	OR (95%CI)	p
Age	1.070 (1.013–1.131)	0.016	1.042 (0.943–1.153)	0.417
Septal tMPI	1.205 (1.091–1.331)	<0.001	0.99 (0.797–1.246)	0.02
Lateral tMPI	1.45 (1.191–1.765)	<0.001	1.28 (0.787–2.083)	0.04
cMPI	1.308 (1.146–1.492)	<0.001	1.198 (0.903–1.591)	0.01

Myocardial performance index (MPI) is a parameter related to both systolic and diastolic performance and is not affected by heart rate, blood pressure, or ventricular geometry. Sasao et al.[11] showed that cMPI had a good correlation with the IR in patients with acute MI and that cMPI was an important indicator for prognosis in this patient group. We investigated the relationship between Selvester score and MPI in determining IS. We also showed a high correlation between the Selvester score and both MPI methods. We believe that the sum of these factors causes an increase in myocardial damage by impairing coronary perfusion and consequently contributes to the increase in the Selvester score. Some limitations of the study also exist. First, this was a single-center study and the number of patients was small. Second, measurements were taken only on the third day after MI and not in the post-discharge period; therefore, the change and correlation of MPI and Selvester score can be seen in the chronic phase. Third, there is a lack of CMR examination to reveal the concordance between these two parameters and CMR.

CONCLUSION

Both MPI and Selvester score are important, easy, reproducible, and inexpensive methods for predicting IS after MI in clinics like ours where CMR is not common. This study also demonstrated a strong correlation between these two methods.

AUTHORS' CONTRIBUTIONS

MK: Conceptualization, Data curation, Formal analysis, Funding acquisition, and Writing – original draft. **CA:** Writing – review & editing, Investigation, Methodology, Resources. **KT:** Software, Supervision, Validation Visualization, and Project administration

REFERENCES

1. Reinstadler SJ, Thiele H, Eitel I. Risk stratification by cardiac magnetic resonance imaging after ST-elevation myocardial infarction. Curr Opin Cardiol. 2015;30(6):681-9. https://doi.org/10.1097/HCO.0000000000000227
2. Sobel BE, Bresnahan GF, Shell WE, Yoder RD. Estimation of infarct size in man and its relation to prognosis. Circulation. 1972;46(4):640. https://doi.org/10.1161/01.cir.46.4.640
3. Selvester RH, Wagner GS, Hindman NB, The Selvester QRS. Scoring system for estimating myocardial infarct size. The development and

application of the system. Arch Intern Med. 1985;145(10):1877-81. PMID: 4037949

4. De Sutter J, Van de Wiele C, Gheeraert P, De Buyzere M, Gevaert S, Taeymans Y, et al. The Selvester 32-point QRS score for evaluation of myocardial infarct size after primary coronary angioplasty. Am J Cardiol. 1999;83(2):255-7, A5. https://doi.org/10.1016/s0002-9149(98)00831-5
5. Fieno DS, Kim RJ, Chen EL, Lomasney JW, Klocke FJ, Judd RM. Contrast-enhanced magnetic resonance imaging of myocardium at risk: distinction between reversible and irreversible injury throughout infarct healing. J Am Coll Cardiol. 2000;36(6):1985-91. https://doi.org/10.1016/s0735-1097(00)00958-x
6. Engblom H, Hedstrom E, Heiberg E, Wagner GS, Pahlm O, Arheden H. Rapid initial reduction of hyperenhanced myocardium after reperfused first myocardial infarction suggests recovery of the periinfarction zone: one-year follow-up by MRI. Circ Cardiovasc Imaging. 2009;2(1):47-55. https://doi.org/10.1161/CIRCIMAGING.108.802199
7. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018;39(2):119-77. https://doi.org/10.1093/eurheartj/ehx393. PMID: 28886621.
8. Wagner GS, Freye CJ, Palmeri ST, Roark SF, Stack NC, Ideker RE, et al. Evaluation of a QRS scoring system for estimating myocardial infarct size. I. Specificity and observer agreement. Circulation. 1982;65(2):342-7. https://doi.org/10.1161/01.cir.65.2.342
9. Rojo EC, Amenabar EO, Mújica OJ, Munayco CV. Disagreement between tissue Doppler imaging and conventional pulsed wave Doppler in the measurement of myocardial performance index. Eur J Echocardiogr. 2006;7(5):356-64. https://doi.org/10.1016/j.euje.2005.08.004
10. Kurisu S, Shimonaga T, Ikenaga H, Watanabe N, Higaki T, Ishibashi K, et al. Selvester QRS score and total perfusion deficit calculated by quantitative gated single-photon emission computed tomography in patients with prior anterior myocardial infarction in the coronary intervention era. Heart Vessels. 2017;32(4):369-75. https://doi.org/10.1007/s00380-016-0884-0
11. Sasao H, Noda R, Hasegawa T, Endo A, Oimatsu H, Takada T. Prognostic value of the Tei index combining systolic and diastolic myocardial performance in patients with acute myocardial infarction treated by successful primary angioplasty. Heart Vessels. 2004;19(2):68-74. https://doi.org/10.1007/s00380-003-0735-7

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20221292

Turkish validity and reliability of the lifestyle questionnaire related to cancer

Neslihan Öner[1*], Arda Borlu[2], Mevlüde Yasemin Akşehirli Seyfeli[3], Tuba Tekin[4]

SUMMARY

OBJECTIVE: The aim of this study was to adapt the lifestyle questionnaire related to cancer in Turkish and investigate its validity and reliability.

METHODS: This methodological study was conducted on 1,196 participants. Cronbach's α was used to assess validity and reliability. The internal consistency was evaluated using item-total correlation.

RESULTS: The normed chi-square in this study was 5.87. The root mean square error of approximation was calculated as 0.051. The comparative fit index and the Tucker-Lewis Index were 0.83 and 0.81, respectively. The split-half method was used to test the reliability of the scale (Part 1 Cronbach's α: 0.826, Part 2 Cronbach's α: 0.812, and Adjusted Cronbach's α: 0.881).

CONCLUSION: The Turkish version of lifestyle questionnaire related to cancer (8 subscales, 41 items) is a reliable and valid measure to evaluate lifestyle behaviors related to cancer in adults.

KEYWORDS: Adult. Behavior. Health. Life style. Primary health care.

INTRODUCTION

Cancer is the second most common cause of death worldwide. It is claimed that cancer will be a huge obstacle to increasing life expectancy. There are many factors implicated in the emergence of cancer; extrinsic factors account for 70–90% of cancer development which can be reduced through lifestyle factors[1,2]. In developed countries, it is observed that the most common types of cancer differ from infection/poverty-related cancers[3] and are mostly associated with Westernization of lifestyle[4,5].

In epidemiological studies, it has been shown that various lifestyle factors such as non-smoking, normal body weight, regular exercise, and a healthy diet could reduce the risk of cancer[6-8]. Healthy lifestyle behaviors, as a means of providing optimal metabolic health and reducing the overall burden of cancer, should be lifelong[9]. Momayyezi et al.[10] constructed a questionnaire named "lifestyle questionnaire related to cancer" (LQ-RC) to examine various aspects of lifestyle related to cancer. The aim of this study was to evaluate the validity and reliability of the LQ-RC in the Turkish population.

METHODS

Study group and procedures

This is a methodological study aiming to evaluate the reliability and validity of the LQ-RC. The sample size is recommended at least 5–10 times the total number of items in the scale when adapting a scale to another culture[11]. Considering that there may be deficiencies or errors in the data, it was planned to use a sample size that was 20 times the total number of items in the original scale. Therefore, the study sample was composed of 1,200 volunteers aged 18–64 years who consulted at primary healthcare centers between February and June 2018. Current dieters, foreigners, and participants who did not completely reply to all questions were excluded from the study. Four participants were removed because of missing data and wrong anthropometric measurements. The study was completed with 1,196 participants. Questionnaires were completed at primary healthcare centers via the face-to-face method.

[1]Erciyes University, Faculty of Health Sciences, Department of Nutrition and Dietetics – Kayseri, Turkey.
[2]Erciyes University, Faculty of Medicine, Department of Public Health –Kayseri, Turkey.
[3]Erciyes University, Faculty of Medicine, Department of Biostatistics – Kayseri, Turkey.
[4]Sivas Cumhuriyet University, Faculty of Health Sciences, Department of Nutrition and Dietetics – Sivas, Turkey.
*Corresponding author: neslihancelik@erciyes.edu.tr
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on September 29, 2022. Accepted on November 21, 2022.

Language adaptation protocol

The language adaptation of the scale was achieved with the back-translation method[12]. The original version of the LQ-RC was translated into target language by the professional translator. Each translated text was administered to 10 participants. Thereafter, another translation from target language back to source language was carried out to compare with the original text. The back-translation comparison process was repeated until the translation and original versions were the same. A pilot study was performed on a small group of 50 participants who had not been included in the main study.

Study instruments

Sociodemographic information and anthropometric measurements of the participants included in the study were recorded on the sociodemographic characteristic form. The original LQ-RC, which was a 4-point Likert-type scale, has 60 items divided into 8 subscales: "physical health, physical activity and exercise, balanced consumption of food, weight control and nutrition, mental health, reproductive health, drug and alcohol avoidance, and environmental pollutants and harmful substances." Cronbach's α of the original scale was 0.87. Items 21, 31, 43, 44, 45, 47, 51, 59, and 60 were reverse scored. High score indicates that individual has healthier lifestyle behaviors that can reduce the risk of cancer.

Ethics statement

This study was performed in accordance with the Helsinki Declaration and has been approved by the Erciyes University Ethics Committee (2017/92). All participants' written consents were obtained. Permission was obtained from the scale developer.

Statistical analysis

Data were analyzed using the IBM SPSS Statistics (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) 22.0 statistical package program and TURCOSA statistical software (Turcosa Analytics Ltd. Co., Turkey). Descriptive statistics were presented as sample size, percentage, mean, and standard deviation. The data were tested with the Kolmogorov-Smirnov test for normal distribution. Cronbach's α was used to test the reliability. Sampling adequacy was tested at Kaiser-Meyer-Olkin (KMO). Bartlett's test was used for factorability. The determination of factor structure was evaluated by principal component analyses. The factor analysis was conducted using the Varimax rotation. Confirmatory factor analysis (CFA) was performed to assess construct validity. Model fit and degrees of freedom were evaluated with goodness-of-fit indices such as the root mean square error (RMSEA), comparative fit index (CFI), and Tucker-Lewis Index (TLI). Cronbach's α criterion was determined as 0.70[13]. The internal consistency was evaluated using item-total correlation. Statistical significance was set at $p<0.05$.

RESULTS

The mean age was 31.17±12.08 years. In total, 71.6% of the participants were female; 43.9% of the participants were high school graduates; 94.5% of the participants were living in urban areas; and nearly half of the participants were single. The mean LQ-RC score of the participants was 65.63±15.74. The sociodemographic characteristics of the study population are shown in Table 1.

Construct validity

To determine the measurement power of each item, all correlation coefficients were examined. A total of 19 items (i.e., 4, 9, 21, 24, 26, 27, 28, 29, 31, 32, 33, 38, 43, 44,

Table 1. Sociodemographic characteristics of the participants.

Characteristics	Values
Age ($\bar{X}$ ± SS)	31.17±12.08 years
Gender, n (%)	
Male	340 (28.4)
Female	856 (71.6)
Education, n (%)	
Illiterate	28 (2.3)
Primary education	219 (18.3)
High school	525 (43.9)
Graduate	363 (30.4)
Postgraduate	61 (5.1)
Employment status, n (%)	
Employed	361 (30.2)
Unemployed	834 (69.7)
Accommodation, n (%)	
Urban	1,130 (94.5)
Rural	66 (5.5)
Marital status, n (%)	
Married	546 (45.6)
Single	612 (51.2)
Divorced	15 (1.3)
Widow	23 (1.9)
LQ-RC score ($\bar{X}$ ± SS)	65.63±15.74

45, 47, 49, 50, and 51) that did not meet this requirement were excluded from the scale. The split-half method was used to test the reliability of the scale consisting of 41 questions and the LQ-RC (Part 1 Cronbach's α: 0.826, Part 2 Cronbach's α: 0.812, and adjusted Cronbach's α: 0.881). The model was found to be compatible (Hotelling T^2 8940.38, p<0.001).

Confirmatory factor analysis

An eight-factorial structure was tested based on the original version for the confirmatory construct validity of the LQ-RC. The desired model fit was analyzed with the TURCOSA statistical software. The normed chi-square (NC) was 5.87 (χ^2/df=3909.482/666). RMSEA was calculated as 0.051. The CFI and TLI were 0.83 and 0.81, respectively.

Internal consistency reliability

The internal consistency of the LQ-RC and its subscales was evaluated by Cronbach's α coefficient. The "stress management" factor yielded 0.834, the "avoiding risky nutrition behaviors" factor yielded 0.716, the "use of preventive health services" factor yielded 0.734, the "physical health" factor yielded 0.712, the "physical activity and exercise" factor yielded 0.684, the "adequate and balanced nutrition" factor yielded 0.608, the "avoidance of hazardous substances" factor yielded 0.584, and the "risk mitigation applications" factor yielded 0.534. As a whole, the LQ-RC had 0.881 (Table 2). Bartlett's test of sphericity was χ^2/df=12951.9/703, p<0.001, while the KMO index was 0.881. The best resolution of the 41 items of the LQ-RC was represented by eight factors corresponding to eight subscales.

The last subscale of the original version is "reproductive health" for women. Due to the fact that approximately half of the participants in this study were single and it is not suitable to question whether single women use birth control because of cultural issues, we excluded this subscale in consultation with the scale developers. Data analysis was carried out with eight factors explaining 51.89% of the variance among the scale items (Table 2). It is suggested that the total explanatory variance of the scale is above 50.0%[14].

DISCUSSION

Cancer awareness and cancer prevention strategies have become popular topics[15]. The aim of this study was to adapt the LQ-RC in Turkish and evaluate its reliability and validity among this population. To the best of our knowledge, the LQ-RC is the first scale to provide information via lifestyle factors related to cancer in adults. This study showed that the Turkish version of the LQ-RC is a reliable and valid measurement tool.

The correlation coefficient of the item analysis is used in the reliability analysis[16]. When a high correlation coefficient is obtained for each item, it is determined that the item is sufficient to measure the targeted item. The recommended item coefficient should be >0.20 or >0.25[16]. According to the correlation coefficient of the split-half method test scores, the LQ-RC scale and its subscales showed internal consistency. No previous culture adaptation study of the LQ-RC has been performed. Therefore, it is not currently possible to compare Cronbach's α values in other cultural adaptations.

The adequacy of the defined subscales to explain the original structure of the scale is determined by CFA. Sampling adequacy was evaluated with KMO sampling adequacy measurement and Bartlett's test. If the sample is sufficient to perform factor analysis, the KMO>0.5[17]. In this study, the KMO value was calculated as 0.881. Regarding the RMSEA values used to determine the model fit, it has been suggested that value <0.05 is good, 0.05–0.08 is acceptable, 0.08–0.10 is marginal, and >0.10 is poor[18]. Finding the RMSEA value at 0.051 indicates that there is an acceptable fit in this study. CFI should be ≥0.80[19] and TLI ≥0.85[20]. The confirmatory analysis of this study revealed that the CFI value is 0.83 and the TLI value is 0.81, so in this study, RMSEA, CFI, and TLI values indicated an adequate fit. Although item 24 in the Turkish version of the LQ-RC was statistically appropriate, it was excluded from the expert's opinion as it was not appropriate to be under the same subscale as the other items. Internal consistency is satisfactory, reflecting the intercorrelation of the items on the scale and the measured construct around it.

CONCLUSION

Although much is known about cancer by the day, there is still no well-established treatment for the many types of cancer. There are more clinical studies about cancer, but lifestyle behaviors are also important. Besides, it is much easier to make lifestyle changes. These results suggest that the Turkish version of the LQ-RC is reliable and valid. With a practical scale, the lifestyle behaviors of individuals could be quickly evaluated, and individuals could be encouraged to have healthier lifestyle behaviors. There are some limitations to this study. The original version of the LQ-RC included reproductive health-related items only for women. Half of the participants in this study were single and probably did not use birth control methods. Future studies may address both genders.

Table 2. Explanatory factor analysis of the lifestyle questionnaire related to cancer.

Items	F1	F2	F3	F4	F5	F6	F7	F8
1	0.185	0.164	0.300	**0.581**	0.121	-0.025	-0.015	0.090
2	0.089	0.074	**0.726**	0.108	0.148	0.087	-0.057	-0.009
3	0.047	0.075	**0.742**	0.089	0.027	0.143	0.101	-0.082
5	0.063	0.117	**0.758**	0.021	0.092	0.082	0.110	0.080
6	0.003	0.255	**0.360**	-0.024	-0.013	0.269	0.022	0.206
7	0.085	0.259	**0.536**	0.051	0.109	0.134	-0.126	0.361
8	0.193	0.158	0.255	**0.683**	0.062	0.024	0.067	-0.027
10	0.374	0.008	0.002	0.254	**0.379**	-0.135	0.155	0.104
11	0.056	0.113	0.110	0.086	**0.756**	0.107	-0.064	0.099
12	0.061	0.029	0.132	0.095	**0.728**	0.063	0.155	0.166
13	0.059	0.121	0.085	0.036	**0.733**	0.174	-0.102	-0.005
14	**0.403**	-0.090	-0.011	0.335	0.264	0.093	0.048	-0.254
15	0.387	-0.048	-0.054	**0.484**	0.172	0.063	0.222	-0.141
16	**0.699**	-0.078	0.140	0.157	-0.027	-0.050	0.115	0.283
17	**0.708**	0.013	0.004	0.241	0.005	0.067	0.023	0.001
18	**0.722**	0.136	0.051	-0.015	0.092	0.079	0.029	0.031
19	**0.726**	0.170	0.008	0.063	0.092	0.081	-0.167	-0.094
20	**0.673**	-0.023	0.017	0.081	0.039	0.078	0.125	-0.103
22	**0.676**	0.126	0.054	0.008	0.025	0.096	0.042	-0.066
24	0.363	-0.083	0.088	-0.017	0.021	-0.005	**0.499**	0.056
25	**0.655**	-0.087	0.132	0.093	-0.044	-0.059	0.174	0.321
30	0.172	**0.464**	-0.024	-0.043	0.012	0.169	0.151	-0.245
34	0.004	**0.613**	0.157	0.129	0.040	-0.013	0.021	0.263
35	0.017	**0.698**	0.153	0.046	0.096	0.085	0.086	0.117
36	0.033	**0.703**	0.108	0.092	0.102	0.114	0.141	0.028
37	0.031	**0.604**	0.094	0.232	0.048	0.023	0.144	0.109
39	0.058	0.375	0.136	0.090	0.168	0.295	0.089	**0.451**
40	0.036	0.338	0.036	0.126	-0.082	0.190	**0.588**	-0.025
41	0.040	0.197	0.018	0.098	0.029	0.072	**0.684**	-0.030
42	0.033	0.300	-0.014	0.036	0.025	0.115	**0.513**	0.255
46	0.065	0.030	-0.032	0.198	0.295	0.023	0.064	**0.519**
48	-0.036	0.309	0.122	-0.107	0.070	0.204	0.057	**0.569**
52	0.038	0.142	-0.097	**0.645**	0.039	0.219	0.059	0.191
53	0.127	0.206	0.017	**0.551**	0.102	0.478	0.046	0.029
54	-0.087	0.229	0.054	0.087	0.330	**0.483**	-0.236	0.117
55	0.136	0.072	0.114	0.106	0.099	**0.642**	0.206	-0.016
56	0.035	0.164	0.150	0.036	0.116	**0.635**	-0.007	0.173
57	0.140	-0.050	0.197	0.111	-0.004	**0.574**	0.244	0.012
Explained variance (%)	10.98	7.63	6.60	6.00	5.96	5.60	4.80	4.31
Total explained variance (%)	10.98	18.62	25.214	31.22	37.17	42.77	47.58	51.89
Cronbach's α	0.834	0.716	0.734	0.712	0.684	0.608	0.584	0.534

Bold indicates the items of the subscales.

AUTHORS' CONTRIBUTIONS

NÖ: Conceptualization, Data analysis, Methodology, Visualization, Writing – original draft, Writing – reviewing & editing. **AB:** Data analysis, Writing – reviewing & editing. **MYAS:** Data analysis, Methodology. **TT:** Conceptualization, Data collection, Methodology, Writing – original draft.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
2. Arem H, Loftfield E. Cancer epidemiology: a survey of modifiable risk factors for prevention and survivorship. Am J Lifestyle Med. 2018;12(3):200-10. https://doi.org/10.1177/1559827617700600
3. Turke KC, Canonaco JS, Artioli T, Sarafyan AHM, Aoki ET, Muszkat D. Perception of risk factors for cancer in the ABC population. Rev Assoc Med Bras (1992). 2020;66(6):757-61. https://doi.org/10.1590/1806-9282.66.6.757
4. Ugai T, Sasamoto N, Lee H-Y, Ando M, Song M, Tamimi RM, et al. Is early-onset cancer an emerging global epidemic? Current evidence and future implications. Nat Rev Clin Oncol. 2022;19:656-73. https://doi.org/10.1038/s41571-022-00672-8
5. Brennan P, Davey-Smith G. Identifying novel causes of cancers to enhance cancer prevention: new strategies are needed. J Natl Cancer Inst. 2022;114(3):353-60. https://doi.org/10.1093/jnci/djab204
6. Elwood PC, Whitmarsh A, Gallacher J, Bayer A, Adams R, Heslop L, et al. Healthy living and cancer: evidence from UK Biobank. Ecancermedicalscience. 2018;12:792. https://doi:10.3332/ecancer.2018.792
7. Stevens C, Vrinten C, Smith CG, Waller J, Beeken RJ. Determinants of willingness to receive healthy lifestyle advice in the context of cancer screening. Br J Cancer. 2018;119(2):251-57. https://doi.org/10.1038/s41416-018-0160-4
8. Sasanfar B, Toorang F, Rostami S, Yeganeh MZ, Ghazi ML, Seyyedsalehi MS, et al. The effect of nutrition education for cancer prevention based on health belief model on nutrition knowledge, attitude, and practice of Iranian women. BMC Women's Health. 2022;22:213. https://doi.org/10.1186/s12905-022-01802-1
9. Kerr J, Anderson C, Lippman SM. Physical activity, sedentary behavior, diet, and cancer: an update and emerging new evidence. Lancet Oncol. 2017;18(8):e457-71. https://doi.org/10.1016/S1470-2045(17)30411-4
10. Momayyezi M, Fallahzadeh H, Momayyezi M. Construction and validation the lifestyle questionnaire related to cancer. Iran J Cancer Prev. 2015;8(5):e3965. https://doi.org/10.17795/ijcp-3965
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. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine (Phila Pa 1976). 2000;25(24):3186-91. https://doi.org/10.1097/00007632-200012150-00014
13. Baş M, Karaca KE, Saglam D, Arıtıcı G, Cengiz E, Köksal S, et al. Turkish version of the Intuitive Eating Scale-2: validity and reliability among university students. Appetite. 2017;114:391-97. http://dx.doi.org/10.1016/j.appet.2017.04.017
14. Arkan B, Bektas M, Özturk C, Özyazıcıoglu N, Vural P. A reliability and validity study of the Turkish version of the parenting scale. J Pediatr Res. 2019;6(1):56-63. https://doi.org/10.4274/jpr.galenos.2018.23855
15. Klein WMP, O'Connell ME, Bloch MH, Czajkowski SM, Green PA, Han PKJ, et al. Behavioral research in cancer prevention and control: emerging challenges and opportunities. J Natl Cancer Inst. 2022;114(2):179-86. https://doi.org/10.1093/jnci/djab139
16. Küçükgüçlü Ö, Mert H, Akpınar B. Reliability and validity of Turkish version of attitudes toward old people scale. J Clin Nurs. 2011;20(21-22):3196-203. https://doi.org/10.1111/j.1365-2702.2011.03764.x
17. Kara M, van der Bijl JJ, Shortridge-Baggett LM, Astı T, Ergüney S. Adaptation of the diabetes management self-efficacy scale for patients with type 2 diabetes mellitus: scale development. Int J Nurs. Stud. 2006;43(5):611-21. https://doi.org/10.1016/j.ijnurstu.2005.07.008
18. Fabrigar LR, Wegener DT, MacCallum RC, Strahan EJ. Evaluating the use of exploratory factor analysis in psychological research. Psychol Methods. 1999;4(3):272-99. https://doi.org/10.1037/1082-989X.4.3.272
19. Byrne BM, Campbell TL. Cross-cultural comparisons and the presumption of equivalent measurement and theoretical structure: a look beneath the surface. J Cross Cult Psychol. 1999;30(5):557-76. https://doi.org/10.1177/0022022199030005001
20. Shadfar M, Malekmohammadi I. Application of structural equation modeling (SEM) in restructuring state intervention strategies toward paddy production development. IJ-ARBSS. 2013;3(12):576-618. https://doi.org/10.6007/IJARBSS/v3-i12/472

ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20221445

Hereditary thrombophilia and low -molecular -weight heparin in women: useful determinants, including thyroid dysfunction, incorporating the management of treatment and outcomes of the entity

Stefan Dugalic[1], Milica Petronijevic[2], Demet Sengul[3], Dzenana A. Detanac[4], Ilker Sengul[5,6], Eduardo Carvalho de Arruda Veiga[7], Tamara Stanisavljevic[2], Maja Macura[1], Jovana Todorovic[8*], Miroslava Gojnic[1,8]

SUMMARY

OBJECTIVE: Our study purposed to examine the complex relationship between low-molecular-weight heparin therapy, multiple pregnancy determinants, and adverse pregnancy outcomes during the third trimester in women with inherited thrombophilia.

METHODS: Patients were selected from a prospective cohort of 358 pregnant patients recruited between 2016 and 2018 at the Clinic for Obstetrics and Gynecology, University Clinical Centre of Serbia, Belgrade.

RESULTS: Gestational age at delivery (β=-0.081, p=0.014), resistance index of the umbilical artery (β=0.601, p=0.039), and D-dimer (β=0.245, p<0.001) between 36th and 38th weeks of gestation presented the direct predictors for adverse pregnancy outcomes. The model fit was examined using the root mean square error of approximation 0.00 (95%CI 0.00–0.18), the goodness-of-fit index was 0.998, and the adjusted goodness-of-fit index was 0.966.

CONCLUSION: There is a need for the introduction of more precise protocols for the assessment of hereditary thrombophilias and the need for the introduction of low-molecular-weight heparin.

KEYWORDS: Thrombophilia, hereditary. Pregnancy. Thyroid gland. Heparin, low-molecular-weight.

INTRODUCTION

Physiologic pregnancy is associated with increased clotting potential and decreased fibrinolysis and anticoagulant activity[1,2], as well as venous stasis in the lower extremities, which are all factors that significantly augment the likelihood of venous thromboembolism (VTE) during pregnancy[3]. The aforementioned risks seem to be accentuated[1], but the association between hereditary thrombophilias and adverse pregnancy outcomes (APO), including fetal loss, preeclampsia, fetal growth restriction, and placental abruption, is being examined among women with hereditary thrombophilias[1,2,4,5]. The disorders of hemostasis, such as hereditary thrombophilias, have been identified as health conditions that can be associated with changes in the hemodynamics of the blood flow to the fetus[6]. Poor uteroplacental blood flow can further lead to thrombi in the placenta, while hereditary thrombophilias are associated with the placental microthrombi[7-9], which leads to infarctions, decreased trophoblast invasion, hypoxia, and overall placental insufficiency associated with the APO like stillbirths and intrauterine growth restriction[7,8,10]. Adequacy of the blood flow can be examined using the Doppler ultrasound and resistance index of the umbilical artery (RiAu)[9].

There is still a lack of evidence for the utility of routine testing for hereditary thrombophilias, although numerous clinicians are ordering it in the past decades[11]. Early identification of hereditary thrombophilias and timely introduction

[1]University Clinical Center of Serbia, Clinic for Gynecology and Obstetrics – Belgrade, Serbia.
[2]Univerzitet u Beogradu, Faculty of Medicine, Department of Gynecology and Obstetrics – Belgrade, Serbia.
[3]Giresun Üniversitesi, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
[4]General Hospital Novi Pazar, Department of Ophthalmology – Novi Pazar, Serbia.
[5]Giresun Üniversitesi, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
[6]Giresun Üniversitesi, Faculty of Medicine, Department of General Surgery – Giresun, Turkey.
[7]Universidade de São Paulo, Faculty of Medicine of Ribeirão Preto, Department of Gynecology and Obstetrics – São Paulo (SP), Brazil.
[8]Univerzitet u Beogradu, Institute of Social Medicine, Faculty of Medicine – Belgrade, Serbia.
*Corresponding author: jovana.todorovic@med.bg.ac.rs
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 27, 2022. Accepted on November 04, 2022.

of low-molecular-weight heparin (LMWH) prophylaxis may improve pregnancy outcomes[5,11,12]. As such, both aspirin and LMWH are proven to decrease the likelihood of APO among women with acquired thrombophilia, mainly antiphospholipid syndrome, but the data are less clear in cases of hereditary thrombophilias[13], and there is insufficient evidence to support the introduction of prophylactic anticoagulant treatment[3]. LMWH is the first choice anticoagulant for pregnant women as it is proven to have adequate bioavailability, predictable dose-response, and safety profile compared to unfractionated heparin[14]. Of note, the guidelines state that the introduction of anticoagulant therapy in pregnant women with hereditary thrombophilias should be based on individual risk assessment and focused on the personal and family history of VTE and risk factors for VTE, such as obesity, prolonged immobility, or cesarean delivery[1,15], relatively frequent in the Balkan region[5].

This study purposed to examine the complex relationship between LMWH therapy, multiple pregnancy determinants, and APO during the third trimester with inherited thrombophilia.

METHODS

In this study, patients had been incorporated from a prospective cohort of 358 pregnant recruited between 2016 and 2018 at the Clinic for Obstetrics and Gynecology, University Clinical Centre of Serbia, Belgrade, Serbia[12]. Briefly, the study endorsed all the referred women with inherited thrombophilia between 11 and 15 weeks of gestation and followed up to the delivery. The examined parameters were laboratory parameters and Doppler flows of the umbilical artery at 28th to 30th, 32nd to 34th, and 36th to 38th gestational weeks (gw), use of LMWH prophylaxis, and obstetric and perinatal outcomes. For this study, we incorporated the cases with the complete data on values of the RiAu between 36th and 38th weeks of gestation and values of D-dimer between 36th and 38th weeks of gestation.

The exclusion criteria were as follows:

(1) age >40 years;
(2) ovarian cell donation;
(3) concurrent hereditary and acquired thrombophilia;
(4) congenital anomalies of the uterus;
(5) multiple pregnancies;
(6) previous gynecological surgery;
(7) presence of perinatal infections (TORCH);
(8) type 1 diabetes, preexisting arterial hypertension;
(9) previous kidney transplantation;
(10) extreme obesity (BMI >40 kg/m^2);
(11) use of LMWH due to any comorbid condition other than hereditary thrombophilia;
(12) abnormal findings in the first-trimester prenatal screening tests, fetal anomalies, central placenta previa, and pathological degree of placental nidation (suspect for placenta acreta, increate, or percreta); and
(13) therapeutic use of LMWH during pregnancy.

The data for the study were drawn from the patient records in the hospital database, including age, comorbid conditions (pulmonary embolism, insulin resistance, thyroid dysfunction), adverse health outcomes in the family history (arterial hypertension, HA; deep venous thrombosis, DVT; myocardial infarction, MI; cerebrovascular insult, CVI; pulmonary embolism, PULME; thyroid dysfunction, THR) if thrombophilia was recognized prior to the current pregnancy, previous APO, type of mutation responsible for thrombophilia (plasminogen-activator inhibitor, PAI; factor V Leiden; MTHFR mutation; prothrombin G20210A; protein S deficiency; factors VII, IX, and XI; or anti-thrombin-related mutation), mode of delivery, APO in the current pregnancy (in our study, we recorded pregnancy losses in the third trimester—intrauterine fetal death—preterm birth, fetal growth restriction), values of resistance index of umbilical artery (RiAu) between 36th and 38th weeks of gestation, and values of D-dimer between 36th and 38th weeks of gestation, the LMWH therapy in the current pregnancy, gestational age at delivery, and fetal sex. The characteristics of the participants from the original cohort are presented elsewhere[12]. From the cohort of 358 pregnant patients, 203 had complete data on values of the RiAu between 36th and 38th weeks of gestation and values of D-dimer between 36th and 38th weeks of gestation and were selected for the analysis. These cases were classified into two groups according to the presence of APO in the current pregnancy: group with APO (33 cases, 16.3%) and group without APO (170 cases, 83.7%).

The statistical analyses were conducted using the methods of descriptive and analytical statistics. To this end, the means, standard deviations, skewness, and kurtosis were calculated for numerical data, and categorical variables were presented by absolute numbers with percentages. The differences between the groups with APO and without APO were analyzed using the chi-square (χ^2) test for categorical variables and the Student's t-test for numerical variables. The path analysis was conducted to examine the relationship between LMWH therapy, previous APO, gestational age at delivery, RiAu between 36th and 38th weeks of gestation, D-dimer value between 36th and 38th weeks of gestation, and APO. Multiple measures were used to assess the adequacy of model fit to the data: the chi-square test and the fit indices such as the comparative fit index (CFI), the normed

fit index (NFI), the adjusted goodness-of-fit index (AGFI), and the root mean square error of approximation (RMSEA). The model consistency was evaluated by the chi-square test, which indicates, when nonsignificant, that the data are consistent. The acceptable model fitting values for fit indices were defined as follows: CFI ≥0.95, NFI≥0.95, AGFI ≥0.95, and RMSEA <0.05. In all the analyses, the significance level was set at 0.05, and the statistical analyses were performed using the Amos 21 (IBM SPSS Inc., Chicago, IL, USA) and IBM SPSS Statistics 25 software.

RESULTS

The groups with and without the APO in the current pregnancy differed significantly in the frequency of the previous APO (p=0.031). The patient history characteristics of the current study sample are presented in Table 1. The women with APO in current pregnancy had significantly higher RiAu between 36th and 38th weeks of gestation (0.69±0.08 vs. 0.57±0.08, p<0.001), significantly higher D-dimer between 36th and 38th weeks of gestation (2.74±1.06 vs. 0.68±0.48, p<0.001), and significantly higher frequency of gestational age at delivery between 36th and 37th weeks (42.4 vs. 2.9%, p<0.001) compared to the women without APO in the current pregnancy. A sum of 27 (13.3%) women had been detected as possessing thyroid dysfunction, and the cases with APO in the current pregnancy had no significance compared to the ones without APO in the current pregnancy. The characteristics of the current pregnancy of patients in both groups are presented in Table 2.

We conducted a path analysis with APO as the target variable. The absolute fit index (χ^2=0.983, df=1, p=0.321) demonstrated a good fit to the data. The values for fit indices NFI (0.998), AGFI (0.966), and CFI (1.000) were above the cutoff value of ≥0.95. The RMSEA value of 0.000 (0.000–0.185) was below the suggested value of ≤0.05. Of note, Figure 1 presents the results from the path analysis, and the path analysis exhibited that the gestational age at delivery (β=-0.081, p=0.014),

Table 1. Characteristics of patient history.

Variables	Total n (%)	APO in current pregnancy n (%)	No APO in current pregnancy n (%)	p-value
Maternal age, years, mean±SD	31.57±5.74	33.68±4.29	33.67±3.96	0.993
Comorbidities, conditions, or previous adverse health events				
Pulmonary embolism	1 (0.5)	0 (0)	1 (0.6)	0.659
Insulin resistance	22 (10.8)	4 (12.1)	18 (10.6)	0.795
Thyroid dysfunction	27 (13.3)	4 (12.1)	23 (13.5)	0.827
Adverse health outcomes in family history, n (%)				
HA	32 (15.8)	2 (6.1)	30 (17.6)	0.095
DVT	4 (2.0)	0 (0)	4 (2.4)	0.373
MI	5 (2.5)	1 (3.0)	4 (2.4)	0.818
CVI	5 (2.5)	1 (3.0)	4 (2.4)	0.818
PULME	2 (1.0)	0 (0)	2 (1.2)	0.531
THR	5 (2.5)	0 (0)	5 (2.9)	0.318
Type of inherited thrombophilia				
PAI-1	27 (23.5)	4 (25.0)	23 (23.2)	
MTHFR	12 (10.4)	1 (6.3)	11 (11.1)	
FVL	9 (7.8)	1 (6.3)	8 (8.1)	
PT	7 (6.1)	0 (0)	7 (7.1)	
Other	4 (3.5)	1 (6.3)	3 (3.0)	
Combined thrombophilia	53 (46.1)	7 (43.8)	46 (46.5)	0.211
Previous APO (any)				
Yes	90 (44.3)	9 (27.3)	81 (47.6)	
No	113 (55.7)	24 (72.7)	89 (52.4)	0.031

RiAu (β=0.601, p=0.039), and D-dimer (β=0.245, p<0.001) between 36th and 38th gw presented the main direct predictors for APO. The important indirect effects on APO were recognized in LMWH therapy via the RiAu between 36th and 38th gw and for previous APO via the D-dimer between 36th and 38th gw.

Table 2. Characteristics of the current pregnancy.

Variables	Total n (%)	APO in current pregnancy n (%)	No APO in current pregnancy n (%)	p-value
Ri 36-38 gw, mean±SD	0.59±0.09	0.69±0.08	0.57±0.08	**<0.001**
D-dimer 36–38 gw, mean±SD	1.02±0.97	2.74±1.06	0.68±0.48	**<0.001**
Delivery				
Vaginal	39 (19.2)	8 (24.2)	31 (18.2)	
Cesarean section	164 (80.8)	25 (75.8)	139 (81.8)	0.423
Gestational age at delivery				
38–39 gw	184 (90.6)	19 (57.6)	165 (97.1)	
36–37 gw	19 (9.4)	14 (42.4)	5 (2.9)	**<0.001**
Fetal sex				
Male	111 (54.7)	17 (51.5)	94 (55.3)	
Female	92 (45.3)	16 (48.5)	76 (44.7)	0.690
LMWH therapy				
Yes	128 (63.1)	17 (51.5)	111 (65.3)	
No	75 (36.9)	16 (48.5)	59 (34.7)	0.133

Bold values indicate statistical significance at the p<0.05 level.

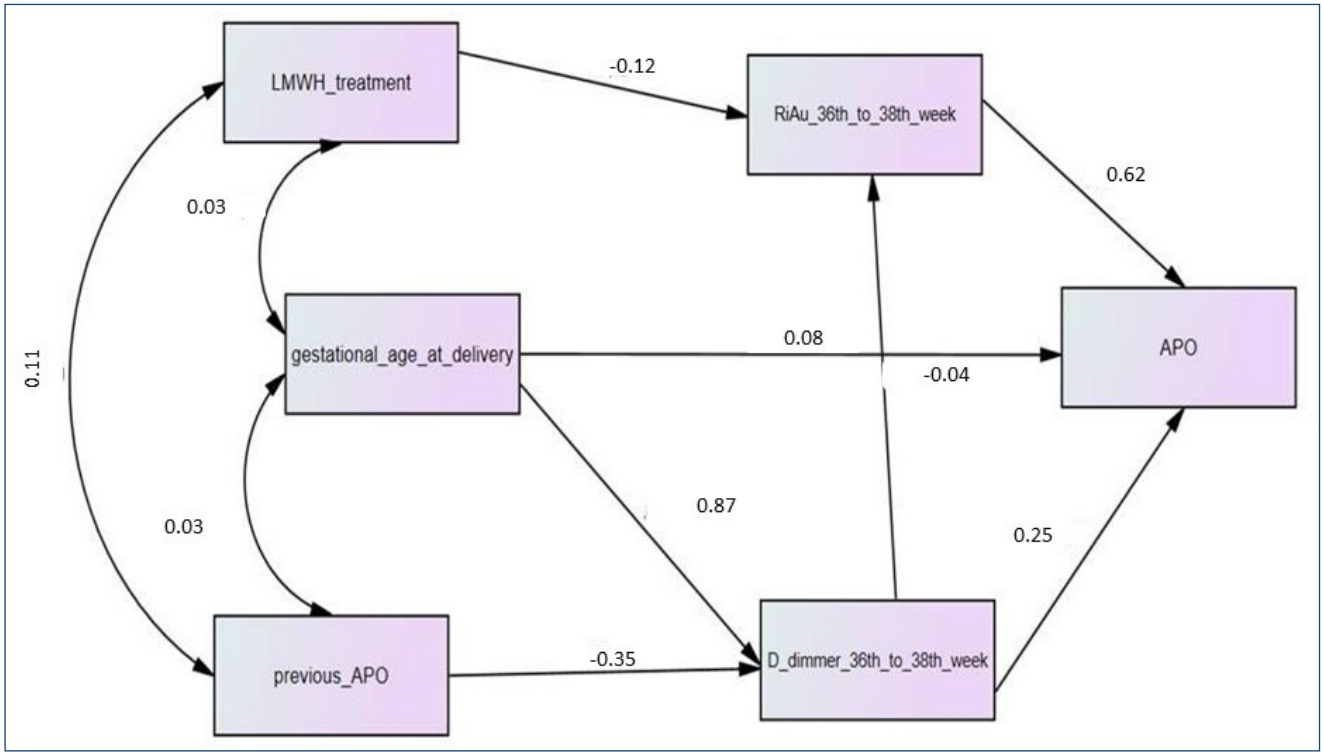

Figure 1. A path model presenting the complex relationship between low-molecular-weight heparin therapy, multiple pregnancy determinants, and adverse pregnancy outcomes during the third trimester in cases with inherited thrombophilia.

DISCUSSION

Some authors suggested that all pregnancies with hereditary thrombophilias should be considered high-risk pregnancies with a high likelihood of APO[16]. However, contradictions remain in the recommendations on their follow-up and prophylactic administration of LMWH, as the routine administration of LMWH is still not recommended[1,3]. The LMWH therapy was, in this study, significantly negatively associated with the RiAu between 36th and 38th gw. The lower gestational age on delivery, higher D-dimer values, and higher RiAu values were associated with APO, and the LMWH therapy had an indirect effect on APO via RiAu between 36th and 38th gw. These findings indicate that previous APO should also be included in the evaluation of the necessity for inclusion of LMWH therapy in order to achieve the attenuation of the occurrence of APO in the current pregnancy[3,12].

There is a need for further randomized controlled trials, as the TIPPS and FRUIT trials, large randomized controlled trials on the use of LMWH, did not show significant benefits. Additionally, some data show that the pathogenic process might originate in the first trimester and that LMWH treatment can have benefits during implantation and placental development. This is why it may be important to evaluate the timing of the initiation of LMWH treatment, especially its introduction in the first trimester, which recommendation is also in accordance with our results as the participants were recruited in the late first or early in the second trimester[17].

It is known that reproductive functions are affected by some conditions, the thyroid hormones, L-thyroxine (3,5,3',5'-tetraiodothyronine, T4), and L-triiodothyronine (3,5,3'-triiodothyronine, T3). These hormones are also known as vital parameters for the normal reproductive function of humans and animals employing regulation of the ovarian, uterine, and placental tissues and metabolism in thyroidology[18]. However, in this study, the cases with APO in the current pregnancy have not revealed any significance compared to the ones without APO in the current pregnancy in terms of thyroid dysfunction.

The RiAu was associated with the LMWH treatment and with APO in the current pregnancy in our study. The higher RiAu was previously shown to be associated with low birth weight and lower fetal weight gain during the third trimester[19]. Of note, the pathophysiology of the low birth weight of the newborns of women with thrombophilias is based on an association of hereditary thrombophilias with placental infarction, abnormal trophoblast invasion, and chronic hypoxia, leading to increase in uterine artery resistance[3]. Attenuation in RiAu in the third trimester may enable the higher fetal growth potential, the higher potential for the adequate duration of the gestational period[19], and decrease the likelihood for APO, as we also found a negative association between the gestational age at birth and APO. In addition, augmented D-dimer values were recognized in the second and third trimesters in most pregnant women[19], but the higher D-dimer values with higher RiAu and a higher likelihood for APO were recognized in this study.

Limitations

The main limitation of our study is in the observational design as our participants were not randomized to the groups examined, and there could be bias in the classification of the participants to the examined groups. However, there were no significant differences in the characteristics of pregnant women from both groups in the age, BMI, and frequencies of most different thrombophilia types.

CONCLUSION

The lower gestational age on delivery, higher D-dimer values, and higher RiAu values during the third trimester were associated with APO. As adequate blood flow allows adequate fetal development through the transition from a high to a low resistive blood flow, and clotting disorders can be considered as the factor associated with the interruption of this process, the adequate values of the RiAu throughout the pregnancy are important for the prevention of the APO. The LMWH therapy had an indirect effect on APO via RiAu, yielding further research on the importance of its timely introduction among pregnant women with hereditary thrombophilias, which can allow for the prevention of APO, their consequences for pregnant women later life, and the consequences of a suboptimal uterine environment for the child's development and future life. *Neque ignorare medicum oportet quæ sit ægri natura.*

ACKNOWLEDGMENTS

The authors thank all the participants who took part in this study.

AUTHORS' CONTRIBUTIONS

SD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **MP:** Investigation, Project administration, Resources, Validation, Visualization, Writing – original draft. **DS:** Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **DAD:** Investigation, Methodology, Software, Validation, Visualization, Writing

– review & editing. **IS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **ECAV:** Investigation, Methodology, Validation, Visualization, Writing – review & editing. **TS:** Data curation, Formal Analysis, Investigation, Project administration, Resources, Validation, Visualization, Writing – original draft. **MM:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **JT:** Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. **MG:** Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft.

REFERENCES

1. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins–Obstetrics. ACOG practice bulletin No. 197: inherited thrombophilias in pregnancy. Obstet Gynecol. 2018;132(1):e18-34. https://doi.org/10.1097/AOG.0000000000002703
2. Trasca LF, Patrascu N, Bruja R, Munteanu O, Cirstoiu M, Vinereanu D. Therapeutic implications of inherited thrombophilia in pregnancy. Am J Ther. 2019;26(3):364-74. https://doi.org/10.1097/MJT.0000000000000985
3. Dłuski D, Mierzyński R, Poniedziałek-Czajkowska E, Leszczyńska-Gorzelak B. Adverse pregnancy outcomes and inherited thrombophilia. J Perinat Med. 2017;46(4):411-7. https://doi.org/10.1515/jpm-2017-0059
4. Hadžić D, Zulić E, Alihodžić H, Softić D, Kovačevic D. Oxygen saturation index for assessment of respiratory failure in neonates. Sanamed. 2018;13(2):123-9. https://doi.org/10.24125/sanamed.v13i2.224
5. Mulrooney JS. Uterine artery Doppler of the gravid uterus as a predictor identifying at-risk pregnancies: a meta-analysis. J Diagnostic Med Sonogr. 2015;31(1):64-9. https://doi.org/10.1177/8756479314563541
6. Vogel JP, Vannevel V, Robbers G, Gwako G, Lavin T, Adanikin AI, et al. Prevalence of abnormal umbilical arterial flow on Doppler ultrasound in low-risk and unselected pregnant women: a systematic review. Reprod Health. 2021;18(1):38. https://doi.org/10.1186/s12978-021-01088-w
7. Ghi T, Contro E, Youssef A, Giorgetta F, Farina A, Pilu G, et al. Persistence of increased uterine artery resistance in the third trimester and pregnancy outcome. Ultrasound Obstet Gynecol. 2010;36(5):577-81. https://doi.org/10.1002/uog.7602
8. Franco C, Walker M, Robertson J, Fitzgerald B, Keating S, McLeod A, et al. Placental infarction and thrombophilia. Obstet Gynecol. 2011;117(4):929-34. https://doi.org/10.1097/AOG.0b013e31820ca040
9. Reynolds LP, Borowicz PP, Caton JS, Vonnahme KA, Luther JS, Buchanan DS, et al. Uteroplacental vascular development and placental function: an update. Int J Dev Biol. 2010;54(2-3):355-66. https://doi.org/10.1387/ijdb.082799lr
10. Bergstrom D, Mital S, Sheaves S, Browne L, O'Reilly D, Nguyen HV. Heritable thrombophilia test utilization and cost savings following guideline-based restrictions: an interrupted time series analysis. Thromb Res. 2020;190:79-85. https://doi.org/10.1016/j.thromres.2020.04.012
11. Dugalic S, Petronijevic M, Stefanovic A, Stefanovic K, Petronijevic SV, Stanisavljevic D, et al. Comparison of 2 approaches in management of pregnant women with inherited thrombophilias: prospective analytical cohort study. Medicine (Baltimore). 2019;98(34):e16883. https://doi.org/10.1097/MD.0000000000016883
12. Simcox LE, Ormesher L, Tower C, Greer IA. Thrombophilia and pregnancy complications. Int J Mol Sci. 2015;16(12):28418-28. https://doi.org/10.3390/ijms161226104
13. Voicu DI, Munteanu O, Gherghiceanu F, Arsene LV, Bohiltea RE, Gradinaru DM, et al. Maternal inherited thrombophilia and pregnancy outcomes. Exp Ther Med. 2020;20(3):2411-4. https://doi.org/10.3892/etm.2020.8747
14. Sengul D, Sengul I, Soares Júnior JM. Caesarean section scar endometriosis: *quo vadis?* Rev Assoc Med Bras (1992). 2022;68(1):1-2. https://doi.org/10.1590/1806-9282.20211074
15. Papadakis E, Pouliakis A, Aktypi A, Christoforidou A, Kotsi P, Anagnostou G, et al. Low molecular weight heparins use in pregnancy: a practice survey from Greece and a review of the literature. Thromb J. 2019;17:23. https://doi.org/10.1186/s12959-019-0213-9
16. Ormesher L, Simcox L, Tower C, Greer IA. Management of inherited thrombophilia in pregnancy. Womens Health (Lond Engl). 2016;12(4):433-41. https://doi.org/10.1177/1745505716653702
17. Sengul D, Sengul I, Soares Júnior JM. Repercussion of thyroid dysfunctions in thyroidology on the reproductive system: ***conditio sine qua non?*** Rev Assoc Med Bras (1992). 2022;68(6):721-2. https://doi.org/10.1590/1806-9282.20220255
18. Kehila M, Touhami O, Hmid RB, Abouda HS, Abdeljelil K, Ayachi A, et al. Correlation between umbilical resistance index and fetal growth: pilot study. J Obstet Gynaecol Res. 2017;43(5):820-4. https://doi.org/10.1111/jog.13274
19. Gutiérrez García I, Pérez Cañadas P, Martínez Uriarte J, García Izquierdo O, Angeles Jódar Pérez M, García Guadiana Romualdo L. D-dimer during pregnancy: establishing trimester-specific reference intervals. Scand J Clin Lab Invest. 2018;78(6):439-42. https://doi.org/10.1080/00365513.2018.1488177

REVIEW ARTICLE

https://doi.org/10.1590/1806-9282.20220810

Self-efficacy in the practice of breastfeeding in adolescent puerperal women

Aline Morais Venancio de Alencar[1*], Roseli Oselka Saccardo Sarni[2,3]

INTRODUCTION

Breast milk is a complex and dynamic food with numerous advantages for the newborn and its mother[1]. The World Health Organization (WHO) and the Ministry of Health recommend breastfeeding for children under 6 months of age exclusively and as supplementation for children up to 2 years or more[2].

The act of breastfeeding goes far beyond nourishing the child, and it involves interaction between mother and child, with an impact on adequate growth, protection against infections, cognitive and emotional development, and future protection against the development of chronic diseases that are common in adults[3,4].

In Brazil, the National Study on Food and Nutrition (ENANI–in Portuguese) conducted in 2019 showed a prevalence of exclusive breastfeeding in infants under 6 months of age and continued breastfeeding in infants between 20 and 23 months of 45.8 and 35.5%, respectively[4].

Maternal age has been identified as an important factor in the early discontinuity of breastfeeding[5]. Several factors interfere with the duration of breastfeeding practiced by adolescent mothers, such as lack of family support and/or information, absence of a partner, return to work or school, early introduction of other foods for the infant, and the use of pacifiers[6].

Another relevant factor that influences a woman's behavior during breastfeeding is the mother's confidence in her ability to breastfeed[7], also called breastfeeding self-efficacy. Several pieces of evidence have shown that the promotion of mothers' self-efficacy in breastfeeding contributes to the prevention of early weaning by promoting holistic, humanized care, contributing to effective assistance, aiming at increasing the time of exclusive breastfeeding, and consequently reducing morbidity and children mortality[8]. Despite this, publications involving teenage mothers, especially in our country, are still scarce in the literature.

Thus, the objective of the present study was to evaluate the self-efficacy of adolescent mothers in their ability to breastfeed, as well as its reflection in practice and adherence to exclusive breastfeeding.

METHODS

The study was approved by the Research Ethics Committee of the Centro Universitário Doutor Leão Sampaio (UNILEÃO – In Portuguese), under opinion No. 3,277,812, following the ethical precepts of resolution 466/2012 of the National Research Ethics Commission. All participants signed the terms of consent to display their information.

Through a descriptive, cross-sectional study with a quantitative, non-probabilistic approach, 30 adolescent puerperal women in the immediate postpartum period (between the first and tenth postpartum day), followed up in the Family Health Strategies (ESF) of the urban area of Juazeiro do North, Ceara, Brazil. Women who were illiterate, with restrictions that made it impossible to understand the instrument, with premature births, or with neonates hospitalized in an intensive care unit for more than 10 days were excluded.

Initially, the researcher made a telephone contact with all nurses working in the FHS to obtain information about pregnant adolescents who were close to childbirth. Professionals were asked to schedule a visit to the postpartum woman's residence during the immediate postpartum period.

Data were collected from August 2019 to January 2020. In the period, 38 adolescent puerperal women attended by the ESF in the municipality and eligible for the study were identified, of which three moved to another municipality with their family members and five gave up the collection, resulting in a total of 30 participants (Figure 1).

[1]Centro Universitário Faculdade de Medicina do ABC, Health Sciences – Santo André (SP), Brazil.
[2]Centro Universitário Faculdade de Medicina do ABC – Santo André (SP), Brazil.
[3]Universidade Federal de São Paulo – São Paulo (SP), Brazil.
*Corresponding author: aline31.venancio@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 04, 2022. Accepted on October 30, 2022.

Data were collected using two instruments. The first instrument was a questionnaire that included sociodemographic and obstetric variables.

The second instrument was the Breastfeeding Self-Efficacy Scale–Short-Form (BSES-SF), which consists of a structured, self-completed Likert scale containing 14 questions divided into two domains: technical and intrapersonal thinking. The instrument allows for assessing the participants' self-efficacy in breastfeeding. Each question has five possible answers that range from 1 to 5, where 1 – totally disagree, 2 – disagree, 3 – sometimes agree, 4 – agree, and 5 – totally agree. The total score of the scale varies from 14 to 70 points, being considered low effectiveness: from 14 to 32 points; medium effectiveness: from 33 to 51 points; and high effectiveness: from 52 to 70 points. The scale was validated in Brazil by Dodt[9] presenting Cronbach's alpha of 0.74, indicating high internal consistency, ratified by the intraclass correlation coefficient that ranged from 0.69 to 0.78, which confirms the reliability of the BSES-SF[9].

Data were entered into an Excel® 2013 spreadsheet, and analyses were performed using the Statistical Package for Social Sciences (SPSS 25.0). Categorical variables were arranged as absolute numbers and percentages and compared using the chi-square or Fisher's exact test. The standard deviation for breastfeeding self-efficacy was evaluated. A significance level of 5% was adopted to reject the null hypothesis.

RESULTS

Table 1 shows the general characterization of adolescent mothers with a predominance of the following characteristics: ages between 17 and 19 years, completed high school, and single.

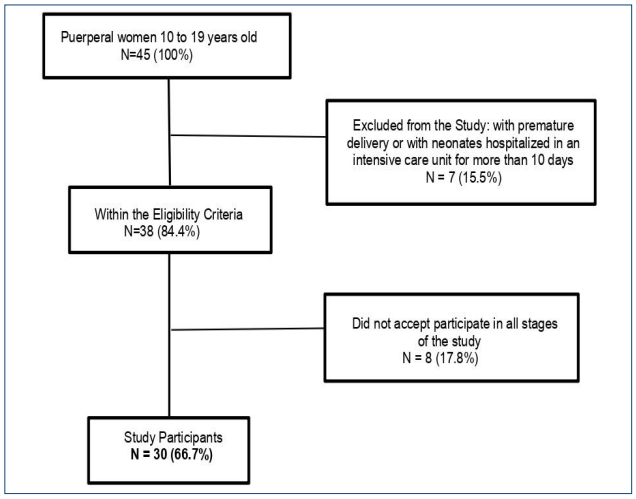

Figure 1. Flow chart of participant selection for the study. Source: Prepared by the author.

Table 1. Association between the effectiveness rates of adolescents during the immediate postpartum period and social and obstetric variables.

Self-efficiency					p
	Moderate	%	High	%	
Age group					
14–16 years	5	62.5	3	37.5	0.4
17–19 years	12	54.6	10	45.4	
Education					
Incomplete elementary	7	77.8	2	22.2	
Complete elementary	4	66.7	2	33.3	0.27
Incomplete high school	4	33.3	8	66.7	
Complete high school	2	66.7	1	33.3	
Religion					
Catholic	10	52.6	9	47.4	0.28
Evangelical	5	62.5	3	37.5	
Does not defined	2	66.7	1	33.3	
Marital status					
Married	3	60	2	40	0.33
Stable union	6	66.7	3	33.3	
Single	8	50	8	50	
Number of people residing in the household					
2 – 4	5	71.4	2	28.6	0.3
5 – 7	9	47.4	10	52.6	
8-10	3	75	1	25	
Planned pregnancy?					
Yes	6	50	6	50	0.4
No	11	61.1	7	38.9	
Had prenatal care?					
Yes	15	53.6	13	46.4	0.32
No	2	100	0	0	
How many appointments?					
0 – 4	3	75	1	25	0.33
5 – 7	5	38.5	8	61.5	
8 – 10	9	69.2	4	30.8	
Received guidance and encouragement to breastfeed ?					
Yes	16	55.2	13	44.8	0.32
No	1	100	0	0	
Who guided/encouraged					
Nurse	14	56	11	44	
Doctor	5	55.6	4	44.4	
Health agent	11	61.1	8	44.4	
Mother	16	64	9	36	0.2
Grandmother	13	76.5	4	23.5	
Sister	5	71.4	2	28.6	
Partner	4	44.4	5	55.6	
Type of delivery					
Cesarean	9	50	9	50	0.37
Vaginal	8	66,7	4	33,3	
Placed the newborn on the chest immediately after delivery?					
Yes	3	50	3	50	0.42
No	14	58.3	10	41.7	
If not at what time did you breastfeed your child?					
After arriving in the room	9	56.2	7	43.8	0.28
The next day	5	83.3	1	16.7	

Juazeiro do Norte, CE, 2020 (n=30). Source: Direct Survey, 2019–2020. p significance level of the chi-square or Fisher's exact test.

Most women did not plan the pregnancy and underwent 5–10 prenatal consultations. The results revealed that there was no statistically significant difference when comparing adolescent mothers with medium and high self-efficacy to breastfeed, considering social and obstetric variables.

The classification and average points based on the score obtained by the questionnaire revealed self-efficacy to breastfeed: medium [17 adolescents (56.6%); 42.2+5.8 points] and high [13 adolescents (43.3%); 56.6+4.6 points]. No postpartum woman showed low self-efficacy.

Table 2 shows the distribution of responses by adolescent mothers according to the technical domain of the BSES-SF questionnaire. In total, 16 (53.3%) of the participants felt that the baby was breastfeeding enough. In addition, 26 (86.7%) adolescents reported not using infant formula or cow's milk as a supplement and practicing exclusive breastfeeding. With regard to latching during the entire feeding, 20 (66.7%) adolescents could not see if it was correct. Eighteen (60%) of the participants reported that they could not control the organization of their routine.

Table 3 shows the distribution of the adolescent mothers' responses according to the intrapersonal thoughts domain of the BSES-SF. In total, 20 (66.6%) of the participants reported dealing with breastfeeding successfully, in the same way that they

Table 2. Distribution of the adolescents' responses during the immediate postpartum period according to the technical domain of the Breastfeeding Self-Efficacy Scale–Short-Form questionnaire.

Domain Technical	Disagree		Sometimes agree		Agree		Totally agree		p
	n	%	n	%	n	%	n	%	
1. I always feel when my baby is getting enough.	5	16.7	9	30	13	43.3	3	10	0.37
3. I always feed my baby without using formula milk as a supplement.	2	6.7	2	6.7	21	70	5	16.7	0.45
4. I always notice that my baby is holding the breast correctly throughout the feed.	3	10	17	56.7	7	23.3	3	10	0.35
6. I can always breastfeed even if my baby is crying.	9	30	6	20	13	43.3	2	6.7	0.37
11. I always breastfeed my baby on one breast and then switch to the other.	2	6.7	8	26.7	13	43.3	7	23.3	0.44
12. I always continue to breastfeed my baby with each feeding. (at each feeding).	0	0	5	16.7	17	56.7	8	26.7	0.47
13. I can always match my needs to the baby's needs. (I organize my bathing, sleeping, feeding needs with the baby's breastfeeding).	5	16.7	13	43.3	8	26.7	4	13.3	0.38
14. I always know when my baby is finished with a feed.	5	16.7	14	46.7	10	33.3	1	3.3	0.36

Juazeiro do Norte, CE, 2019-2020 (n=30). Source: Direct Survey, 2019–2020.

Table 3. Distribution of adolescents' responses during the immediate postpartum period according to the intrapersonal thoughts domain of the Breastfeeding Self-Efficacy Scale–Short-Form questionnaire.

Domain intrapersonal thoughts	Totally disagree		Disagree		Sometimes Agree		Agree		Totally agree		p
	n	%	n	%	n	%	n	%	n	%	
2. I always deal with breastfeeding successfully, just as I deal with other challenges. (Successfully overcome breastfeeding and other life situations).	0	0	3	10	7	23.3	16	53.3	4	13.3	0.42
5. I always handle breastfeeding in ways that satisfy me.	1	3.3	7	23.3	8	26.7	12	40	2	6.7	0.38
7. I always feel like continuing to breastfeed.	0	0	4	13.3	6	20	18	60	2	6.7	0.41
8. I can always breastfeed comfortably in front of my family.	0	0	7	23.3	7	23.3	13	43.3	3	10	0.39
9. I am always satisfied with my breastfeeding experience.	0	0	1	3.3	17	56.7	11	36.7	1	3.3	0.39
10. I can always deal with the fact that breastfeeding takes time. (Even consuming my time I want to breastfeed).	0	0	5	16.7	8	26.7	14	46.7	3	10	0.40

Juazeiro do Norte, CE, 2019–2020 (n=30). Source: Direct Survey, 2019–2020.

dealt with other challenges and expressed a desire to continue breastfeeding. In total, 16 (53.3%) felt comfortable breastfeeding in front of family members, but satisfaction with breastfeeding was mentioned by only 12 (40%) of the participants.

DISCUSSION

The present study revealed that all the evaluated adolescent mothers had medium to high scores of self-efficacy to breastfeed. The analysis of the domains of the scale made it possible to identify aspects that can make the breastfeeding process difficult and that are subject to intervention. The sociodemographic and obstetric variables did not differ in the comparison between the groups with medium and high efficacies to breastfeed.

The identification of confidence to breastfeed among adolescents can contribute to the understanding of their situational context and to the removal of social and structural obstacles that may interfere with the woman's ability to breastfeed in a confident and peaceful way. It is noteworthy the practicality of the BSES-SF instrument applied in the present study with scientific evidence of reliability and validity in all age groups[10].

A Brazilian study carried out in the Northeast region with 172 adolescent mothers using the same instrument used by us showed a predominance of high self-efficacy to breastfeed in 84% of the participants, higher than that observed by us[11].

The absence of mothers with low breastfeeding self-efficacy in our study and in the one mentioned above can be attributed to the important role of the ESF in the dissemination of guidelines on breastfeeding during prenatal care. A previous study showed that the ESF was heavily involved in various activities in the community, with an emphasis on guidance provided during prenatal care for mothers living in the areas covered[7].

It is known that the socioeconomic context in which the adolescent mothers are inserted has a strong relationship with the knowledge, attitudes, and practices of breastfeeding in low- and middle-income countries. Therefore, these factors must be considered in the work of health teams, especially in screening and monitoring, since the limits imposed by poverty, especially in the north and northeast regions of Brazil, imply access to information, care, and adherence to healthy behaviors[12].

In the present study, 50% of the adolescents had completed or discontinued high school. Mothers with a higher level of education tend to be able to effectively breastfeed their babies, as they are able to analyze the external factors that influence this practice in a more conscious and coherent way, preventing them from interfering in the breastfeeding process[13].

The survey revealed that most participants were single. Contrasting the data of the present research, some studies reveal that single mothers have greater difficulty in performing the practice of breastfeeding, due to the physical and emotional exhaustion they face when they feel alone in this process and are unable to maintain breastfeeding[14]. Teenagers' mothers have the greatest influence on their breastfeeding experience, from making the decision to breastfeed or not, to maintaining to continuing[15].

Identifying and recognizing the influences on a woman's decision to breastfeed, in particular, the confidence to breastfeed among adolescent mothers, makes it possible to optimize the support and encouragement of breastfeeding, contributing to the understanding of its social and structural contexts that may impair a woman's ability to breastfeed[16].

Regarding aspects of self-efficacy for breastfeeding, the results of the present study indicated that mothers showed greater adherence to items related to the breastfeeding technique. On the contrary, there was lower adherence regarding the difficulty of breastfeeding the baby when he was crying, highlighting the need for professionals to work on these aspects related to tolerance and seeking to improve the adolescents' self-confidence[17].

The study limitations include the sample size, the fact that it was carried out in a single municipality in the interior of Ceará, and the cross-sectional design that makes it impossible to establish causal relationships, limiting the findings to the population. It is worth noting that the present work contributed relevant information to the practical field of collective health, given the existence of incipient information on the self-efficacy of adolescent mothers monitored by the ESF for the management of breastfeeding. It also made it possible to identify in which aspects mothers need to be primarily helped, using them in the planning of actions in primary health care and in the identification of groups with greater vulnerability to early weaning.

CONCLUSION

It was found that the adolescent mothers accompanied by the ESF during prenatal care presented medium and high self-efficacy in breastfeeding in the immediate postpartum period, evidencing new knowledge in relation to vulnerability to breastfeeding for this specific public, which resides in a municipality in the interior of Ceará, in general.

Factors that interfere with maternal self-efficacy, when identified early and carefully addressed by the health professional with family support, are more easily resolved, encouraging the puerperal woman to maintain breastfeeding, increasing the duration of exclusive breastfeeding, and, consequently, reducing infant morbidity and mortality.

It is suggested that further studies be carried out using the BSES-SF, as this instrument can help health professionals in prenatal and childcare consultations (longitudinal studies) in order to help plan actions and decisions for qualified and effective care.

ETHICAL ASPECTS

The study was approved by the Research Ethics Committee of the Centro Universitário Doutor Leão Sampaio (UNILEÃO) with the opinion number: 3.277.812. Families received information, risks, and benefits from the study. The adolescent puerperal women received term of assente and their responsible a TCLE.

AUTHORS' CONTRIBUTIONS

AMVA: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **ROSS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

REFERENCES

1 Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st Century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475-90. https://doi.org/10.1016/S0140-6736(15)01024-7

2 Ministério da Saúde. Secretaria de atenção à saúde; departamento de atenção básica. saúde da criança: aleitamento materno e alimentação complementa. 2nd ed. Brasília: Ministério da Saúde; 2015. p. 184. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/saude_crianca_nutricao_aleitamento_alimentacao.pdf

3 Ministério da Saúde. Secretaria de atenção primaria à saúde; Departamento de Promoção da Saúde. Guia alimentar para crianças brasileiras menores de 2 anos. Brasília: Ministério da Saúde; 2019. p. 265. Available from: http://189.28.128.100/dab/docs/portaldab/publicacoes/guia_alimentar_2anos.pdf

4 Universidade Federal do Rio de Janeiro. Aleitamento materno: prevalência e práticas de aleitamento materno em crianças Brasileiras menores de 2 anos 4: ENANI 2019. Rio de Janeiro, RJ: UFRJ; 2021. Available from: https://enani.nutricao.ufrj.br/index.php/relatorios/

5 Carreiro JA, Francisco AA, Abrão ACFV, Marcacine KO, Abuchaim ESV, Coca KP. Dificuldades relacionadas ao aleitamento materno: análise de um serviço especializado em amamentação. Acta Paul Enferm. julho de 2018;31(4):430-8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-21002018000400430&lng=pt&tlng=pt

6 Margotti E, Margotti W. Fatores relacionados ao aleitamento materno exclusivo em bebês nascidos em hospital amigo da criança em uma capital do norte Brasileiro. Saúde Debate. 2017;41(114):860-71. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103- 11042017000300860&lng=pt&tlng=pt

7 Pereira LB, Ferreira TN, Silva MFFS, Souza AAM. Autoeficácia em amamentação e fatores interligados. Rev Rene. 2018;19:1-7. Available from: https://www.redalyc.org/articulo.oa?id=324054783006

8 Noronha DD, Moura LP e, Oliveira JM, Torres JDRV, Oliveira KCF, Teles MAB. Percepção de mães cadastradas em uma estratégia saúde da família sobre aleitamento materno exclusivo. Revista de Enfermagem UFPE Online. 2017;11(3):1403-9. Available from: https://periodicos.ufpe.br/revistas/revistaenfermagem/article/view/13983

9 Dodt RCM. Aplicação e validação da breastfeeding self-efficacy scale-short form (Bses-sf); 2008. Available from: http://www.repositorio.ufc.br/handle/riufc/2018

10 Dodt RCM, Ximenes LB, Almeida PC, Batista Oriá MO, Dennis CL. Psychometric and maternal sociodemographic assessment of the breastfeeding self-efficacy scale - short form in a Brazilian sample. JNEP. 2012;2(3):66. Available from: http://www.sciedu.ca/journal/index.php/jnep/article/view/627

11 Bizerra RL, Carnaúba JP, Chaves AFL, Rocha RS, Vasconcelos HCA, Oriá MOB. Autoeficácia em amamentar entre mães adolescentes. Rev Eletr Enf. 2015;17(3). Available from: http://revistas.ufg.emnuvens.com.br/fen/article/view/31061

12 Abreu LM, Filipini R, da Costa Aguiar Alves B, da Veiga GL, Fonseca FLA. Evaluation of breastfeeding self-efficacy of puerperal women in shared rooming units. Heliyon. 2018;4(10):e00900. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405844018307618

13 Ferreira HLOC, Oliveira MF, Bernardo EBR, Almeida PC de, Aquino PS, Pinheiro AKB. Fatores associados à adesão ao aleitamento materno exclusivo. Ciênc Saúde Coletiva. 2018;23(3):683-90. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413- 81232018000300683&lng=pt&tlng=pt

14 Turke KC, Santos LR dos, Matsumura LS, Sarni ROS. Risk factors for the lack of adherence to breastfeeding. Rev Assoc Med Bras. 2021;67(1):107-14. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-42302021000100107&tlng=en

15 Fernandes RC, Höfelmann DA. Intenção de amamentar entre gestantes: associação com trabalho, fumo e experiência prévia de amamentação. Ciênc Saúde Coletiva. 2020;25(3):1061-72. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413- 81232020000301061&tlng=pt

16 Coca KP, Pinto VL, Westphal F, Mania PNA, Abrão ACFV. Conjunto de medidas para o incentivo do aleitamento materno exclusivo intra-hospitalar: evidências de revisões sistemáticas. Rev Paul Pediatr. 2018;36(2):214-20. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-05822018000200214&lng=pt&tlng=pt

17 Viana RMS, Cassino L. Aleitamento materno: fortalecedor do vínculo afetivo entre mãe e filho. Revista Brasileira de Ciências da Vida. 2017;5(2):1-25. Available from: http://jornalold.faculdadecienciasdavida.com.br/index.php/RBCV/article/view/22

REVIEW ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20220938

Efficacy of invasive techniques in physical therapy for migraine treatment and prevention: a systematic review of randomized controlled trials

Giorgia Lonzar[1], Vanesa Abuín-Porras[1], Jose A Del-Blanco-Muñiz[1], Ángel González-de-la-Flor[1], Guillermo García-Pérez-de-Sevilla[1], Diego Domínguez-Balmaseda[1,2*]

INTRODUCTION

Approximately 14% of the world population suffers from migraine, a highly debilitating idiopathic primary headache[1].

The classification and diagnosis of migraines are carried out according to the International Classification of Headache Disorders (ICHD-3), based primarily on monthly migraine frequency and the manifestation of aura. More than 15 attacks per month is considered chronic and ≤15 episodic[2].

The etiology of this autonomic dysfunction is unclear, but a plausible hypothesis suggests peripheral and/or central sensitization. Generally, a migraine attack develops in three or four successive stages in which the onset of pain is determined by the trigeminal-vascular system. Parasympathetic activity increases in the intracranial arteries and fires the first-order neurons of this structure, sending nociceptive information from the durometer to the trigeminal ganglion. The information is then forwarded to the brainstem, in the spinal trigeminal nucleus (STN), via second-order trigeminal vascular neurons. The trigeminovascular neurons of the third-order STN are located in the thalamus, and from there, the nociceptive information is finally sent to the somatic-sensory cortex[3].

Migraine medication is known to induce moderate-to-severe adverse effects, and the prophylactic treatments' effectiveness is only 50–60%, further decreasing in the chronic modality[4,5]. Nonpharmacological treatments for migraine prophylaxis include physical activity, relaxation, and physiotherapy. Physiotherapy, in particular, includes treatments such as neuromodulation, acupuncture, and myofascial release techniques.

Neuromodulation refers to any intervention (drug or physical agent) that can induce a stimulating or inhibiting effect on a neurological function[6].

Another treatment with neuromodulatory effects is acupuncture—a Traditional Chinese medicine therapy that obtains therapeutical benefits by stimulating specific points in the body (acupoints)[5]. Acupoints usually correspond with nerve fibers and terminals, which, when stimulated, modulate the information they emit[7].

Myofascial treatment is another physiotherapy approach used for migraine prevention. This therapy, through techniques such as dry needling (DN), treats pain induced by myofascial trigger points (TrPs), and hyperirritable loci caused by dysfunctional motor endplates. When palpated or when the muscle harboring them gets activated or stretched, TrPs induce referred and/or local pain[8]. TrPs in the craniocervical area constantly emit nociceptive inputs to the STN, facilitating its sensitization and, therefore, predisposing both the onset and chronification of migraine[1].

Despite being a recent systematic review on the subject, the articles included are not recent, which justifies an update on the subject.

OBJECTIVES

The main objective of this systematic review was to update scientific knowledge regarding invasive physiotherapy techniques for migraine prophylaxis. Secondary objectives include identifying which of the therapies has greater clinical relevance and implies the best risk-benefit assessment. A comparison among techniques, and, possibly, with drug therapies, will be made.

METHODS

Study design

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements[9].

[1]Universidad Europea de Madrid, Faculty of Sport Sciences, Department of Physiotherapy – Madrid, Spain.
[2]Universidad Europea de Madrid, Faculty of Health Sciences, Masmicrobiota Group – Madrid, Spain.
*Corresponding author: diego.dominguez@universidadeuropea.es
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on October 14, 2022. Accepted on October 14, 2022.

This systematic review has been registered in PROSPERO (International prospective register of systematic reviews) in November 2021, with registration number CRD42021287200.

Literature search strategy

Randomized controlled trials (RCTs) included in the review were selected from three different databases: Academic Search Ultimate, CINAHL with Full Text, and Medline Complete. Initially, PubMed, Cochrane, and Google Scholar were searched to identify the latest systematic reviews on migraine prevention via invasive physiotherapy techniques using key terms such as migraine prevention/prophylaxis, invasive physiotherapy, dry needling, acupuncture, and percutaneous electrostimulation. References from included studies were also searched for any relevant study.

The utilized MESH terms for identifying RCTs were acupuncture OR electroacupuncture OR dry needling OR percutaneous nerve stimulation AND migraine.

Inclusion criteria

RCTs published in the last 5 years and available on Academic Search Ultimate, CINAHL with Full Text, and Medline Complete were identified.

Inclusion criteria were the following:

1. RCTs including participants aged between 18 and 80 years and diagnosed with episodic or chronic migraine with or without aura according to the ICHD-3.
2. PEDro score ≥6/10.
3. Main purpose of the study: to evaluate the preventive effects of invasive physiotherapy techniques compared with other treatments (placebo or pharmacology).
4. Type of interventions: acupuncture, DN, and percutaneous electrostimulation.
5. Types of outcome measures: change in monthly migraine days (frequency, intensity, and duration), acute medication intake, adverse events, pain pressure threshold, cervical range of motion (ROM), or muscle thickness.

Data extraction

Two researchers (GL, DDB) autonomously carried out the data selection and extraction. If disagreement occurred at any stage, a third author considered the available information, or if necessary, the study authors were contacted for clarification. When eligibility could not be determined in cases of disagreement, both researchers discussed the study based on its relevance to inclusion and exclusion criteria, interventions used, and outcomes measured to reach an accord. We obtained the aid of a third reviewer in instances when common ground could not be attained.

Quality assessment

RCT quality was assessed based on the PEDro scale criteria. All studies scored ≤6/10 on PEDro.

RESULTS

Study selection

A total of 1,465 articles were identified using the keywords and MESH terms; 190 articles were dismissed in the primary phase, and later 370 titles and abstracts were analyzed. Finally, nine articles satisfied eligibility criteria (Figure 1).

Study characteristics

This review included nine randomized controlled trials that comprehensively analyzed 1,054 participants. Acupuncture was compared with pharmacotherapy in three studies, combined with pharmacotherapy in two, and compared with sham or no treatment in four studies. Overall results outlined acupuncture to be significantly effective in reducing migraine frequency. Acupuncture's effectiveness was significantly higher than sham or no treatment; compared to pharmacotherapy, it was at least as effective.

Finally, both DN and percutaneous electrostimulation obtained similar results for decreasing migraine frequency compared to sham treatment. Table 1 describes a detailed summary of this section, in which we can make a detailed comparison of the most relevant studies of invasive physiotherapy treatment for migraine.

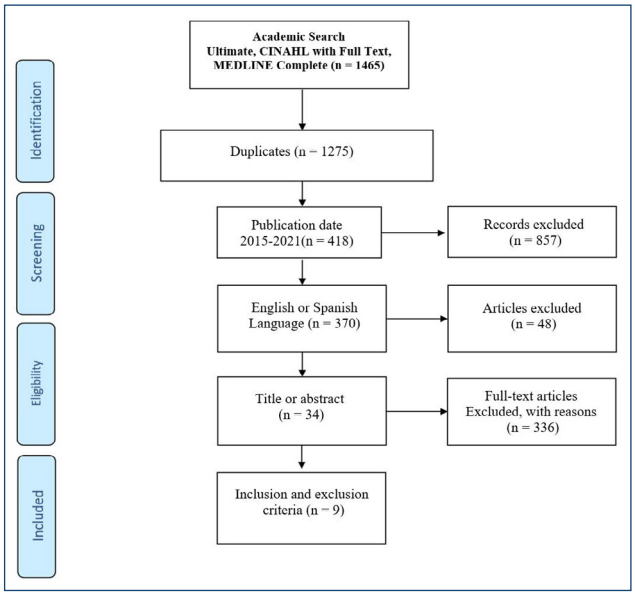

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart.

Table 1. Summary of invasive physiotherapy for migraine prevention.

Authors	Study	Participants	Outcomes	Interventions	Results
Li and Xu[17]	RCT	n=62 Monthly migraine frequency ≥2 Migraine history >3 months Age 18–70 years	Migraine days/month. Episodes/month RR 50% per month Headache days/month. Acute medication intake/month. Follow-up immediately post treatment.	12 weeks treatment. Group 1: PES 30'/day, 5 days/week´12 weeks. Group 2: placebo PES 30'/day, 5 days/weeks´12 weeks.	PES: ↓ migraine frequency versus placebo and baseline.
Rezaeian et al.[12]	RCT	n=40 Migraine of myofascial origin.	Monthly frequency, intensity, and migraine duration. Acute medication intake. Muscle thickness of SCM. Pain pressure threshold (PPT) in the SCM. Active cervical ROM. Follow-up up to 1-month post treatment.	Group 1: 3 DN sessions in the sternocleidomastoid muscle (SCM) in 1 week. 48 h between sessions. 8–10 functions/session. Group 2: 3 sham DN sessions (no penetration). Same frequency as group 1.	Group 1: migraine frequency, intensity, duration ↓, PPT ↑, active cervical ROM ↑ and muscle thickness of SCM ↑ compared to baseline and to CG. Results persisted in the 1-month follow-up.
Musil et al.[19]	RCT	n=86 Migraine history ≥12 months ≥4 migraines in the last 4 weeks. Migraine with and without aura.	Migraine days/4 weeks. RR 50%. Acute medication intake/4 weeks. Quality of Life (MIDAS) 6 months post-treatment follow-up.	*Preventive drugs allowed in both groups. 12 w of treatment. Group 1: 14 sessions of 25' semi-standardized acupuncture. 2 sessions/w in w 1–4, 1 session/w in w 5–8, 1 session/14 days in w 9–12. Group 2: waiting list.	Group1: migraine frequency ↓, acute medication intake ↓, 50% RR ↑ versus group 2 and baseline.
Wang et al.[20]	RCT	n=50 Migraine history ≥12 months ≥5 migraine days/4 weeks. Age of 18–80 years.	Migraine days/4 weeks. Migraine duration. Migraine intensity. RR 50%. Acute medication intake. Quality of life (McGill questionnaire and MSQ). Pressure pain threshold. 1-year post-treatment follow-up.	20 w treatment. Preventive drugs allowed in both groups. Group 1: 16 25' semi-standardized acupuncture reaching "de qi." 2 sessions/1 in w 1–4, 1 session/w in w 5–8, 1 session/2 w in w 9–12, 1 session/ month in w 13–20. Group 2: same protocol of sham acupuncture (insertion and no insertion).	Group 1: migraine days ↓, attack intensity ↓, 50% RR ↑, pressure pain threshold ↑. Results persisted in the 3 months follow-up but not in the 1-year follow-up.
Zhao et al.[5]	RCT	n=249 2–8 migraines/month Age 18–65 years	Change in migraine frequency from baseline to w 16. Migraine days/month. Average migraine intensity/month. Average acute medication intake/every 4 weeks for 24 weeks. Anxiety (SAS) Depression (SDS) Quality of life (MSQ). 20 weeks post-treatment follow-up.	4 w of treatment; no preventive drugs allowed Group 1: 20 30' semi standardized electro-acupuncture, reaching De qi. 5 session/w – 2 days' rest´4 w. Frequency 2/100 Hz (alternating every 3 s) intensity 0.1–1.0 mA. Group 2: sham electro-acupuncture in not acupoints. Same parameters of electrostimulation as group 1, but without reaching de qi. Group 3: waiting list.	True acupuncture: migraine frequency ↓ versus sham and baseline.
Xu et al.[21]	RCT	n=147 Migraine without aura, Age 15–65 years. Migraine history >12 months. Onset <50 years. 2–8 attacks/month. Not being familiarized with acupuncture.	Change in number of migraine days/4 weeks cycles from baseline to week 20 after randomization. RR 50% in weeks 17–20. Attack intensity. Quality of life (MSQ and MIDAS) Sleep quality (PSQI) Anxiety (BAI) Depression (BDI-II) Change in acute medication intake from baseline to week 20. Expectations on acupuncture efficacy. 3 months post-treatment follow-up.	8 w of treatment. Group 1: 20´30' session of semi-standardized acupuncture. Frequency: 10 days alternating/9 days rest/10 days alternating + usual care. Group 2: 20´30' sham acupuncture without needle penetration. Usual care. Same protocol as group 1. Group 3: usual care.	Real acupuncture: quality of life ↑, migraine days ↓, migraine attacks ↓ versus baseline and other groups.

Continue...

Table 1. Continuation.

Authors	Study	Participants	Outcomes	Interventions	Results
Naderinabi et al.[16]	RCT	n=150 Chronic migraines. Ages 20–60 years. No liver or coagulation dysfunctions.	Pain intensity. Migraine days/ month. Lost days (work, school, family) due to migraine. Acute medication intake. Associated symptoms. Follow-up 1-, 2-, and 3-months post-treatment.	127 days duration. Group 1 and 2 without drug prevention. Group 1: 60´30' sessions of manual acupuncture. Reaching De qi. 2 cycles of 30 sessions carried out in 60 days. 1 w rest between cycles. Group 2: botulinum toxin in 31 facial and pericraneal acupoints. Group 3: sodium valproate 500 mg/day for 3 months.	Group 1: average pain intensity ↓, migraine days ↓ vs group 2 and 3. *All 3 groups significantly benefitted versus baseline: group 1 demonstrated more efficacy and less AEs.
Nie et al.[23]	RCT	n = 135 Migraine with or without aura. migraine history ≥12 months. Frequency of ≥2. ≤6 attacks/month. Onset < 50 years of age. Age 18–65 years.	Monthly migraine frequency. Average migraine duration/ month. Migraine intensity. Acute medication intake. Associate symptoms. Quality of life (PRO). Clinical efficacy. Follow-up 1-month post-treatment.	12 w duration. Group 1: 14´30' semi-standardized acupuncture reaching de qi + 10' Tuina massage therapy. 2 sessions/w in w 1–4, 1 session/w in w 5–8 and 1 session/14 days in w 9–12. Group 2: only acupuncture, same protocol as group 1. Group 3: drug prevention.	All outcomes improved significantly in all groups. Groups 1 and 2 improved significantly more than group 3. Group 1 improved significantly more than group 2. Tuina therapy improves acupuncture's efficacy.
Giannini et al.[18]	RCT	n=135 Episodic migraines with or without aura. No previous preventive treatment in the last 3 months. Ages>18 years.	Headache frequency. Migraine frequency. Acute medication intake. Quality of life and of migraines (MIDAS y SF-36). Treatment satisfaction. RR 50%. Withdrawals from trial. Depression and anxiety (SDS y SAS). 3 y 6-month follow-up.	4 months duration. Group 1: 12 semi-standardized acupuncture sessions. 2 in the 1st week and then 1 session/w. Group 2: personalized preventive drugs 4 months.	Migraine and headache frequency ↓ significantly in both groups, (↔ difference between groups). In the 3rd and 6th months follow-up benefits persisted significantly ↑ in the acupuncture group. No AEs in group 1.

RCT: randomized controlled trials; N: number of patients; AEs: adverse effects; PES: percutaneous electro-stimulation; RR 50%": 50% responders' rate; ROM: range of movement; SCM: sternocleidomastoid; MSQ: migraine-specific quality of life questionnaire; SAS: Zung self-rating anxiety scale; PSQUI: Pittsburg quality sleep index; BAI: Beck anxiety inventory; BDI-II: Beck depression inventory-II; SF-36: short form health survey 36 items; PRO: patient reported outcome (for quality of life); PPT: pressure pain threshold; CG: control group; MIDAS: migraine disability assessment score.

DISCUSSION

Despite having relatively low effectiveness and the common adverse effects it implies, pharmacological treatment is still the first-line therapy for migraine prophylaxis and acute management. The low tolerability of this therapy is the reason why researchers have been seeking for alternatives in the past years. In this regard, specific invasive physiotherapy techniques have been the subject of study for migraine prevention; these therapies include neuromodulation via PES, acupuncture, and DN[6-8].

TrPs are more preponderant and appear to be contributing notably to migraine[10]. Their treatment in muscles associated with the STN has demonstrated to be effective in improving conditions of migraine patients[11].

The study included in this review supports DN for migraine prophylaxis as it significantly reduced migraine frequency and was significantly superior to placebo. In addition, DN improved cervical ROM, muscle belly thickness, and reduced acute medication intake[12].

Considering migraine frequency and comparing DN with acupuncture and PES, DN obtained significant benefits with only three sessions performed in 1 week, which, in addition, persisted throughout the follow-up month. Considering the abovementioned techniques, DN appears to be the fastest to decrease migraine frequency and acute medication intake when compared to the other analyzed techniques.

PES is one of the most validated neuromodulation techniques for migraine prevention[6]. The study included in this review[13] used PES on the Taiyang EX-HN 5 acupoint, coinciding with the zygomaticotemporal nerve derived from the zygomatic nerve (branch of CN V2). Most acupoints of the face and forehead correspond to cutaneous or terminal branches of the facial and trigeminal nerves, both anatomically related to migraine[14].

Analgesic electrostimulation applied in these locations stimulates the production of serotonin and substances

analogous to endogenous morphine, lacking in migraine patients and essential to improve their condition[15]. The analyzed study did not consider such a parameter, but it is likely that the reduction in migraine frequency was favored by it. This study had only a short-term follow-up and demonstrated that PES significantly improved migraine frequency and 50% of the RR parameters.

The main limitation is that the implemented protocol had an extremely high volume of sessions (60) carried out with a 5 days/week frequency for 12 weeks. Surprisingly, the abandon rate was only ≈11%. An acupuncture study[16] shared the total number of sessions, but diluted them in 4.5 months, impacting less on daily life.

Acupuncture differs from conventional neuromodulation due to its different clinical reasoning and foundational theories. Among its various application modalities are manual and electroacupuncture. The latter seems to achieve faster and longer-lasting analgesia[17]. In the analyzed study[5], electroacupuncture induced significant preventive effects for migraine in only 4 weeks; these persisted up to 5 months post-treatment. Compared to manual acupuncture studies[18-20], even though the total number of sessions (20) and its duration (25–30 min) were similar, electroacupuncture prescribed a higher treatment frequency (5 days/week) during a shorter intervention period (4 weeks). Additionally, in contrast to other acupuncture protocols, which gradually diluted treatment frequency[18-20], the intervention was interrupted abruptly.

Considering efficacy compared to placebo, manual acupuncture is significantly superior to sham acupuncture after 12–13 weeks from baseline[5,20,21]; meanwhile, electroacupuncture is superior already after 4 weeks[5]. Concerning significant improvements from baseline, both manual[22] and electroacupuncture[7] are effective after approximately 4 weeks, but with different statistical values ($p=0.026$ and $p<0.001$, respectively).

According to the reviewed acupuncture studies[16,18-20,23], this therapy significantly decreased acute medication intake in all cases except one, in which baseline levels were already extremely low[18]. On the contrary, drug intake improved in the short term and during the 1-month follow-up in all analyzed studies. These results persisted in the long term, except for one study, in which the dropout rate in follow-up was particularly high[20]. As for DN[12] and PES[13], which also effectively improved this parameter, benefits were traceable only in the short term due to a lack of long-term follow-up.

Some of the acupuncture studies included in the current review[5,13,21] also analyzed parameters related to quality of life and migraine impact on life. In this regard, variables such as sleep quality and migraine-related missed workdays[16] were also included.

Quality of life was measured either with MSQ (*Migraine-specific quality of life questionnaire)* or MIDAS *(migraine disability assessment scale*). All analyzed acupuncture protocols recorded improvements of such parameters.

PES and DN studies did not include quality of life or migraine impact on life as dependent variables[12,13].

LIMITATIONS

This review has a number of limitations. First, some studies only include patients whose migraine depended directly on the TrPs of the sternocleidomastoid. Second, the terms of the use of prophylactic drugs were not specified; a detail that could mainly distort the results.

CONCLUSION

According to the analyzed studies, all investigated techniques are promising options for migraine prophylaxis, either in combination with or in substitution of pharmacotherapy.

Since most of the studies assessed acupuncture, a rigorous comparison with the other considered therapies was not viable.

ACKNOWLEDGMENTS

We would like to thank the European University of Madrid for having facilitated this study.

AUTHORS' CONTRIBUTIONS

GL: Investigation (equal), Methodology (equal), Writing – original draft (equal). **VAP:** Formal Analysis (equal), Investigation (equal), Project administration (equal), Software (equal), Writing – review & editing (equal). Á**GF:** Methodology (equal), Writing – review & editing (equal). **GGPS:** Formal Analysis (equal), Investigation (equal), Writing – review & editing (equal). **JADBM:** Methodology (equal), Writing – review & editing (equal), Supervision (equal). **DDB:** Methodology (equal), Supervision (equal), Writing – review & editing (equal).

REFERENCES

1. Burch RC, Buse DC, Lipton RB. Migraine: epidemiology, burden, and comorbidity. Neurol Clin. 2019;37(4):631-49. https://doi.org/10.1016/j.ncl.2019.06.001
2. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38(1):1-211. https://doi.org/10.1177/0333102417738202
3. Burstein R, Noseda R, Borsook D. Migraine: multiple processes, complex pathophysiology. J Neurosci. 2015;35(17):6619-29. https://doi.org/10.1523/JNEUROSCI.0373-15.2015
4. Urits I, Patel M, Putz ME, Monteferrante NR, Nguyen D, An D, et al. Acupuncture and its role in the treatment of migraine headaches. Neurol Ther. 2020;9(2):375-94. https://doi.org/10.1007/s40120-020-00216-1
5. Zhao L, Chen J, Li Y, Sun X, Chang X, Zheng H, et al. The long-term effect of acupuncture for migraine prophylaxis: a randomized clinical trial. JAMA Intern Med. 2017;177(4):508-15. https://doi.org/10.1001/jamainternmed.2016.9378
6. Moisset X, Pereira B, Ciampi de Andrade D, Fontaine D, Lantéri-Minet M, Mawet J. Neuromodulation techniques for acute and preventive migraine treatment: a systematic review and meta-analysis of randomized controlled trials. J Headache Pain. 2020;21(1):142. https://doi.org/10.1186/s10194-020-01204-4
7. Zhou W, Benharash P. Effects and mechanisms of acupuncture based on the principle of meridians. J Acupunct Meridian Stud. 2014;7(4):190-3. https://doi.org/10.1016/j.jams.2014.02.007
8. Barbero M, Schneebeli A, Koetsier E, Maino P. Myofascial pain syndrome and trigger points: evaluation and treatment in patients with musculoskeletal pain. Curr Opin Support Palliat Care. 2019;13(3):270-6. https://doi.org/10.1097/SPC.0000000000000445
9. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. https://doi.org/10.1136/bmj.n71
10. Do TP, Heldarskard GF, Kolding LT, Hvedstrup J, Schytz HW. Myofascial trigger points in migraine and tension-type headache. J Headache Pain. 2018;19(1):84. https://doi.org/10.1186/s10194-018-0913-8
11. Gandolfi M, Geroin C, Valè N, Marchioretto F, Turrina A, Dimitrova E, et al. Does myofascial and trigger point treatment reduce pain and analgesic intake in patients undergoing onabotulinumtoxinA injection due to chronic intractable migraine? Eur J Phys Rehabil Med. 2018;54(1):1-12. https://doi.org/10.23736/S1973-9087.17.04568-3
12. Rezaeian T, Mosallanezhad Z, Nourbakhsh MR, Noroozi M, Sajedi F. Effects of dry needling technique into trigger points of the sternocleidomastoid muscle in migraine headache: a randomized controlled trial. Am J Phys Med Rehabil. 2020;99(12):1129-37. https://doi.org/10.1097/PHM.0000000000001504
13. Li H, Xu QR. Effect of percutaneous electrical nerve stimulation for the treatment of migraine. Medicine (Baltimore). 2017;96(39):e8108. https://doi.org/10.1097/MD.0000000000008108
14. Schwedt TJ, Vargas B. Neurostimulation for treatment of migraine and cluster headache. Pain Med. 2015;16(9):1827-34. https://doi.org/10.1111/pme.12792
15. Biçer M, Bozkurt D, Çabalar M, Işıksaçan N, Gedikbaşı A, Bajrami A, et al. The clinical efficiency of acupuncture in preventing migraine attacks and its effect on serotonin levels. Türkiye Fiz Tıp ve Rehabil Derg. 2017;63(1):59-65. https://doi.org/10.5606/tftrd.2017.45578
16. Naderinabi B, Saberi A, Hashemi M, Haghighi M, Biazar G, Abolhasan Gharehdaghi F, et al. Acupuncture and botulinum toxin A injection in the treatment of chronic migraine: a randomized controlled study. Caspian J Intern Med. 2017;8(3):196-204. https://doi.org/10.22088/cjim.8.3.196
17. Schliessbach J, Van Der Klift E, Arendt-Nielsen L, Curatolo M, Streitberger K. The effect of brief electrical and manual acupuncture stimulation on mechanical experimental pain. Pain Med. 2011;12(2):268-75. https://doi.org/10.1111/j.1526-4637.2010.01051.x
18. Giannini G, Favoni V, Merli E, Nicodemo M, Torelli P, Matrà A, et al. A randomized clinical trial on acupuncture versus best medical therapy in episodic migraine prophylaxis: the ACUMIGRAN study. Front Neurol. 2021;11:570335. https://doi.org/10.3389/fneur.2020.570335
19. Musil F, Pokladnikova J, Pavelek Z, Wang B, Guan X, Valis M. Acupuncture in migraine prophylaxis in Czech patients: an open-label randomized controlled trial. Neuropsychiatr Dis Treat. 2018;14:1221-8. https://doi.org/10.2147/NDT.S155119
20. Wang Y, Xue CC, Helme R, Da Costa C, Zheng Z. Acupuncture for frequent migraine: a randomized, patient/assessor blinded, controlled trial with one-year follow-up. Evid Based Complement Alternat Med. 2015;2015:920353. https://doi.org/10.1155/2015/920353
21. Xu S, Yu L, Luo X, Wang M, Chen G, Zhang Q, et al. Manual acupuncture versus sham acupuncture and usual care for prophylaxis of episodic migraine without aura: multicentre, randomised clinical trial. BMJ. 2020;368:m697. https://doi.org/10.1136/bmj.m697
22. Linde K, Allais G, Brinkhaus B, Fei Y, Mehring M, Vertosick EA, et al. Acupuncture for the prevention of episodic migraine. Cochrane Database Syst Rev. 2016;2016(6):CD001218. https://doi.org/10.1002/14651858.CD001218
23. Nie L, Cheng J, Wen Y, Li J. The effectiveness of acupuncture combined with tuina therapy in patients with migraine. Complement Med Res. 2019;26(3):182-94. https://doi.org/10.1159/000496032

REVIEW ARTICLE

AOP

https://doi.org/10.1590/1806-9282.20221374

Endofibrosis as a cause of peripheral artery disease: a comprehensive review and proposal of two novel algorithms for diagnosis and treatment

Tereza Mazurová[1,2*], Ilker Sengul[3,4], Daniel Toman[2,5], Anton Pelikán[2,5,6], Demet Sengul[7], Miloslav Mazur[1,2], Petr Vávra[2,5], Václav Procházka[8,9]

INTRODUCTION

Endofibrosis is a rare condition affecting blood vessels, occurring mainly among young healthy athletes. This condition arises as progressive stenosis of the iliac arteries, which attenuates the blood circulation of the limb, thus leading to pain during movement. Iliac artery compression was first described in 1984 among professional cyclists[1]. Some authors have reported that up to 10–20% of top athletes are affected[2,3]. Endofibrosis is one of the rare causes of peripheral artery disease, in which the exact prevalence is unknown, possessing no any accurate data.

This comprehensive review is purposed to sum up the current knowledge of endofibrosis and ensure concise information about its etiology, and diagnostic and treatment modalities. In addition, two cases including imagery are purposed to be presented to illustrate the perioperative findings.

METHODS

Input data for endofibrosis have been limited. Our search was carried out in Cochrane, PubMed, EMBASE, and UpToDate databases using keywords "endofibrosis," "iliac artery compression," and "cyclists." A total of 233 articles were selected. Of these, 183 articles had not been relevant for the study. Also, 30 case or original studies with minimal groups of the patients were excluded from the study. The remaining 20 articles had been included in our review.

Epidemiology and etiology

Endofibrosis is characterized by iliac artery stenosis, with a predilection for the external iliac artery (EIA). It mostly affects athletes, of which 80% of the cases are performance cyclists, but runners, football players, cross-country skiers, and others can also be affected[4]. The disease occurs in men 8–10 times more often than in women.

Stenosis often occurs due to anatomic, mechanic, and postural causes[5]. Repeated hip hyperflexion causes trauma to the vessel wall and hypertrophy of the psoas muscle, leading to the psoas compression of the artery, thereby causing stenosis[6]. The affected vessel restricts the blood supply to the limb, and the leg becomes ischemic, which leads to pain during sports activities.

Symptoms

Patients with endofibrosis are often entirely asymptomatic during routine activities, with difficulties appearing only at the maximal limb stress. It can manifest as femoral claudication, limb weakness, numbness, or, less frequently, swelling[7].

Diagnosis

Early diagnosis is the cornerstone of successful treatment. Although the top athletes have their own doctors, these physicians might have minimal experience with endofibrosis. In addition, the available literature data suggest that the vascular surgeon is the most relevant physician for this phenomenon. A detailed

[1]AGEL Ostrava Vitkovice Hospital, Department of Surgery – Ostrava, Czech Republic.
[2]Ostravská Univerzita, Faculty of Medicine, Department of Surgery – Ostrava, Czech Republic.
[3]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
[4]Giresun University, Faculty of Medicine, Department of Surgery – Giresun, Turkey.
[5]University Hospital Ostrava, Department of Surgery – Ostrava, Czech Republic.
[6]Univerzita Tomáše Bati ve Zlíně, Department of Surgery – Zlin, Czech Republic.
[7]Giresun University, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
[8]Ostravská Univerzita, Faculty of Medicine, Department of Surgery – Ostrava, Czech Republic.
[9]University Hospital Ostrava, Department of Radiology – Ostrava, Czech Republic.
*Corresponding author: demet.sengul.52@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on: October 13, 2022. Accepted on October 13, 2022.

personal history, signs and symptoms, low ankle-brachial index (ABI), and Doppler sonographic examination of the lower limb arteries are essential to determine the correct diagnosis of endofibrosis[8]. Lim et al.[9] highlighted the importance of the ABI measurements before and within 1 min after an exercise activity. The authors described a significant attenuation in the blood pressure by 21–40 mmHg in patients with endofibrosis. Furthermore, the specific cycle ergometer-based protocols are more appropriate for the diagnosis of endofibrosis than a standard treadmill[9].

Pathological ABI values appear in 85% of patients with endofibrosis after exercise, with the sensitivity being high[10]. If the ABI value drops by 0.5 within the first minute of the exercise, the sensitivity is 80–85% (but the specificity was not reported)[11]. If the drop of ABI is by 0.66 or more after 1 min of physical exercise, the sensitivity increases to 90%, with a specificity of 87%[12]. At this end, the importance of abundance for pathological ABI data is an emerging knowledge for physicians.

Color Doppler sonography is the method of choice in diagnosing endofibrosis, mostly due to its noninvasive nature and high sensitivity of up to 85%[13]. The results are relatively normal during rest examination; however, after provocative maneuvers, such as examination in hip flexion or immediately after exercise, the results become pathological. Peak systolic velocity (PSV) is significantly higher in the symptomatic limb, which supports the high sensitivity of this method in diagnosing endofibrosis[4]. Other available imaging modalities include digital subtraction angiography (DSA), computed tomography angiography (CTA), and magnetic resonance imaging (MRI)[14]. Our novel proposal for the diagnostic algorithm of this entity, according to the available literature, is depicted in Figure 1.

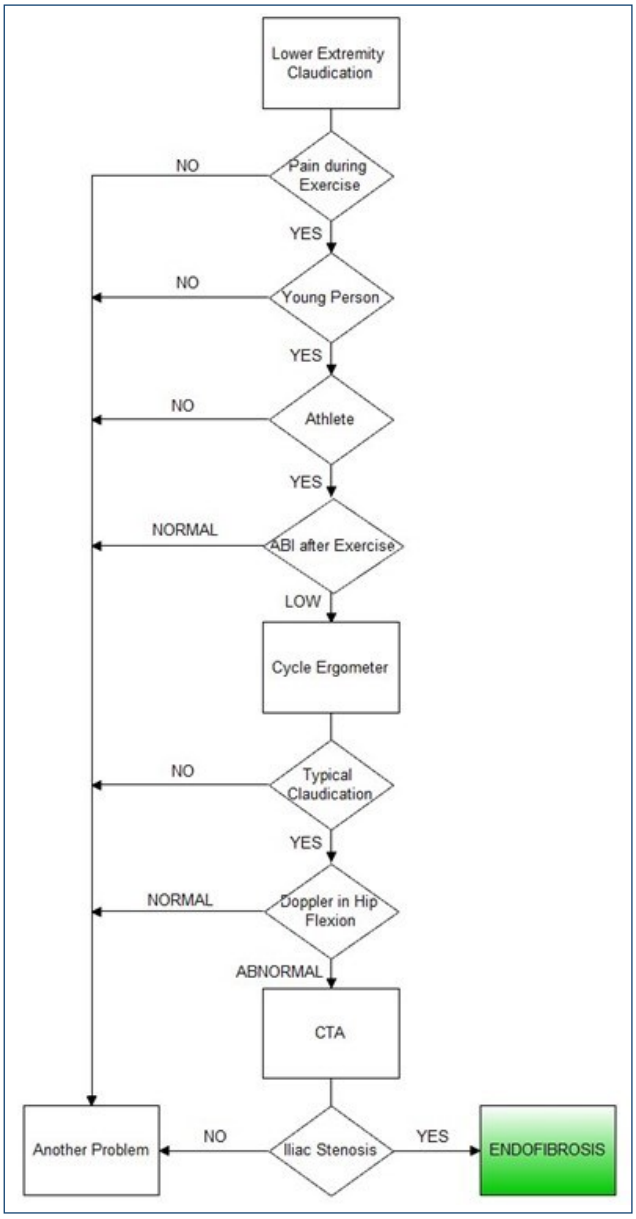

Figure 1. Proposal for the diagnostic algorithm.

Our experience

To date, there are no official guidelines on how to treat a patient with endofibrosis. Based on our research and international evidence-based medicine, our second proposal in this work, our novel proposal for the treatment algorithm of this phenomenon, is depicted in Figure 2. If the patient diagnosed with endofibrosis is a professional athlete, then he should undergo surgery. Even though the patient is not a professional athlete but very limited in life, he should also undergo surgery. In case with no significant limitation in life, the patient should stop the provocative activity and follow the rules for atherosclerosis risk reduction. Regarding surgery, endofibrosectomy and thrombectomy with a venous patch, in case of fibrotic stenosis, and with a venous bypass, in case of chronic obliteration of EIA, are the best options. In this surgical process, the iliac artery is usually attached to the surrounding structures, and surgeons need to release the artery so that they can operate.

Two cases of endofibrosis, both professional cyclists, had been managed in the Vitkovice Hospital. The first case, a 29-year-old woman, possessed the rapid and sudden onset of symptoms, including pain in the right leg, numbness, and paresthesia. She was originally diagnosed with embolism. Later, she was diagnosed with endofibrosis. During surgery, the EIA was released from the psoas muscle and then thrombectomy, endofibrosectomy, and patch reconstruction with a great saphenous vein (GSV) were performed. Histopathological examination of the excised artery revealed the preserved endothelium, in places with a fibrin

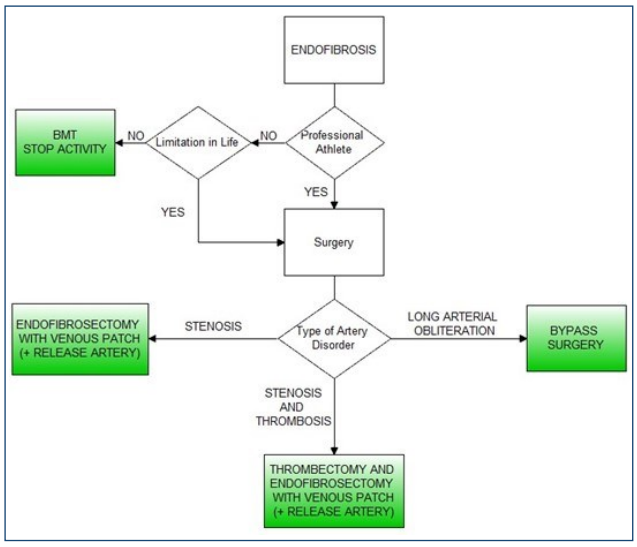

Figure 2. Proposal for the treatment algorithm.

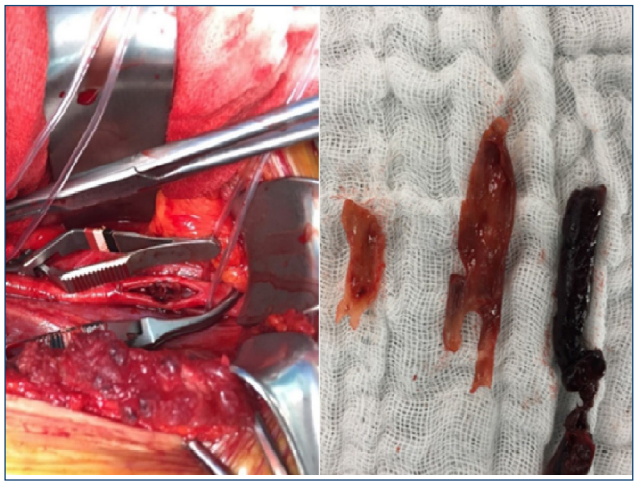

Figure 3. External iliac artery with a thrombus, endofibrotic tissue (left, center), and thrombus from external iliac artery (right).

thrombus. The media appeared to be normal and the patient returned to cycling within 2 months (Figure 3). The second case, a 31-year-old woman, presented with post-exercise pain in the left leg. Her ABI level was revealed at a normal range at rest (1.0) and dropped to 0.42 after exercise. The Doppler sonography exhibited a normal triphasic waveform at rest, replaced with the pathological monophasic waveforms after an exercise activity. Her CTA confirmed the EIA stenosis, and the diagnosis of endofibrosis was established. The patient underwent a surgical procedure involving the release of EIA and endofibrosectomy with a GSV venous patch. The patient returned to cycling in 6 weeks. The current follow-up is 48 and 29 months, respectively. The current primary patency is 100%, and both cases are still professional cyclists without limitations.

DISCUSSION

A protocol, describing the diagnosis and management of endofibrosis of the iliac artery, was published in 2016. Experts have agreed that the recommended best medical treatment may not be sufficient in terms of the therapeutic approach for the entity. As a method of choice, surgical modalities should be recommended for patients in whom endofibrosis leads to a reduced quality of life. They also agreed that endovascular therapy has not been placed in the treatment of endofibrosis[8]. Schep et al.[4] recommended that if the patient is not a professional athlete, they should give up sports; this change in lifestyle should be combined with conservative treatment. Although the etiology of endofibrosis is not related to atherosclerosis, it is recommended that patients should follow the general rules for atherosclerosis risk reduction[15].

Only a short-term effect is described in patients undergoing angioplasty. In most cases, the symptoms recurred within 8 weeks[16]. Giannoukas et al., on the contrary, reported that angioplasty is less invasive, with faster recovery and less tissue damage than surgical treatment[17]. Arterial dissection and recurrence of symptoms are the most common complications, and stent implantation is not recommended because of the risk of migration or fracture[7].

The outcomes of most studies favor surgical therapy. Of note, in case of diagnosed endofibrosis, some authors recommend endarterectomy (endofibrosectomy) with a venous patch[18-20], whereas others prefer resection of the affected EIA and a venous iliofemoral bypass graft from the GSV[13]. It is not recommended to use a prosthetic patch due to the risk of infection and pseudoaneurysm formation. Feugier et al.[21] included a total of 56 women and 435 men treated between 1991 and 2013 with an absolute majority of cyclists (87%). Endofibrosectomy was performed in 322 limbs and venous iliofemoral bypass in 202. One case, aged 28 years, died of iliac artery rupture 3 weeks after the surgery due to a premature return to sport. A sum of 97% of the cases returned to the original sport on average after 3.2±1.5 months. Five years after the surgery, the symptoms improved in 96% of the patients. The primary patency of endofibrosectomy and iliofemoral bypass after 5 years was 94 and 98%, respectively, and the secondary patency was 100% for both types of reconstruction.

We propose that, based on the outcomes of the literature review, iliac endofibrosis should always be considered a possibility in the case of claudication in athletes. Early diagnosis, per se, will attenuate unnecessary examinations, prevent disease progression, and offer early treatment. As such, the patient's history, stress test, Doppler sonography, and, possibly, other imaging modalities are essential tools instrumental

in the diagnosis of endofibrosis[22]. Reviewed publications suggest that the cases who are not top athletes should start with a conservative approach and abstain from exercise causing difficulty, thus reducing the risk factors of atherosclerosis. On the contrary, surgical treatment is a primary recommendation for professional athletes. In general, it is recommended to avoid endovascular procedures and the use of artificial materials in surgical treatment. However, specific recommendations and guidelines for the management of this phenomenon are still missing. Endofibrosis, per se, is described as a progressive disease, and the regeneration process remains controversial with the cases frequently suffering for an unnecessarily long period of time[23,24]. As such, professional athletes, in particular, cyclists, with this entity should be considered in an occupational disease condition[25].

CONCLUSION

So far, there are no complete guidelines that we should follow up on, which leads to relatively late diagnosis. As endofibrosis is a progressive disease, patients often suffer for an unnecessarily long time[23]. It would be advantageous to create a registry of cases treated for endofibrosis and to develop prospective studies with long-term follow-up. This could in effect lead to preparing international guidelines valid for the diagnosis and management of this disease. It is also worth considering that in the case of professional athletes or (in particular) cyclists, endofibrosis should be classified as an occupational disease[24]. In this article, we have also demonstrated the use of surgical procedures that agree with the up-to-date literature knowledge in two patients.

ACKNOWLEDGMENTS

The authors thank all the participants who took part in this study.

AUTHORS' CONTRIBUTIONS

TM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **IS**: Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. **DT**: Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization. **AP**: Investigation, Methodology, Project administration, Validation, Visualization. **DS**: Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. **MM**: Data curation, Formal Analysis, Investigation, Project administration, Visualization, Writing – original draft. **PV**: Methodology, Project administration, Resources, Supervision, Visualization. **VP**: Methodology, Project administration, Resources, Supervision, Visualization.

REFERENCES

1. Walder J, Mosimann F, Van Melle G, Mosimann R. A propos de l'endofibrose iliaque chez deux coureurs cyclistes. Helvet Chir Acta. 1985;51(6):793-5. PMID: 3972637
2. Schep G, Schmikli SL, Bender MH, Mosterd WL, Hammacher ER, Wijn PF. Recognising vascular causes of leg complaints in endurance athletes. Part 1: validation of a decision algorithm. Int J Sports Med. 2002;23(5):313-21. https://doi.org/10.1055/s-2002-33141
3. Feugier P, Chevalier J. Endofibrosis of the iliac arteries: an underestimated problem. Acta Chir Belg. 2004;104(6):635-40. https://doi.org/10.1080/00015458.2004.11679635
4. Peach G, Schep G, Palfreeman R, Beard JD, Thompson MM, Hinchliffe RJ. Endofibrosis and kinking of the iliac arteries in athletes: a systematic review. Eur J Vasc Endovasc Surg. 2012;43(2):208-17. https://doi.org/10.1016/j.ejvs.2011.11.019
5. Fisher AT, Tran K, Dossabhoy SS, Sorondo S, Fereydooni A, Lee JT. Anatomic factors contributing to external iliac artery endofibrosis in high-performance athletes. Ann Vasc Surg. 2022;87:181-7. https://doi.org/10.1016/j.avsg.2022.05.011
6. Czihal M, Banafsche R, Hoffmann U, Koeppel T. Vascular compression syndromes. Vasa. 2015;44(6):419-34. https://doi.org/10.1024/0301-1526/a000465
7. Ford SJ, Rehman A, Bradbury AW. External iliac endofibrosis in endurance athletes: a novel case in an endurance runner and a review of the literature. Eur J Vasc Endovasc Surg. 2003;26(6):629-34. https://doi.org/10.1016/j.ejvs.2003.08.003
8. INSITE Collaborators (INternational Study group for Identification and Treatment of Endofibrosis). Diagnosis and management of iliac artery endofibrosis: results of a Delphi consensus study. Eur J Vasc Endovasc Surg. 2016;52(1):90-8. https://doi.org/10.1016/j.ejvs.2016.04.004
9. Lim CS, Gohel MS, Shepherd AC, Davies AH. Iliac artery compression in cyclists: mechanisms, diagnosis and treatment. Eur J Vasc Endovasc Surg. 2009;38(2):180-6. https://doi.org/10.1016/j.ejvs.2009.03.024
10. Abraham P, Saumet JL, Chevalier JM. External iliac artery endofibrosis in athletes. Sports Med. 1997;24(4):221-6. https://doi.org/10.2165/00007256-199724040-00001
11. Abraham P, Chevalier JM, Leftheriotis G, Saumet JL. Lower extremity arterial disease in sports. Am J Sports Med. 1997;25(4):581-4. https://doi.org/10.1177/036354659702500424
12. Abraham P, Bickert S, Vielle B, Chevalier JM, Saumet JL. Pressure measurements at rest and after heavy exercise to detect moderate arterial lesions in athletes. J Vasc Surg. 2001;33(4):721-7. https://doi.org/10.1067/mva.2001.112802
13. Alimi YS, Accrocca F, Barthelemy P, Hartung O, Dubuc M, Boufi M. Comparison between duplex scanning and angiographic findings in the evaluation of functional iliac obstruction in top endurance athletes. Eur J Vasc Endovasc Surg. 2004;28(5):513-9. https://doi.org/10.1016/j.ejvs.2004.08.008

14. Perrier L, Feugier P, Goutain-Majorel C, Girouin N, Boutier R, Papillard M, et al. Arterial endofibrosis in endurance athletes: prospective comparison of the diagnostic accuracy of intra-arterial digital subtraction angiography and computed tomography angiography. Diagn Interv Imaging. 2020;101(7-8):463-71. https://doi.org/10.1016/j.diii.2020.03.003
15. Schep G, Bender MH, Kaandorp D, Hammacher E, de Vries WR. Flow limitations in the iliac arteries in endurance athletes. Current knowledge and directions for the future. Int J Sports Med. 1999;20(7):421-8. https://doi.org/10.1055/s-1999-8826
16. Wijesinghe LD, Coughlin PA, Robertson I, Kessel D, Kent PJ, Kester RC. Cyclist's iliac syndrome: temporary relief by balloon angioplasty. Br J Sports Med. 2001;35(1):70-1. https://doi.org/10.1136/bjsm.35.1.70
17. Giannoukas AD, Berczi V, Anoop U, Cleveland TJ, Beard JD, Gaines PA. Endofibrosis of iliac arteries in high-performance athletes: diagnostic approach and minimally invasive endovascular treatment. Cardiovasc Intervent Radiol. 2006;29(5):866-9. https://doi.org/10.1007/s00270-004-0202-6
18. Korsten-Reck U, Röcker K, Schmidt-Trucksäss A, Schumacher YO, Striegel H, Rimpler H, et al. External iliac artery occlusion in a young female cyclist. J Sports Med Phys Fitness. 2007;47(1):91-5. PMID: 17369804
19. O'Ceallaigh P, Burns P, McLaughlin R, Leader M, Bouchier-Hayes D. Complete external iliac artery occlusion in a 34-year-old cyclist. Eur J Vasc Endovasc Surg. 2002;23(4):376-7. https://doi.org/10.1053/ejvs.2001.1587
20. Wille J, de Jong JR, Moll FL, Hammacher ER, van der Zwan A, Fick TE, et al. Endofibrosis of the external iliac artery in sportsmen clinical review and four new cases. Vasc Endovasc Surg. 1998;32(4):323-8. https://doi.org/10.1177/153857449803200405
21. Feugier P, Rouviere O, Della Schiava N, Millon A, Lermusiaux P, Chevalier JM. Long-term results of iliofemoral revascularizations in high-performance athletes. Eur J Vasc Edovasc Surg. 2015;50(3):394. https://doi.org/10.1016/j.ejvs.2015.06.040
22. Lindo FA, Lee JT, Morta J, Ross E, Shub Y, Wilson C. Diagnosis and management of external iliac endofibrosis: a case report. J Vasc Nurs. 2019;37(2):86-90. https://doi.org/10.1016/j.jvn.2018.11.008
23. Peake LK, D'Abate F, Farrah J, Morgan M, Hinchliffe RJ. The investigation and management of iliac artery endofibrosis: lessons learned from a case series. Eur J Vasc Endovasc Surg. 2018;55(4):577-83. https://doi.org/10.1016/j.ejvs.2018.01.018
24. Sengul D, Sengul I. Connection of reactive oxygen species as an essential actor for the mechanism of phenomena; ischemic preconditioning and postconditioning: come to age or ripening? North Clin Istanb. 2021;8(6):644-9. https://doi.org/10.14744/nci.2021.78466
25. Hinchliffe RJ. Iliac artery endofibrosis. Eur J Vasc Endovasc Surg. 2016;52(1):1-2. https://doi.org/10.1016/j.ejvs.2016.04.006