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Burnout in pediatric critical care medicine: more challenging days during the COVID-19 pandemic

Isadora Souza Rodriguez[1]
Pollyana Coelho Pessoa Santos[1]
Artur Figueiredo Delgado[1]
Werther Brunow de Carvalho[1]

1. Instituto da Criança e do Adolescente (ICr), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil.

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KEYWORDS: *Coronavirus Infections. Pandemics. Critical Care. Burnout, Professional. Estresse Psicológico. Intensive Care Units, Pediatric.*

INTRODUCTION

Physicians have more night shifts and consecutive workdays in COVID-19 times[1]. Burnout syndrome is a global burden experienced in pediatric critical care medicine (PCCM) as well as other specialties and by professionals in the healthcare practice. Despite the lack of robust evidence in this topic, the medical literature has shown a prevalence of 40-70% of this condition among critical care physicians. Along with its social burden, burnout also has a negative relationship with individual clinician productivity that can impact in patient safety, as it can be associated with higher error rates and lower patient satisfaction[2,3]. Burnout, depression, and other mental health problems such as anxiety and stress are highly correlated, and severe/extreme cases have been described in up to 20% of residents in a Brazilian study performed in our university[4].

Among physicians, PCCM fellows need to be under surveillance for signs of burnout. This syndrome is characterized by impairment in personal and professional achievement, depersonalization, and emotional exhaustion. Suttle et al.[1] described risk factors such as: lower satisfaction with PCCM, educational debits, second year of training, lower comfort level of seeking co-fellow and faculty help. Resident physicians and fellows are young doctors, in general, and satisfaction with the career choice in pediatric critical care and comfort discussing patient care or educational topics are essential for personal accomplishment. Other factors such as spirituality and having a stress outlet can be useful, as emphasized by the mentioned study[1].

It is interesting to notice that even in different hospitals, there seems to be a similar pattern of behavior regarding burnout, indicating that this is a national - even global - problem, and can be identified among residents during the pediatric intensive care unit (PICU) routine[1,5,6]. The potential differences between countries are associated with individual experiences during fellowship programs, and in overwhelming periods some resident physicians or fellows are at high risk of moral distress and other situations that predispose burnout. In Brasil, for example, the first year of the program is considered the most stressful because many students have to move to another city

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CORRESPONDING AUTHOR: Werther Brunow de Carvalho
Department of Pediatrics, Faculdade de Medicina da USP, Av. Dr. Enéas Carvalho Aguiar, 647, São Paulo, SP, Brasil – 05403-900
E-mail: werther.brunow@hc.fm.usp.br

or even state. Leaving home is an additional challenge such as initiating a new fellowship program and adapting to a different hospital. Besides this, all fellows bear the responsibility of caring for critically ill children, participating in decision-making processes, and discussing complex cases with faculty.

In the last few months, the novel coronavirus disease of 2019 (COVID-19) pandemic has resulted in a real cascade of new cases of depression and anxiety, and an exacerbation of existing mental health issues[6]. When experiencing burnout symptoms, health care professionals may develop a sense of detachment from work, both physically and psychologically[7]. During this pandemic, an emergency situation filled with unpredictability, some elements based on autonomy, competency, and relatedness should be restored[6,7].

Burnout should be an institutional concern and all fellowship programs must stimulate adequate preventive measures, mainly family support, hobbies, solitude/company[8]. It's very important to promote maximum well-being for all healthcare professionals, but at the same time, it is not easy to establish mechanisms and strategies within our intensive care units to attempt to put well-being concepts into practice. All of us should have well-being initiatives to impact health professionals and patients. Several successful approaches have been carried out, such as comfortable and safe space for the entire team, supporting coffee breaks, promoting good mental health (reinforcing the positive), maintaining pride in being an intensivist, promoting safe virtual forums to discuss anxiety and concerns of the entire team with the psychology staff[6,9].

The use of questionnaires with simple tools such as Google Forms was adopted in our hospital. We think that this instrument can help all PICU to perform a rapid evaluation of stress and anxiety levels. This assessment can be useful for acute initiatives to implement postures that promote well-being.

We reckon that clinician productivity could be improved with psychological support, avoiding moral distress and burnout in common or pandemic days. We have to care for those who are caring for our patients.

PALAVRAS-CHAVE: *Infecções por Coronavirus. Pandemias. Cuidados Críticos. Esgotamento Profissional. Stress, Psychological. Unidades de Terapia Intensiva Pediátrica.*

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Symptomatic cholelithiasis may be the first sign of sarcoidosis

Murat Akalın[1]
Semra Demirli Atıcı[1]
Dudu Solakoğlu Kahraman[2]
Cem Tuğmen[1]

1. University of Health Sciences Tepecik Training and Research Hospital, Department of General Surgery, Izmir, Turkey
2. University of Health Sciences Tepecik Training and Research Hospital, Department of Pathology, Izmir, Turkey

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SUMMARY

Sarcoidosis is a multisystemic noncaseating granulomatous disease that rarely affects the gastrointestinal system. The initial diagnosis of sarcoidosis with gallbladder/gallbladder-associated lymph node involvement is a very rare condition in the literature. Herein, we aimed to report a case of newly diagnosed sarcoidosis with lymph node involvement associated with the gallbladder.

KEYWORDS: *Sarcoidosis. Cholelithiasis. Gallbladder.*

INTRODUCTION

Sarcoidosis is a chronic, multisystemic inflammatory disorder of unknown etiology. The disease is more commonly seen in females than males and may affect all organs at various rates[1,2]. Although the disease mostly affects the pulmonary and lymphoid systems, it rarely affects the gastrointestinal system. Sarcoidosis of the gallbladder and lymph node associated with the gallbladder is an extremely rare clinical entity.

Herein, we aimed to report a case of newly diagnosed sarcoidosis with a postoperative examination of the cholecystectomy specimen.

Case Presentation

A 69-year-old female patient presented to our clinic with symptoms of nausea, vomiting, and right upper quadrant pain. She had type 2 diabetes mellitus and a history of operation for left breast carcinoma and endometrial carcinoma. She did not receive chemotherapy or radiotherapy in the postoperative follow-up period. The patient was admitted to the emergency department twice due to biliary colic symptoms. Gastroscopy was performed because of the history of oncological operation. Gastroscopy was unremarkable and showed no pathology. Abdominal ultrasonography showed multiple stones of 3-4 mm in the gallbladder lumen. Laboratory tests were unremarkable with normal range liver function tests and normal white blood cell levels. The patient was scheduled for elective surgery due to symptomatic cholelithiasis. Preoperative chest X-Ray was normal without parenchymal lesions or hilar/mediastinal lymphadenopathy. Laparoscopic

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CORRESPONDING AUTHOR: Murat Akalın
S.B.Ü. Tepecik Eğitim ve Araştırma Hastanesi Güney Mahallesi, 1140/1. Sk. No:1, 35180 Yenişehir/Konak/İzmir, Turkey – Tel: +905321773270
E-mail: akalinmurat@yandex.com

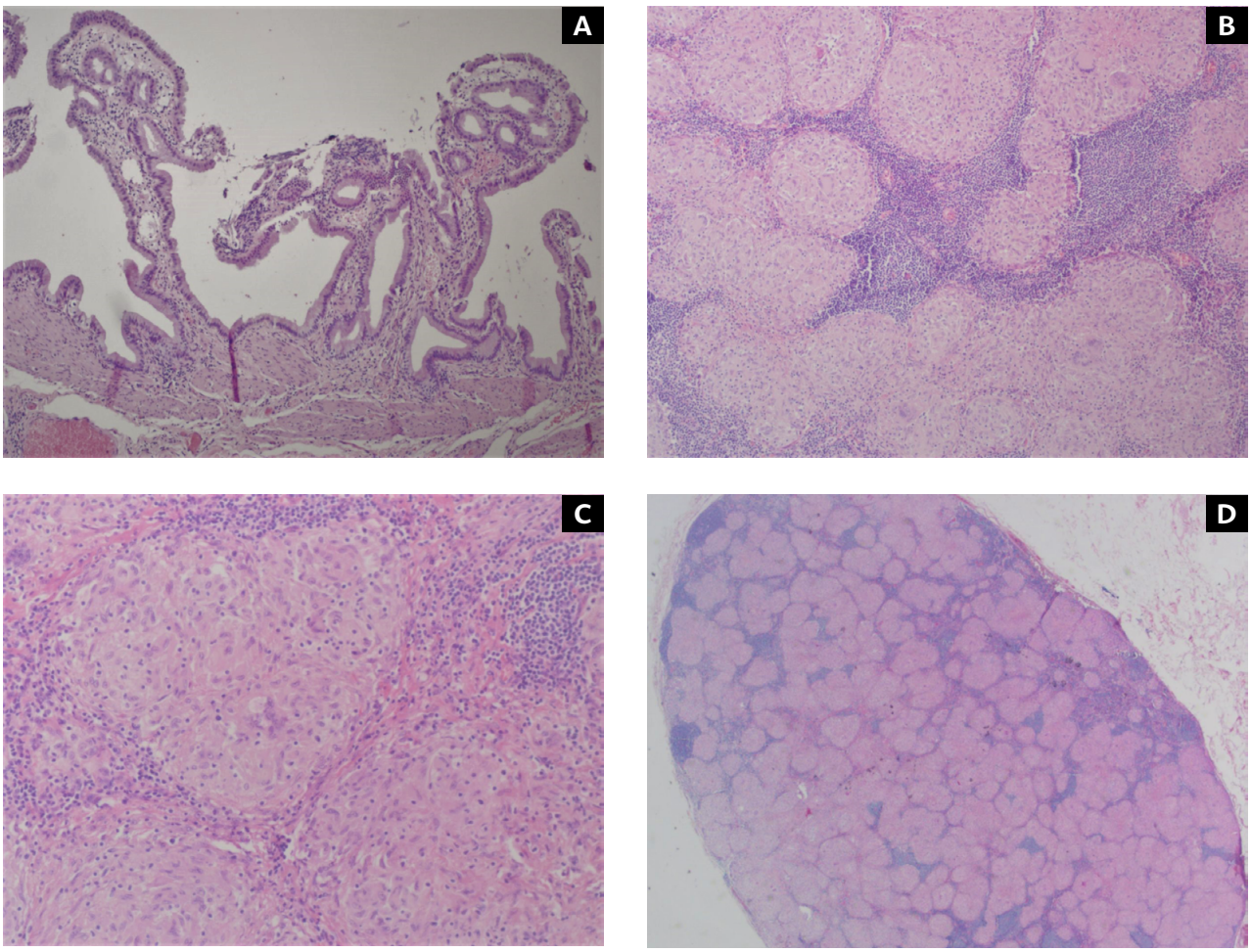

FIGURE 1. SARCOIDOSIS OF THE GALLBLADDER LYMPH NODE.

A: Low-power view of a lymph node from the gallbladder neck, showing numerous characteristic confluent granulomas (H&E, x20). B and C: High-power view of a non-caseating granuloma composed of epithelioid histiocytes and multinucleated giant cells with an asteroid body (arrow) (H&E, x40,x200). D: Histopathologic findings of chronic cholecystitis and cholesterolosis in the gallbladder (H&E, x100).

surgery was planned but during the operation, open cholecystectomy was performed because of widespread intra-abdominal adhesions secondary to the previous operation. The postoperative follow-up period was uneventful and she was discharged postoperatively on the second day.

The postoperative histopathological examination of the gallbladder wall showed signs of chronic cholecystitis and cholesterolosis. The lymph node in the gallbladder neck revealed numerous granuloma structures. The granulomas were associated with each other and they consisted of small size, non-necrotized, epithelioid histiocytes and multinuclear giant cells (Figure 1).

An asteroid body (small, intracytoplasmic, eosinophilic star-shaped structure) was seen in the giant cell cytoplasm. Non-necrotizing granulomatous lymphadenitis was identified at the lymph node of the gallbladder. Acid-resistant bacilli (AFB) were negative. The findings suggested sarcoidosis. Postoperative, the patient was referred to the pulmonary disease unit.

DISCUSSION

Sarcoidosis is a multisystemic noncaseating granulomatous disease with a prevalence of 10-20 per 100,000 individuals and frequently occurs between the second and fourth decades [of life1,2]. The most affected system in sarcoidosis is the lung, with lymph node involvement[1]. Less than 4% of sarcoidosis cases have gastrointestinal and hepatic involvement[1]. Sarcoidosis of the gallbladder and its associated lymph node has been reported in the literature by eight case reports[1,3]. Among these eight cases, only two of them

presented gallbladder-associated lymph node involvement, as in our case[1].

About half of patients with gallbladder sarcoidosis are usually asymptomatic and often diagnosed accidentally by other methods, such as chest x-ray and histopathological examination, when investigating another cause[1]. Symptomatic patients may present different symptoms mimicking benign or malignant conditions such as biliary colic, chronic or acute cholecystitis, chronic cholestasis, biliary fibrosis/cirrhosis, portal hypertension, Budd Chiari syndrome, obstructive jaundice[1,2]. Inflammation involving the biliary tree and lymph nodes may also cause extrinsic compression of the cystic canal and consequently increased jaundice. In addition, sarcoidosis may often mimic cholangiocarcinoma due to the strictures in the extrahepatic ducts[2,4].

The diagnosis of gallbladder sarcoidosis should be supported by laboratory findings. Diseases that may cause non-caseified granuloma should be considered and excluded in the differential diagnosis. As in our case, tuberculosis must be excluded by performing an acid-fast bacilli (AFB) test through Ziehl-Neelsen staining.

Although in our case the patient was diagnosed after cholecystectomy operation with postoperative specimen pathology, the diagnosis of sarcoidosis is established by clinical, radiologic, and histopathologic findings.

Prednisolone is the most prominent drug for the treatment of systemic sarcoidosis. However, there is limited literature based on case reports about the specific treatment of gallbladder sarcoidosis[1,2]. Laparoscopic or conventional cholecystectomy is the treatment of choice for patients with gallbladder sarcoidosis.

CONCLUSION

Cholecystectomy must be performed to patients with symptomatic cholelithiasis with or without a previous diagnosis of sarcoidosis. Symptomatic cholelithiasis may be the first sign of sarcoidosis. It is important to follow up on the postoperative specimen material.

Informed Consent

Informed consent was obtained from the patient for publication of this case report

Conflict of Interest

No conflict of interest was declared by all the authors.

Financial Disclosure

The authors declare that this study has received no financial support.

RESUMO

A sarcoidose é uma doença granulomatosa multissistêmica não-caseosa que raramente afeta o sistema gastrointestinal. O diagnóstico inicial de sarcoidose com envolvimento de linfonodo da vesícula biliar ou associado à vesícula biliar é muito raro na literatura. Aqui, o nosso objetivo foi relatar um caso de sarcoidose recém-diagnosticado com envolvimento de linfonodos associados à vesícula biliar.

PALAVRAS-CHAVE: *Sarcoidose. Colelitíase. Vesícula biliar.*

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Chiari malformation Type I - effect of the section of the filum terminale

Jerônimo Buzetti Milano[1]
Alecio Cristino Evangelista Santos Barcelos[1]
Jefferson Walter Daniel[1]
Andrei Fernandes Joaquim[1]
Fernando Luiz Rolemberg Dantas[1]
Roger Schmidt Brock[1]
Eloy Rusafa Neto[1]
Eduardo de Freitas Bertolini[1]
Marcelo Luis Mudo[1]
Franz Jooji Onishi[1]
Ricardo Santos Oliveira[1]
Ricardo Vieira Botelho[1]

Created on: July 2020

1. Sociedade Brasileira de Neurocirurgia, São Paulo, SP, Brasil
E-mail: wmbernardo@usp.br

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize producers to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

METHODOLOGY FOR EVIDENCE COLLECTION

This guideline followed the standards for a systematic review with evidence collected based on the Evidence-Based Medicine movement. We used the structured method to formulate the question, synthesized by the P.I.O. acronym, in which: P - corresponds to patients diagnosed with Chiari malformation type I; I - section of the *filum terminale*, O - robust measures of relevant clinical prognosis. The clinical question was: "What is the effect of the section of the *filum terminale* in the treatment of Chiari malformation type I symptoms?" From this structured question, we identified the descriptors used to search for evidence in the *Medline-Pubmed* databases. A total of 21 abstracts and titles were considered eligible for analysis, in addition to 10 studies obtained through cross-references. After applying the eligibility criteria (inclusion and exclusion), only two studies were included to answer the structured question (Annex 1).

CLINICAL QUESTION

Does the section of the *filum terminale* improve the functional prognosis of patients with Chiari malformation type I?

GRADE FOR RECOMMENDATION AND LEVEL OF EVIDENCE

A: Experimental or observational studies of higher consistency.

B: Experimental or observational studies of lower consistency.

C: Uncontrolled studies/case reports.

D: Opinion deprived of critical evaluation, based on consensus, physiological studies, or animal models.

OBJECTIVE

This guideline aims to analyze the effect of the section of the *filum terminale* in the treatment of Chiari malformation type I symptoms.

CONFLICT OF INTEREST

There is no conflict of interest related to this review that can be declared by any of the authors.

INTRODUCTION

Chiari malformation type I (CM) is a congenital dysplasia of the posterior cranial fossa which results in herniations of the cerebellar tonsils through the foramen magnum (Beijani, 2001). The clinical scenario may involve headache, which worsens with the Valsalva maneuver, dizziness, tinnitus, dysphagia, dysphonia, caused by compression of the lower cranial nerves, in addition to the impairment of sensory and motor tracts, which manifests as unbalance, ataxic gait, paresthesias, or paresis. Magnetic resonance imaging (MRI) is the gold standard to confirm the diagnosis, demonstrating the absence of the cisterna magna due to tonsillar herniation (McRae, 1960; Nishikawa, 1997).

The most widely accepted theory to explain the physiopathology of CM is based on the disproportion between the *continent*, represented by the posterior cranial fossa, delimited by the clivus, the petrous portion of the temporal bone, occipital bone, and cerebellar tentorium, and the *content*, comprising the cerebellum, brainstem, cranial nerves (III to XII), and vascular structures. Thus, the cerebellar tonsils migrate caudally and impact the foramen magnum, compromising the flow of cerebrospinal fluid between the cranium and spinal canal (Schady W. et al., 1987; Nishikawa M. et al., 1997; Karagöz F. et al., 2002; Milhorat TH. et al., 2010).

The widely accepted treatment of CM is the decompression of the posterior fossa through suboccipital craniectomies, opening the foramen magnum, with or without magnification of the dura mater, associated with resection of the posterior arch of the atlas and, more rarely, of the axis lamina to decompress the cerebellar tonsils and restore the cerebrospinal fluid flow through the foramen magnum (Oliveira et al., 2018; Zhao et al., 2016; Steinmetz et al., 2003).

However, some authors have proposed the theory that the caudal migration of the cerebellar tonsils occurs due to the caudal traction of the spinal cord and, consequently, of the brainstem and cerebellum, resulting in *occult tethered cord syndrome* (Tubbs et al., 2004; Wehby et al., 2004). Therefore, the section of the *filum terminale* was proposed as a therapeutic approach for CM. According to the proponents of this theory, this technique, which is already used on the treatment of *filum terminale* lipomas and other spinal dysraphisms, could improve CM symptoms, with lower risks of complications than the classical technique (Royo-Salvador, 1997; Tubbs et al., 2004; Wehby et al., 2004; Royo-Salvador et al., 2005;).

RESULTS OF THE SELECTED EVIDENCE

Does the section of the *filum terminale* improve the functional prognosis of patients with Chiari malformation type I?

This systematic review was based on two case series published by the same group of authors (Royo-Salvador, 1997; Royo-Salvador et al., 2005). It was not possible to define if the cases of the first study were included in the second. Thus, both studies were evaluated. The methodological qualities of both studies, according to the criteria proposed by MINORS, were low (3 and 4, respectively, considering the 16 points) (Slim et al., 2003). These are retrospective studies with small samples of non-consecutive patients, without a standardized analysis of outcomes, with data collection carried out by the surgical team, with a follow-up time not clearly defined.

Therefore, considering the scientific literature available, it is not possible to determine if the section of the *filum terminale* improves the functional prognosis of patients with Chiari malformation type I.

SYNTHESIS OF EVIDENCE

The theory that presents occult tethered cord as the genesis of CM, as well as the section of the *filum terminale* as the treatment for this condition, is controversial (Massimi et al., 2011). In addition, the fact that the classically established treatment for this disease, which consists in the decompression of the posterior fossa, demonstrates clinical outcomes that are satisfactory

and reproducible in several centers reinforces as the physiopathology of CM the theory of reduced volume of the posterior fossa during its formation in the embryonic stage (Zhao et al., 2016; Oliveira et al., 2018; Beijani G, McRae, 1960, Nishikawa et al., 1997, Karagöz F. et al., 2002, Pang et al., 2011).

The analysis of the 31 excluded studies obtained in the initial search (21) and from cross-references (10), resulted in the exclusion of 29. These studies included patients with a diagnosis of tethered spinal cord or other spinal dysraphisms, case reports or review studies, in addition to the studies in which it was not possible to specify whether the authors treated patients with Chiari malformation type I or Type II (Millorat et al., 2010).

Both studies included present evidence level 4 (case series of low quality according to the criteria proposed by Oxford) (available on Http://www.cebm.net/oxford-centre-evidence-based-medicine-levelsevidence-march-2009; Royo-Salvador, 1997; Royo-Salvador et al., 2005).

RECOMMENDATION

It is not possible to recommend the section of the *filum terminale* in the treatment of Chiari malformation type I based on the findings of this systematic review.

The section of the *filum terminale* for treating Chiari malformation can be considered an experimental treatment.

ANNEX I

Structured question

P - patients with Chiari malformation type I
I - section of the *filum terminale*
O - robust measures of clinical prognosis

Methodology for Evidence Search

PubMed-Medline

((*arnold chiari malformation*) OR (chiari 1) OR (type 1 chiari)) AND (*filum terminal**)

First batch of studies retrieved: 25 titles of original studies

Studies retrieved

The evidence used was retrieved by the following steps: elaboration of the clinical question, structuring of the question, search for evidence, presentation of results, and recommendations.

We reviewed articles from the MEDLINE (PubMed) databases, with no time limit.

The studies retrieved during the search were initially evaluated based on their titles, then their abstracts, and, finally, the studies selected were evaluated in full. Two authors were responsible for the independent evaluation of the results and all disagreements were resolved through discussions between them (JWD and FO). Cross-references obtained from the primary articles were evaluated.

The search was conducted on 1st January 2019 and 21 papers were obtained, in addition to 10 obtained through cross-references, which had their abstracts evaluated. Of this total of 31 papers, 13 were excluded because their content was not related to the object of study or they were case reports (Figure 1). Among the 18 papers evaluated in full, 16 were excluded for various reasons (Table 1). Only two studies were included for the final analysis.

Inclusion criteria

4.1. According to study designs

The search primarily targeted randomized clinical trials; in their absence, non-randomized clinical trials, controlled comparative studies, and, finally, a case series, successively.

4.2. Language

We included articles in English, Spanish, and Portuguese.

4.3. According to publication

Only studies with texts available in its entirety were considered for critical evaluation.

Method for critical evaluation

For the review protocol, the PRISMA flowchart (REF) was used to describe the flow of tracking, eligibility, and final selection of papers (Figure 1).

Extraction of results

The results extracted are described in Annex II and the recommendations were drawn based on their discussion according to the Oxford grade for recommendation (REF).

Quality assessment

The methodological quality was assessed with the aid of the MINORS (Methodological Items for Non-Randomized Studies) instrument; Slim et al., 2003).

APPENDIX II

FIGURE 1. FLOWCHART OF THE SEARCH MECHANISM ACCORDING TO THE PRISMA RECOMMENDATIONS FOR SYSTEMATIC REVIEWS (SLIM ET AL., 2003; MOHER ET AL., 2009). PRISMA 2009 FLOW DIAGRAM

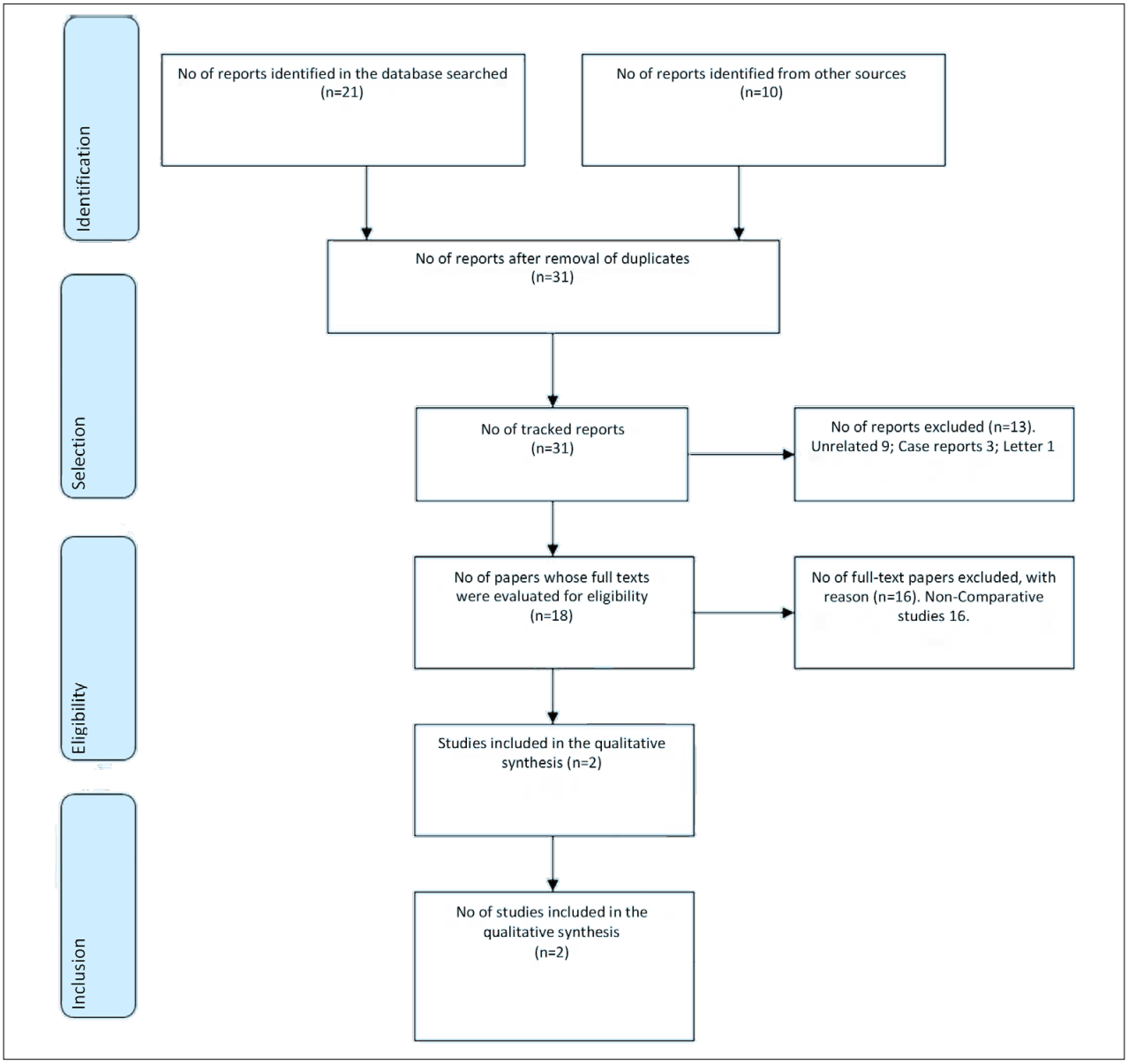

TABLE 1. EXTRACTION OF DATA ON THE SECTION OF THE FILUM TERMINALE* FOR TREATING CHIARI MALFORMATION TYPE I

Study/ Type of study	Patients	Follow-up/ Prognosis*	Conclusion
Royo-Salvador, et al./ 1997/ Case series	N = 5 Scoliosis 1 (20%); Syringomyelia 2 (40%); Chiari type I 1 (20%), Associated 1 (20%) Mean age: 33.8 years	Improvement in 5 patients (100%) Follo-up not informe	SFT is a useful strategy in the treatment of scoliosis, syringomyelia, and Chiari malformation type I
Royo-Salvador, et al./ 2005/ Case series	N = 20 Scoliosis 8 (40%); Syringomyelia 5 (25%), Chiari type I 2 (10%); Associated 5 (25%) Mean age: 33.5 years	Improvement in 9 (45%); Without improvement in 7 (35%); Unknown in 4 (20%) Follow-up of 4 months to 11 years (mean 4.8 years)	SFT is a useful strategy in the treatment of scoliosis, syringomyelia, and Chiari malformation type I

* only intervention carried out; there was no control group for comparison

TABLE 2. MINORS (METHODOLOGICAL ITEMS FOR NON-RANDOMIZED STUDIES) OF THE PAPERS INCLUDED IN THIS SYSTEMATIC REVIEW (SLIM ET AL., 2003)

Study / Items	Royo-Salvador (1997)	Items Score	Royo-Salvador (2005)	Items Score
Objective clearly established	Yes. The objective was to report (evaluate) the results of cases operated with a surgical technique (an intervention)	2	Yes. The objective was to report (evaluate) the results of cases operated with a surgical technique (an intervention)	2
Consecutive inclusion of patients	No. Non-consecutive patients	0	No. Non-consecutive patients	0
Prospective data collection	No. Retrospective collection	0	No. Retrospective collection	0
Appropriate outcomes for the objective of the study	No. The author described the clinical improvements of each patient, without standardization of data collection	0	No. The author described the percentages of clinical improvement for each patient, without standardization of data collection	1
Unbiased analysis of the study outcome	No. Although it was not described, it is suggested that the surgical team collected the data	0	No. Although it was not described, it is suggested that the surgical team collected the data	0
Appropriate follow-up time for the objective of the study	Uncertain. There is no description of long-term follow-up.	1	Uncertain. Patients operated between 1993 and 2013. Table 1 suggests that the formal outcomes were collected in September and October 2014.	1
Prospective calculation of study sample size	No. This is a case series with a small sample of patients. Only patients 3 and 4 were suggestive of or compatible with Chiari malformation type I	0	No. This is a case series with a small sample of patients. Only patients 4, 5, and 11 had Chiari malformation type I	0
Total score		3*		4*

*The maximum MINORS score for non-randomized studies is 16 points. Therefore, the methodological quality of both studies selected is low.

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Systemic treatment and surgery versus systemic treatment alone for metastatic breast cancer

Glauce Romeiro de Almeida[1]
Antônio Silvinato[1]
Wanderley Marques Bernardo[2]

1. Programa Diretrizes da Associacao Medica Brasileira, Sao Paulo, SP, Brasil
2. Coordenador do Programa Diretrizes da Associacao Medica Brasileira, Sao Paulo, SP, Brasil

E-mail: wmbernardo@usp.br

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QUESTION: What is the impact of systemic therapy with surgery in the treatment of patients with metastatic breast cancer on overall mortality outcomes (death from any cause) and quality of life, compared to systemic therapy alone?

Answer: There In women with metastatic breast cancer, breast surgery (mastectomy: removing the whole breast, including the nipple and areola, or Lumpectomy: removing the tumor and breast tissue around it, preserving the nipple and the areola) combined with medical treatment (such as chemotherapy and hormone therapy) compared with medical treatment alone:

- Does not improve the overall survival. The quality of the evidence is very low.
- Does not improve local progression-free survival. The quality of the evidence is very low.
- Abbreviates distant progression-free survival. Moderate quality of evidence.
- Does not improve or alter the quality of life. The quality of the evidence is very low.

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Coinfection of SARS-CoV-2 and Measles morbillivirus in a front-line health worker in Rio de Janeiro, Brasil

Jacqueline Stephanie Fernandes do Nascimento[1]
Renata R. T. Castro[1,4]
Janie Kelly Fernandes do Nascimento[1]
Brunno Bastos Knoploch[2]
Patrícia Maria Carla Osório Duque[3]
Marco Antônio Orsini Neves[4]

1. Faculdade de Medicina, Universidade Iguaçu, Nova Iguaçu, RJ, Brasil.
2. Sistema Único de Saúde,Rio de Janeiro, RJ, Brasil.
3. Universidade Estácio de Sá, Rio de Janeiro, RJ, Brasil.
4. Universidade Federal Fluminense, Niterói, RJ, Brasil.

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KEYWORDS: *Coronavirus Infections. Coinfection. Measles. Vaccines.*

Healthcare workers (HCW) are at high risk of contracting the Coronavirus disease 2019 (COVID-19), even with the use of personal protective equipment, due to the greater exposure, work intensity, lack of rest, inadequate training, and while undressing[1]. In China, at least 3,387 HCW were infected, with 22 deaths[1]. Sadly, numbers are still growing, but by March 2020, Italy had reported the infection of over 2,600 HCW and, in April, the United States estimated more than 9,200 infected HCW.

Currently, there are no vaccines or specific antiviral drugs for COVID-19. Its frequent mutations facilitate fast transmissibility and difficult the designing of a vaccine[2]. Vaccination exposes the body to antigens and activates the immune system without causing disease. However, even in a highly vaccinated population, outbreaks may still happen. In this letter, we describe a case of co-infection with SARS-CoV-2 and Measles morbillivirus in a front-line health worker in Brasil. The case report was approved by the Ethics Research Committee on March 15, 2020, under the number 012342020. The patient signed an informed consent form.

CASE REPORT

A 25-year-old male caucasian physician with mild asthma history and not in use of any medication, fighting in the front-line against COVID-19, presented prostration, sleeping difficulty, and fever (37.8ºC), for which he took antipyretics and analgesics. On the following two days, he evolved with odynophagia, dysphagia, worsening of prostration, productive cough, and anosmia. On the 3rd day, the patient reported dry cough and rhinorrhea. A nasopharyngeal swab was used and sent for RT-PCR COVID-19 testing. On the

DATE OF SUBMISSION: 07-Jun-2020
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CORRESPONDING AUTHOR: Renata Castro
Av. Abílio Augusto Távora, 2134, Jardim Nova Era, Nova Iguaçu, RJ, Brasil – 26275-580
E-mail: castrorrt@gmail.com

5th day, new symptoms were added (table 1): tachydyspnea, conjunctival hyperemia, morbilliform erythematous maculopapular non-itching rash on his face, trunk, limbs, and on the palms of both hands. (figure 1). A physical exam also revealed palpable lymph nodes in the bilateral occipital and spinal accessory chains and clustered white lesions on the buccal mucosa (Koplik's sign). Azithromycin 500mg, prednisone 20mg, antiemetic, antiallergic, and analgesic drugs were prescribed. On the next day, the patient presented desaturation and needed supplementary oxygen. Three days later, the patient had no rashes. The last symptoms to disappear were cough and anosmia (table).

RT-PCR were positive for COVID-19 and Measles morbilivirus. Serologies for measles were also positive [IgG: 3687 mUI / mL (positive reference value> 200 mUI / mL); IgM: 524 U / mL (positive reference value> 15 U / mL). The patient reported vaccination with three doses of MMR vaccine during childhood.

DISCUSSION

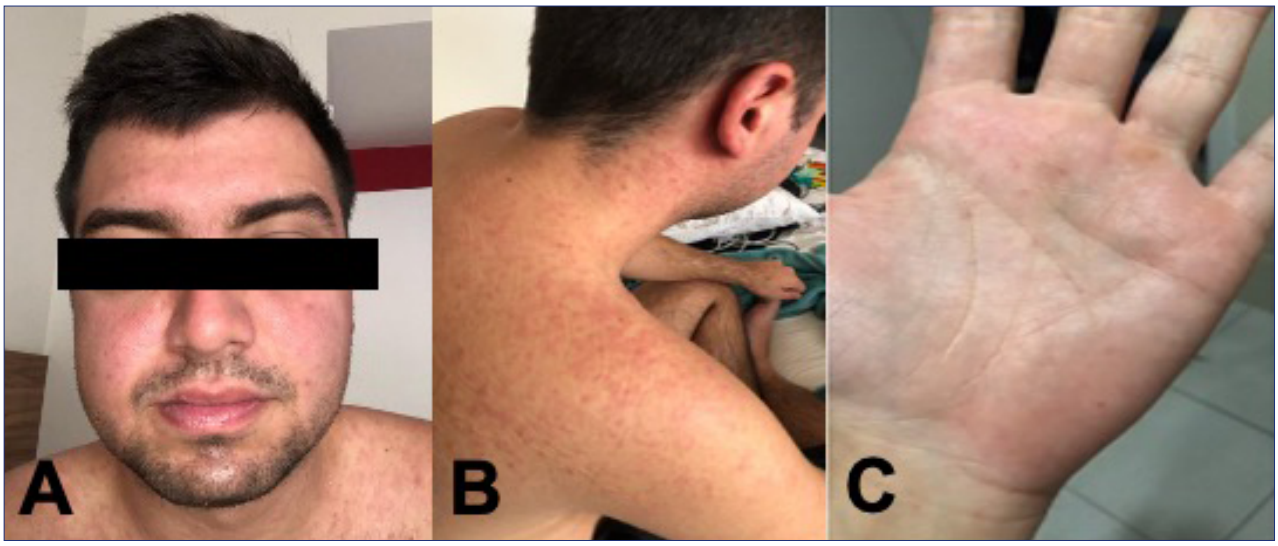
Measles is a highly contagious disease and still a common threat in the underdeveloped world[3]. Live attenuated measles vaccine is considered to have high protective efficacy, especially after two doses (94% efficacy)[4]. In countries with high vaccine coverage, as in Brasil, measles outbreaks happen mainly due to importation or vaccination failure[5]. Nevertheless, the infection may happen in vaccinated patients due to suboptimal measles vaccine-induced humoral

TABLE 1. SYMPTOM TIMELINE

SWAB + PCR — AZITHROMYCIN — SOROLOGIES

Symptomatologic Chronology	29/3 D1	30/3 D2	31/3 D3	01/4 D4	02/4 D5	03/4 D6	04/4 D7	05/4 D8	06/4 D9	07/4 D10	08/4 D11	09/4 D12	10/4 D13	11/4 D14	12/4 D15	13/4 D16
Prostration/ Nausea																
Fever																
Odinophagy																
Cough																
Anosmia																
Ageusia																
face edema																
Skin rash/ Conjunctival hyperemia																
Tachypnea																
Desaturation																

FIGURE 1. ERYTHEMATOUS MACULOPAPULAR RASH MORBILLIFORM ON THE FACE (A), TRUNK (B), AND HANDS (C).



immunity and/or waning immunity in a low measles-exposure environment[6].

Coronavirus and Measles morbillivirus have an evolutionary connection, as they share core proteins[7]. Measles vaccination may increase the ability of the immune system to fight COVID-19[7]. Coinfections may facilitate genetic exchange and generate recombinant viruses, influencing viral evolution, sensitivity to antiviral therapy, and disease course[8,9].

Our patient presented a relatively benign course of both infections, which may be explained by competitive suppression between the viruses. In this case, similar viruses interfere with the replication of one another or there may have been a competition between the viruses for metabolites, replication sites, or host factors necessary for virus replication[8].

Physicians are trained to think of a single virus causing a clinical syndrome. During the pandemic, it is clear that COVID-19 is going to be the first diagnostic hypothesis for most respiratory syndromes. But we must be aware of the similarities between viruses. Future studies should increase the knowledge about how frequent coinfections happen and how they interfere in the disease course.

Conflicts of Interest

The authors declare no conflicts of interest.

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Author's Contribution

JSFN, JKFN, and MAON devised the project, the main conceptual ideas, and collected the data.

All authors worked on technical details, literature review, and discussion.

RRTC and MAON wrote the manuscript

PALAVRAS-CHAVE: *Infecções por Coronavirus. Coinfecção. Sarampo. Vacinas.*

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ORIGINAL ARTICLE

Comparison of tru-cut biopsy and fine-needle aspiration cytology in an experimental alcoholic liver disease model

Yasemen Adalı[1]
Hüseyin Avni Eroğlu[2]
Mustafa Makav[3]
Sunay Sibel Karayol[4]
Gülname Fındık Güvendi[5]
Mustafa Gök[6]

1. Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Pathology, Çanakkale, Turkey.
2. Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Physiology, Çanakkale, Turkey.
3. Kafkas University, Faculty of Veterinary Medicine, Department of Physiology, Kars, Turkey.
4. Harran University, Faculty of Medicine, Department of Radiology, Şanlıurfa, Turkey.
5. Rize Recep Tayyip Erdoğan University, Faculty of Medicine, Department of Pathology, Rize, Turkey.
6. Aydın Adnan Menderes University, Faculty of Medicine, Department of Radiology, Aydın, Turkey.

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SUMMARY

INTRODUCTION: *Liver biopsies such as tru-cut (sharp needle) and fine-needle aspiration cytology (FNAC) are the most commonly preferred techniques to detect the grade and stage of certain liver diseases. In this study, we aimed to compare the efficiency of USG-guided tru-cut biopsy and fine-needle aspiration cytology in an experimental alcoholic liver disease model.*

METHODS: *Thirty-six female Wistar albino rats, 4-6 months old, and weighing from 190 to 250 g, were used in this study. The animals were randomly divided into six equal groups: G1 (control), G2 (tru-cut control), G3 (FNAC control), G4 (Alcoholic liver disease model), G5 (Alcoholic liver disease model + FNAC), and G6 (Alcoholic liver disease model + tru-cut biopsy). After a histopathological evaluation by light microscopy, the sensitivity, specificity, positive and negative predictive values of FNAC and tru-cut biopsy for the diagnosis of liver lesions were calculated.*

RESULTS: *No pathology was detected in G1 except for mild congestion. On the other hand, hepatocyte damage, periportal inflammation, congestion, and fatty changes were detected in all liver tissues of the alcoholic liver disease groups. The sensitivity of hepatocyte damage, inflammation, congestion, and fatty change parameters for FNAC were 33.3%, 80%, 0%, and 0%, respectively, while the sensitivity of the same variables for tru-cut were 66.7%, 40%, 100%, and 20%, respectively.*

DISCUSSION: *Both techniques were superior in some aspects. FNAC can be an attractive alternative to tru-cut biopsy and applied in routine practice in the diagnosis of non-tumoral liver diseases.*

KEYWORDS: *Liver diseases, alcoholic. Biopsy, fine-needle. Biopsy. Aspiration Biopsy.*

INTRODUCTION

Alcoholic liver disease (ALD) is known as a progressive disease that worsens with the prolonged use of alcohol[1]. The main risk factors of ALD are genetic and metabolic traits, sex, obesity, and volume and duration

DATE OF SUBMISSION: 26-Jan-2020
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CORRESPONDING AUTHOR: Yasemen Adalı
Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Pathology, Çanakkale, Turkey
Tel: +90 506 343-7199
E-mail: yasemenadali@hotmail.com

of alcohol use[2]. ALD represents a wide-range spectrum of liver pathologies such as steatosis, steatohepatitis, steatofibrosis-cirrhosis, cholestasis, alcoholic foamy degeneration, megamitochondria, perivenular fibrosis-central hyaline sclerosis, and siderosis[3]. Steatosis (fatty changes), which is generally seen in 90% of heavy drinkers, is the first liver response to alcohol[4]. Alcohol shows its pathogenesis in different pathways by increasing NADH/NAD+ in the hepatocytes, disrupting fatty acid oxidation, increasing triglyceride synthesis, upregulating lipogenic enzymes, leading to lymphocyte recruitment, increasing gut permeability, translocation of bacterial products such as LPS into the portal circulation, triggering neutrophilic infiltration, secretion of cytokines and chemokines, and inhibiting the anti-fibrotic action of natural killer cells[4-6].

Ultrasonographic imaging (USG), computed tomography (CT), or magnetic resonance imaging (MRI) are frequently and effectively used techniques to evaluate benign and malignant diseases of the liver, including in the diagnosis and grading of alcoholic liver disease[7,8]. However, histopathological examinations are more reliable in cases where a radiological examination is insufficient to guide diagnosis and treatment[9]. On the other hand, liver biopsies such as tru-cut (sharp needle) biopsy and fine-needle aspiration cytology (FNAC) are the most commonly preferred and performed techniques to detect the grade and stage of certain liver diseases. Therefore, these methods are candidates for the "gold standard method"[10,11]. The role of liver biopsy in alcoholic liver disease is to provide accurate clinical data, facilitate diagnosis, and predict the severity, grade, and stage of the disease by using semi-quantitative tools[3].

FNAC is a simple, rapid, less expensive, and relatively safe investigation, while tru-cut biopsy is more expensive and takes more time[12]. Additionally, FNAC causes less tissue damage and has fewer complications than tru-cut biopsy. Compared to FNAC, the risk of complications such as hemorrhage, tumor seeding, infection, fistula formation, bleeding, perforation, and pain can be encountered more in tru-cut biopsy[11]. Despite its advantages, such as the high sensitivity and specificity rates and reducing the need for other biopsy techniques such as tru-cut, in some types of lesions, FNAC may not provide enough cellular details[13].

Considering the current literature, FNAC is generally used for lesions suspected of malignancy in the liver, not in benign lesions[14,15]. Therefore, in this study, we aimed to compare the efficiency of USG-guided tru-cut biopsy and fine-needle aspiration cytology concerning some histological parameters such as inflammation, hepatocyte damage, congestion, and fatty change and intended to detect the sensitivity, specificity, positive/negative predictive values, and diagnostic accuracy of the two methods in an experimental alcoholic liver disease model.

METHODS

After the study was approved by the Local Ethics Committee of Animal Experiments of the Kafkas University (Ethical Approval Date 17.02.2016 and Number: 2016/053), thirty-six female Wistar albino rats, 4-6 months old and weighing 190 to 250g, were purchased from the Ataturk University Medical Experimental Research and Application Center and used in this study. Female rats are chosen for the study because they are more prone to alcoholic damage[16].

The rats were housed in an animal room maintained at a temperature of 22-25 °C, in 12-hours light periods, and were fed ad-libitum. Then, the rats were randomly divided into the following six experimental groups (n=6 per group):

Group 1: (1st control group) Nothing was done. The animals were only fed ad-libitum for 28 days (n=6).

Group 2: (2nd control group) Nothing was applied. The animals were only fed ad-libitum for 28 days, followed by tru-cut biopsy (n=6).

Group 3: (3rd control group) Nothing was applied. The animals were only fed ad-libitum for 28 days, followed by FNAC (n=6).

Group 4: Alcoholic liver disease was inducted by applying Ethanol (7 g/kg/day) + Water (50%) for 28 days (n=6).

Group 5: Alcoholic liver disease was inducted by applying Ethanol (7 g/kg/day) + Water (50%) for 28 days, followed by FNAC (n=6).

Group 6: Alcoholic liver disease was inducted by applying Ethanol (7 g/kg/day) + Water (50%) for 28 days, followed by tru-cut biopsy (n=6).

Ethanol was administered considering the time to create subchronic damage using the dosage modified per Bharrhan et al.'s study[17]. At the end of the 28-days, the rats were not fed overnight. After applying the anesthesia protocol, the samples were taken by USG-guided tru-cut biopsy or FNAC. After the interventions, the rats were subjected to cervical vertebra dislocation according to ethical rules.

Imaging during FNAC

The samples were obtained by the FNAC technique following local anesthesia using a 20 cc injection syringe with 21G needle from the non-vascular area of the liver with the guide of an ultrasonography (USG) device (Toshiba Aplio XG).

Imaging during tru-cut biopsy

The samples were obtained by the tru-cut biopsy technique following local anesthesia using a 18G tru-cut needle from the liver tissue with the guidance of USG (Toshiba Aplio XG).

The harvested tru-cut biopsy and hepatectomy samples were fixed in 10% buffered formaldehyde solution for 24 hours for pathological examinations. They were dehydrated in graded alcohol series and embedded in paraffin wax. Then, four-µm thick sections of paraffin blocks were obtained and stained with hematoxylin & eosin (H&E). The sections were evaluated by light microscopy (Clinical Microscope BX46, Olympus, Japan). The cytological materials were also analyzed by light microscopy both as direct smear and thin-prep method, which is a cell replication method. Since direct smears revealed more diagnostic features than the thin-prep method during the microscopic evaluation, direct smear was used for cytology findings that constitute the main framework of the study. The hepatectomy materials, tru-cut biopsies, and FNAC preparations of all experimental groups were evaluated for histopathological assessments of the following parameters: congestion, inflammation, hepatocyte damage, and steatosis (fatty change).

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values of FNAC and tru-cut biopsy in the diagnosis of liver lesions were determined using histopathological diagnoses of the liver tissues as the gold standard.

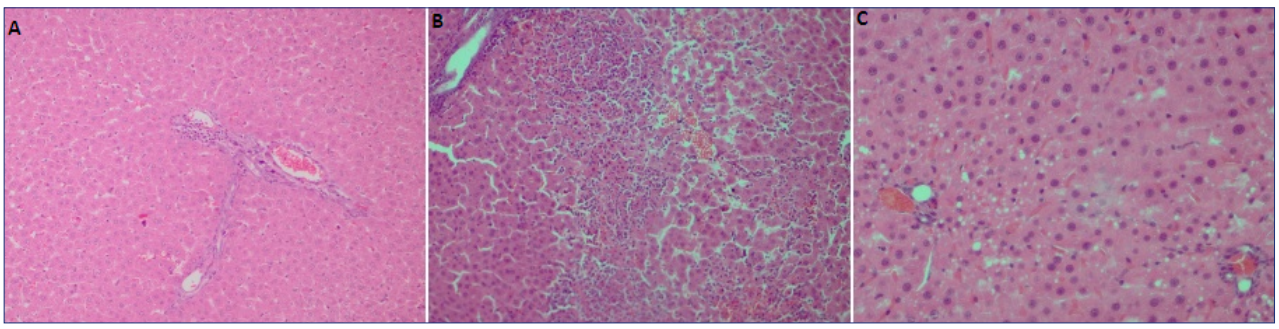
RESULTS

Per the histopathological evaluation, no histopathological changes were detected in Group 1 (1st control group) except for mild congestion (Figure 1A). Group 2 and Group 3 had similar histopathological properties to Group 1 in terms of hepatocyte damage, periportal inflammation, congestion, and changes in fatty parameters (no histopathological changes were detected except mild congestion). On the other hand, hepatocyte damage, periportal inflammation, congestion, and fatty changes were detected in the liver tissues of the ethanol-administrated group (Group 4) (Figure 1B-C).

The sensitivity, specificity, diagnostic accuracy, positive and negative predictive values of the histopathological parameters (hepatocyte damage, inflammation, congestion, and fatty change) of Group 5 and Group 6 (Figure 2A-B) are shown in Table 1 and Table 2.

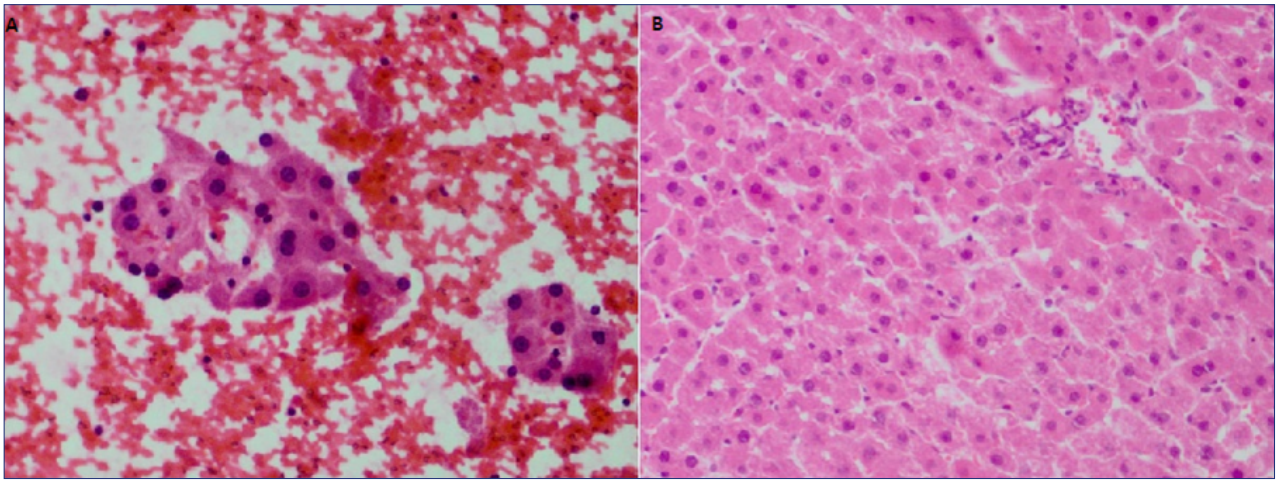
The sensitivities of hepatocyte damage, inflammation, congestion, and changes in fatty parameters for FNAC were 33.3%, 80%, 0%, and 0%, respectively, while, the sensitivities of the same variables for the tru-cut biopsy were 66.7%, 40%, 100%, and 20%, respectively. On the other hand, the diagnostic accuracy of congestion was the same both for tru-cut and FNAC (100%). The diagnostic accuracies of tru-cut biopsy regarding fatty changes (33.3% vs. 16.7%) and hepatocyte damage (66.7% vs. 33.3%) were higher than those of FNAC, while the diagnostic accuracy of FNAC concerning inflammation (83.3% vs. 50%) was higher than the tru-cut biopsy method.

FIGURE 1. THE HISTOPATHOLOGICAL MICROGRAPHS OF GROUP 1 AND 4.



A: Group 1, mild congestion (H&E, 200x); B: Group 4, periportal inflammation (H&E, 200x); C: Group 4, microvesicular steatosis and hepatocyte damage (H&E, 400x).

FIGURE 2. THE HISTOPATHOLOGICAL MICROGRAPHS OF GROUP 5 AND 6.



A: Group 5, hepatocyte damage (H&E, 400x); B: Group 6, hepatocyte damage (H&E, 400x).

The positive predictive values of FNAC in terms of hepatocyte damage, inflammation, and congestion were 100%, whereas it was 0% regarding fatty changes. However, the positive predictive values of the tru-cut biopsy method were 100% for all histopathological parameters. On the other hand, the negative predictive values of hepatocyte and congestion of both techniques were 0%, while negative predictive values of inflammation (50% vs. 25%) and fatty changes (33.3% vs. 20%) were higher in the FNAC compared to the tru-cut biopsy method.

DISCUSSION

Only a few studies compare FNAC and tru-cut biopsy in non-tumoral liver diseases. In this study, we performed tru-cut biopsy and FNAC to rats with experimental alcoholic liver disease and observed that tru-cut biopsy had higher sensitivity and diagnostic accuracy concerning hepatocyte damage when compared to FNAC. However, positive predictive values were similar for both methods. For inflammation, FNAC had higher sensitivity, diagnostic accuracy, and negative predictive value than tru-cut biopsy. For congestion and changes in fatty parameters, it was detected that tru-cut biopsy had higher sensitivity, diagnostic accuracy, and positive predictive value.

For any hepatic disease, the radiological, serological (such as alpha-fetoprotein), and clinical findings, among others, are not precisely and reliably able to differentiate benign and malign lesions;

TABLE 1. THE SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES OF GROUP 5 (ALD WITH THE FNAC-APPLIED GROUP)

	Hepatocyte damage	Inflammation	Congestion	Fatty change
Sensitivity (%)	33.3	80	0	0
Specificity (%)	0	100	0	100
Diagnostic accuracy (%)	33.3	83.3	100	16.7
Positive predictivity (%)	100	100	100	0
Negative predictivity (%)	0	50	0	33.3

TABLE 2. THE SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES OF GROUP 6 (ALD WITH THE TRU-CUT-APPLIED GROUP)

	Hepatocyte damage	Inflammation	Congestion	Fatty change
Sensitivity (%)	66.7	40	100	20
Specificity (%)	0	100	0	100
Diagnostic accuracy (%)	66.7	50	100	33.3
Positive predictivity (%)	100	100	100	100
Negative predictivity (%)	0	25	0	20

these methods can only increase the accuracy of the diagnosis[14]. At some point, liver biopsies become inevitable for the evaluation of patients and the management of the diseases[18]. Most liver biopsies are applied under radiological guidance, and the choice of the biopsy needle depends on the radiologist. Naturally, the experience of the radiologist may influence management. This decision also depends on many variants including the size and location of the tumor, and the risk of possible complications[19]. Currently, there are two commonly accepted and used techniques to obtain diagnostic material, namely, fine needle aspiration cytology (FNAC) and tru-cut biopsy. The FNAC technique usually provides a sample for cytological examination, whereas tru-cut biopsy primarily delivers a sample for histological assessment. Each method has different advantages and disadvantages, and both are considered safe[19]. Therefore, the sensitivity and specificity rates of these two techniques should be available for choosing the appropriate one.

There have been adequate scientific data about both tru-cut biopsy and FNAC techniques in the literature reporting the advantages and complications, as well as the rates of sensitivity and specificity. Ding et al.[20] performed a study with 46 hepatocellular carcinoma patients to distinguish primary and metastatic tumors and emphasized that FNAC is a useful technique. Li et al.[11] performed a tru-cut biopsy and FNAC techniques with 18G and 21G needles, respectively, on 94 patients with unresectable malignant tumors, and reported that the 21G FNAC and 18G tru-cut biopsy procedures were substantially similar. However, the safety of the 21G FNAC was found superior to that of 18G tru-cut biopsy. Tissues obtained by either of these two techniques are sufficient for any pathological and molecular diagnosis. Another study performed by Kaçar Özkara et al.[21] in 2013 pointed out that FNAC had a high sensitivity rate, especially in hepatic neoplasia. However, they also stressed that combined cyto-histopathology is superior to FNAC.

Most of the related studies generally report that FNAC is a successful technique and can be used for the diagnosis of hepatic tumors. It was stated that FNAC is a suitable method to be used for open biopsy or when surgery is not possible[22]. Sattar et al.[23] performed a study on 450 patients with focal hepatic lesions and determined that USG-guided FNAC was a rapid, safe, easy, and uncomplicated method for diagnosis. A comprehensive study[24] performed in 2015 on 755 patients with malignant and benign hepatic lesions detected that the diagnostic accuracies of FNAC and tru-cut biopsy were in the range of 58.8%-98.9%. Additionally, no complication had been reported in this study. On the other hand, Nazir et al.[15] detected a diagnostic accuracy and specificity between 95.2% and 100%. According to these authors, FNAC was a cheap, easy, and safe method. However, they claimed that tru-cut biopsy should be the gold-standard method.

In light of these findings, we observed that these tests were each superior in some aspects and equivalent regarding different features. We consider that the selection of the diagnostic method in alcoholic liver disease according to the histopathological parameters is a central point for a patient-based individualized approach. For instance, if the "inflammation" parameter is to be evaluated, FNAC should be preferred, while tru-cut biopsy should be at the forefront for the assessment of "fatty changes." This way, it will be possible to make a more suitable selection of the biopsy methods in terms of patient comfort, complication risk, ease of application, and cost.

In conclusion, we think that FNAC can be an attractive alternative to the tru-cut biopsy method and applied in routine practice in the diagnosis of non-tumoral liver diseases. To get the best results, a combined approach of FNAC and tru-cut biopsy may also be used.

Study Limitation

This study has some limitations. First, the biopsy techniques could also be performed on other liver diseases such as hepatocellular carcinoma and/or cirrhosis, or any other hepatic illness in addition to alcoholic liver disease. Second, a prospective study on patients with hepatic diseases could yield better results. Third, clinical, radiological, and serological findings could be obtained and compared with the biopsy results. Lastly, we did not perform a detailed observation of the complications because we had cervical dislocation immediately after the procedures.

Conflict of Interest

The authors have no financial disclosure or conflict of interest in this study.

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Author's Contribution

Y.A. and M.G. conceived the presented idea. Y.A., H.A.E., M.M, S.S.K., G.F.G., and M.G. planned the experiments Y.A., H.A.E., M.M, S.S.K., and G.F.G. carried out the experiments. Y.A., H.A.E., M.M, S.S.K., and G.F.G. contributed to sample preparation and planned the experiments. Y.A., H.A.E., M.M, S.S.K., and G.F.G. contributed to the interpretation of the results. Y.A. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

RESUMO

INTRODUÇÃO: *Biópsias hepáticas tais como por agulha tru-cut e por citologia aspirativa por agulha fina (CAAF) são as técnicas frequentemente preferidas para detectar o grau e o estágio de certas doenças hepáticas. Neste estudo, nosso objetivo foi comparar a eficiência da biopsia com agulha tru-cut guiada por ultrassom e a citologia aspirativa por agulha fina em um modelo experimental de doença hepática alcoólica.*

MÉTODOS: *Trinta e seis ratos Wistar albinos fêmeas, de 4 a 6 meses de idade e pesando entre 190 e 250g, foram utilizados neste estudo. Os animais foram divididos aleatoriamente em seis grupos: G1 (controle), G2 (controle tru-cut), G3 (CAAF), G4 (modelo de doença hepática alcoólica), G5 (modelo de doença hepática alcoólica + CAAF) e G6 (modelo de doença hepática alcoólica + biópsia tru-cut). Após uma avaliação histopatológica por microscopia de luz, foram calculados a sensibilidade, especificidade e os valores preditivos positivos e negativos da CAAF e biópsia por tru-cut para o diagnóstico de lesões hepáticas.*

RESULTADOS: *Nenhuma patologia foi detectada no G1, apenas leve congestão. Por outro lado, detectamos danos nos hepatócitos, inflamação periportal, congestão e alterações nos ácidos graxos nos tecidos hepáticos de todos os grupos de doença hepática alcoólica. As sensibilidades encontradas para os danos nos hepatócitos, inflamação, congestão e alterações nos parâmetros de ácidos graxos para a CAAF foram 33,3%, 80%, 0% e 0%, respectivamente, enquanto que as sensibilidades das mesmas variáveis para o método tru-cut foram 66,7%, 40%, 100% e 20%, respectivamente.*

DISCUSSÃO: *Ambas as técnicas foram superiores em alguns aspectos. A CAAF pode ser uma alternativa atraente à biópsia por tru-cut e aplicada como prática de rotina no diagnóstico de doenças hepáticas não tumorais.*

PALAVRAS-CHAVE: *Hepatopatias alcoólicas. Biópsia por agulha fina. Biópsia.*

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ORIGINAL ARTICLE

Diagnosis and referral flow in the single health system for climacteric women

Isabel Cristina Esposito Sorpreso[1,2]
Francisco Winter dos Santos Figueiredo[1,3]
Adna Thaysa Marcial da Silva[1]
Juliana Zangirolami-Raimundo[1,2]
Bhárbara Karolline Rodrigues Silva[3]
Fernando Adami[3]
Lea Tami Suzuki Zuchelo[1]
Edmund Chada Baracat[1]
José Maria Soares Júnior[1]
Luiz Carlos de Abreu [2,4]

1. Disciplina de Ginecologia, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, Brasil
2. Laboratório de Delineamentos de Estudos e Escrita Científica, Centro Universitário Saúde ABC (FMABC), Santo André, SP, Brasil
3. Laboratório de Epidemiologia e Análise de Dados, Centro Universitário Saúde ABC (FMABC), Santo André, SP, Brasil
4. Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (Emescam), Vitória, ES Brasil

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SUMMARY

OBJECTIVE: *The association between gynecological diagnoses and their distribution across healthcare sectors benefits health promotion and the identification of topics for continued education of gynecological care. This study aimed to identify healthcare diagnoses and referral flow in climacteric women.*

METHODS: *This is a cross-sectional study conducted at the Women's Health Clinic of the University Hospital, University of São Paulo, with a reference to gynecology and training for Residents of Family and Community Medicine, between 2017 and 2018. The medical records of 242 women whose sociodemographic and clinical information, gynecological diagnoses, and distribution of healthcare services (primary, secondary, and tertiary) had been processed were collected. Statistical analysis included the chi-square test and odds ratio.*

RESULTS: *Smoking (OR = 2.27, 95% CI 1.05–4.89; p = 0.035) was associated with the referral of climacteric women to higher complexity services. Considering the distribution of non-oncological diagnoses in climacteric patients, the chance of women being referred to medium- and high-complexity health services presented a 2-fold increase in cases of breast diseases, a 2.35-fold increase in cases of noninflammatory disorders of the female genital tract, and a 3-fold increase in cases of inflammatory diseases of the pelvic organs.*

CONCLUSION: *Climacteric women aged over 55 years, postmenopausal women, and smoking women were most frequently referred to medium- and high-complexity outpatient surgery.*

KEYWORDS: *health services; gynecology; women's health; health care levels; health systems.*

INTRODUCTION

Evaluating medium-complexity health services is important to ensure adequate care, guidance, and training for primary care network professionals and to maintain a balance between health care levels[1]. The evaluation process also allows for the analysis of procedures between providers and users (referencing) and

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CORRESPONDING AUTHOR: Isabel Cristina Esposito Sorpreso
Address: Dr. Enéas de Carvalho Aguiar Avenue, n 255, 10. floor, room 10166 - 05403-000, São Paulo, SP, Brasil. Tel.: +55 (11) 2661 7621
E-mail: icesorpreso@usp.br

of the final health situation (individual or collective) as a result of the complex between providers and consumers of healthcare interventions[2].

The characterization of the assisted population at different levels of health services improves the quality of care. Consequently, topics relevant to women's health in interdisciplinary training in outpatient settings[3] are fundamental for the hierarchization of healthcare services, in which health promotion and treatment measures remain incipient. Some studies have reported that women who were referred to medium-complexity healthcare services had non-oncological gynecological diagnoses that were inadequately clinically managed in primary care[4,5].

The climacteric period is the phase in a woman's life when prevention and promotion actions are expected to happen in primary care[6,7]. A projection study indicated that by 2020, the demand for specific women's healthcare services will have grown by 6-10%, in both developed and developing countries[8]. Studies associating gynecological diagnoses and their distribution at different complexities and healthcare service levels are rare, but they improve health promotion, continued and interdisciplinary medical education, and resource rationalization[9-11]. Thus, such studies significantly improve the various fields of action of a multidisciplinary team, with marked effects on public health, resulting in direct benefits to women's health[3].

The objective of this study, which was conducted in a women's health assistance service that included a residency supervision in Family and Community Medicine, was to identify healthcare diagnoses and the referral flow of climacteric women.

METHODS

Study design, location and period

This study was conducted as a cross-sectional study at the Women's Health Outpatient Clinic, University Hospital of the University of São Paulo (ASM-HU/USP), from January 2017 to December 2018. The outpatient clinic is intended for gynecological care, teaching, learning, training, and supervision service for first-year Family and Community Medicine residents. It receives women with unsatisfactory or unexpected previous clinical management referred from basic health clinics to the medium-complexity reference outpatient clinic in the so-called Western Region Project (PRO, in the Portuguese acronym).

The PRO was an agreement between the University and the city government of São Paulo, Brasil, for the provision of assistance, education, and research; the development of joint activities with undergraduate Medicine, Speech Therapy, Physical Therapy, and Occupational Therapy courses, and for training graduate Family and Community Medicine, Psychiatry, Pediatrics, and General Medicine students. The basic health clinics in the PRO implement the Family Health Strategy of the Brazilian Unified Health System (SUS) in the western region of the city of São Paulo, which comprises the neighborhoods of Jardim Boa Vista, Butantã, Jardim d'Abril, Jardim São Jorge, Vila Dalva, Jardim Jaqueline, Vila Sônia, and Paulo VI.

Study participants

This is a secondary analysis of previously published data[3] in which the medical records of 428 initial consultations were collected by convenience sampling. This study selected medical records of 242 women aged between 40 and 65 years.

Data collection source

The study used secondary data from the Medical Records Storage Service of the University Hospital of the University of São Paulo (SAME/HU/USP), and information from the first consultations of patients treated at the Women's Health Clinic was extracted.

Data collection procedure

All sociodemographic, clinical, and gynecological data, including information on the type of treatment, referral, and counter-referral, were collected using a standardized form and entered into a Microsoft Excel spreadsheet (*.xls*). Data were checked for consistency, and, in case of differences, the medical records were reread.

Sociodemographic variables considered were age, ethnicity, economic activity, and origin. The clinical variables included multiple morbidities (presence of two or more concomitant diseases), age at first sexual intercourse, age at menarche, age at last menstruation (age at menopause), sexual activity, parity, current smoking habit, and type of treatment (clinical or surgical).

The gynecological diagnoses were standardized according to the 10th revision of the International Classification of Diseases (ICD-10, 2011) and grouped into five categories[3,11], namely, urinary tract diseases (N30–N39), breast diseases (N60–N64), inflammatory diseases of the female pelvic organs (N70–N77),

TABLE 1. CHARACTERIZATION AND TYPES OF GYNECOLOGICAL DIAGNOSES IN CLIMACTERIC WOMEN TREATED AT THE WOMEN'S HEALTH OUTPATIENT CLINIC IN THE UNIVERSITY HOSPITAL OF THE UNIVERSITY OF SÃO PAULO (ASM/HU/USP).

Characteristics	N	%
Age (years)		
40–44	70	28.92
45–54	99	40.91
55–64	68	28.10
65	5	2.06
Race		
White	142	62.83
Not white	84	37.17
Employment		
No	100	58.14
Yes	72	41.86
Multimorbidity		
No	83	35.78
Yes	149	64.22
Alcohol consumption		
No	182	95.79
Yes	8	4.21
Menarche (years)		
8–12	95	39.09
13–16	100	41.56
> 16	14	5.76
Menopause		
No	146	60.33
Yes	96	39.67
Natural age at menopause (years)		
< 40	9	9.47
40–45	20	21.05
46–54	51	53.68
> 55	15	15.79
Smoker		
No	154	75.12
Yes	51	24.88
Parity		
Nulliparous	21	8.64
1–2 pregnancies	92	37.86
3 or more pregnancies	129	53.50
Active sex life		
No	64	32.49
Yes	133	67.51
Types of diagnoses		
Urinary tract diseases (N30–N39)	59	24.28
Breast diseases (N60–N64)	9	3.70
Inflammatory diseases of the female pelvic organs (N70–N77)	16	6.58
Noninflammatory disorders of the female genital tract (N80–N99)	181	74.49
General examination, contraception, and procreation (Z00–31)	9	3.70

noninflammatory disorders of the female genital tract (N80–N99), and general examination and investigation of patients without complaints (Z00–31). The grouping was based on clinical symptoms and similar diagnostic evaluations. When a patient had two or more diagnoses, each one was described separately. The exclusion criteria were pregnancy, childbirth, puerperium, ectopic pregnancy, and cancer, as well as incomplete information. Oncological diagnoses were not referred to the outpatient clinic but to oncological treatment services in the healthcare network.

Healthcare services were characterized according to the type of assistance provided and the complexity as primary sector or low complexity (basic healthcare clinics, teaching healthcare centers, women's outpatient clinics), secondary sector or medium complexity (the university hospital and other medium-complexity hospitals and surgical specialty clinics), and tertiary sector or high complexity (high-complexity hospitals and oncological support hospitals). The flow of the healthcare service distribution started at the referral and counter-referral services, i.e., the place of origin and the place where the patient received the treatment or returned to the place of origin.

Ethical aspects

This research was analyzed and approved by the Ethics Committee of the University of São Paulo School of Medicine under opinion No. 228/13.

Statistical analysis

Study variables were grouped by absolute and relative frequency and odds ratio, as described in tables. The odds ratio was estimated using logistic regression. Stata® software (StataCorp, LC) version 11.0 was used for statistical analysis, and the significance level was 5%.

RESULTS

A total of 428 women were observed and registered at the outpatient clinic in the University of ASM - HU/USP during the study period, with 242 climacteric women referred from the basic healthcare clinics (UBS) of Jardim São Jorge (46.28%, n = 112), Jardim Boa Vista (14.87%, n = 36), Vila Dalva (10.33%, n = 25), Jardim Jaqueline (7.85%, n = 19), Butantã (7.02%, n = 17), Jardim d'Abril (4.96%, n = 12), and Vila Sônia (0.41%, n = 1), as well as the staff at the University Hospital and other health centers (8.26%, n = 20).

Of the 242 cases, only 54.3% were low-complexity cases of possible resolution in the referred outpatient clinic (clinical management), 42% required other interventions (outpatient or hospital) and were referred to medium-complexity services, and 3.7% were referred to high-complexity services (Figure 1).

The main gynecological and non-oncological diagnoses found in patients treated at 'the University of ASM - HU/USP were noninflammatory disorders of the female genital tract (N80–N99), 74.49% (181); urinary tract diseases (N30–N39), 24.28% (59); inflammatory diseases of the female pelvic organs (N70–N77), 6.58% (16); breast diseases (N60–N64), 3.70% (9); and general examination, contraception, and procreation (Z00–31), 3.70% (9).

As shown in Table 2, of the sociodemographic factors and clinical history, smoking (OR = 2.27, 95%CI 1.05–4.89; p = 0.035) was associated with the referral of climacteric women to higher-complexity services.

The distribution of non-oncological diagnoses in climacteric women (Table 2) shows that the chance of a woman being referred to medium- and high-complexity services was 2 times higher in cases of breast diseases, 2.35 times higher in cases of noninflammatory disorders of the female genital tract, and 3 times higher in cases of inflammatory diseases of the pelvic organs, with no statistically significant difference.

DISCUSSION

Healthcare service evaluation studies characterize the assisted population, identify the referral flow of patients at different levels of healthcare services, and improve the quality of care.

Clinical, sociodemographic, and gynecological and obstetric characteristics of patients treated at the Women's Outpatient Health Clinic are similar to those found[3,12-14] in São Paulo and in the Southern Brasil regions. Our results describe climacteric women in transition to late menopause and in the first post-menopause years, with at least two or more associated clinical diseases (hypertension, diabetes, or hypothyroidism), who were multiparous, unemployed, and smokers.

The main healthcare diagnoses in gynecology found in this study were noninflammatory disorders of the female genital tract and diseases of the urinary tract, reflecting the healthcare reality of climacteric women in basic health clinics[15,16].

Noninflammatory disorders of the female genital tract, including abnormal uterine bleeding, have a prevalence of 40-60% in the reproductive period, which may worsen in the late reproductive period due to progressive ovarian[17,18] and physiological dysfunction. Symptoms related to changes in the menstrual cycle can lead to anemia[19], which implies morbidity and mortality[20]. Furthermore, these disorders can affect women's health and cause imbalances in their sexual activity[15,17,18].

Adequate clinical management in primary care becomes relevant to avoid worsening women's health[1,7,21]. In addition, noninflammatory disorders of the female genital tract are the most common non-oncological diagnoses in tertiary care, showing an important financial impact on healthcare systems[3,22]. Thus, clinical outpatient control of these patients through the incorporation of drug therapy (such as levonorgestrel-releasing intrauterine devices and others not yet available in the market, such as progesterone receptor analogues) can be performed in low-complexity services, which is essential for avoiding high costs and reducing morbidity.

Demands related to urogenital dysfunction are common and have negative effects on different aspects of a woman's lives. In this study sample, these demands were assisted at the primary and secondary care levels[16,22]. Health professionals working with women's health in low-complexity settings should be aware and trained in assisting women with non-oncological gynecological diagnoses so that these women can receive adequate clinical management, thereby improving their reproductive and sexual health[7,13].

Inflammatory diseases of the female pelvic organs, breast disorders, general physical examination, contraception, and reproduction were also reported

FIGURE 1. DISTRIBUTION OF RECORDS OF WOMEN RESPONSIVE TO PRIMARY CARE AND THOSE REQUIRING TERTIARY CARE.

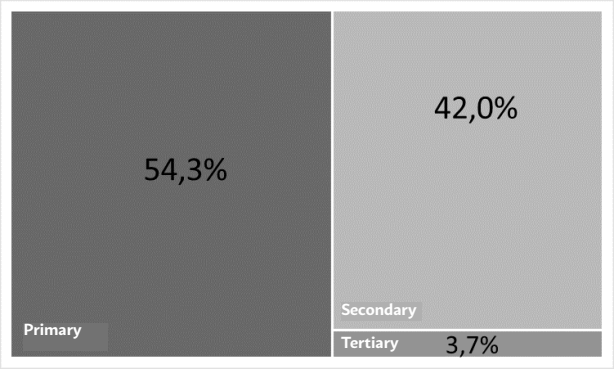

TABLE 2. FACTORS ASSOCIATED WITH THE REFERRAL OF CLIMACTERIC WOMEN TO MEDIUM- AND HIGH-COMPLEXITY SERVICES AT THE WOMEN'S HEALTH OUTPATIENT CLINIC IN THE UNIVERSITY HOSPITAL OF THE UNIVERSITY OF SÃO PAULO (ASM/HU/USP).

Characteristics	Odds	OR (95% CI)	p*
Age (years)			
40–44	1.80	ref.	ref.
45–54	1.56	0.86 (0.46–1.63)	0.664
55–64	3.85	2.14 (0.99–4.60)	0.051
65	1.50	0.83 (0.13–5.32)	0.847
Race			
White	2.02	ref.	ref.
Not white	2.00	0.98 (0.55–1.75)	0.971
Employment			
No	2.70	ref.	ref.
Yes	2.00	0.74 (0.38–1.43)	0.370
Multimorbidity			
No	1.67	ref.	ref.
Yes	2.31	1.37 (0.78–2.42)	0.267
Alcohol consumption			
No	2.08	ref.	ref.
Yes	1.66	0.79 (0.18–3.45)	0.765
Menarche (years)			
8–12	1.50	ref.	ref.
13–16	2.15	1.43 (0.79–2.58)	0.225
>16	3.66	2.44 (0.63–9.34)	0.191
Menopause			
No	1.77	ref.	ref.
Yes	2.55	1.44 (0.82–251)	0.199
Natural age at menopause (years)			
> 40	3.50	ref.	ref.
40–45	4.00	1.14 (0.16–7.76)	0.891
46–54	2.40	0.68 (0.12–3.69)	0.660
55–65	1.50	0.42 (0.06–2.81)	0.377
Smoker			
No	1.80	ref.	ref.
Yes	4.10	2.27 (1.05–4.89)	0.035
Parity			
Nulliparous	1.33	ref.	ref.
1–2 pregnancies	2.17	1.62 (0.61–4.29)	0.324
3 or more pregnancies	2.09	1.57 (0.61–4.02)	0.346
Active sex life			
No	2.20	ref.	ref.
Yes	2.02	0.91 (0.48–1.74)	0.797
Types of diagnoses			
Urinary tract diseases (N30–N39)			
No	1.09	ref.	ref.
Yes	1.56	0.70 (0.38–1.29)	0.256
Breast Diseases (N60–N64)			
No	1.96	ref.	ref.
Yes	2.00	0.98 (0.23–4.02)	0.979
Inflammatory diseases of female pelvic organs (N70–N77)			
No	4.53	ref.	ref.
Yes	3.00	1.51 (0.47–4.83)	0.488
Non-inflammatory disorders of the female genital tract (N80–N99)			
No	4.00	ref.	ref.
Yes	2.35	1.69 (0.93–3.08)	0.082
General examination, contraception, and procreation (Z00–31)			
No	0.75	ref.	ref.
Yes	1.25	0.60 (0.15–2.30)	0.458

OR: odds ratio; ref: reference category; *logistic regression; **Absence of this diagnosis as reference.

by patients in this study, which corresponds with demands related to the late reproductive period and to sexual activity[5,13].

Benign breast diseases are more likely to be diagnosed during the nonreproductive period. However, it is important to highlight that only clinical complaints of benign breast disorders were evaluated in this outpatient clinic, and clinical management was performed mainly in medium- and high-complexity facilities. This may corroborate the specificity of this entity and emphasize the importance of specialized physicians in healthcare[23].

Patient survey was conducted in a training program with emphasis on women's health and the main areas of investment were identified, such as health education for professionals and encouragement of multidisciplinary work[3-5]. Thus, this study reinforces the importance of training health professionals in basic healthcare clinics on the climacteric period and on the clinical management of abnormal uterine bleeding and urogenital dysfunction.

The distribution of healthcare service utilization was considered appropriate, according to the literature in countries using hierarchical healthcare levels and universal access, such as the United Kingdom and Canada[24]. The final referral of patients, mostly to the primary sector, is considered satisfactory and expected in a hierarchical health system. However, special attention should be given to the need to optimize services and referral flows at various healthcare system levels to improve healthcare quality, especially regarding women's health[10,11].

A Brazilian study on a medical audit of the prenatal care program in the Southern region of the country showed that the use of epidemiological methods to organize healthcare services is important for improving the quality of care[25].

Descriptive and retrospective studies have their own limitations, especially considering the quality of sociodemographic information records, indeterminate racial classification in Brasil, and clinical data recorded with heterogeneous criteria.

Furthermore, the local demands and use of health services for women's care described in this study may not represent the demands in the Brazilian Unified Health System, because the analyzed outpatient clinic is accredited to a teaching and learning unit.

The novelty of this study lies in its correlation between the main diagnoses in climacteric women's health and the hierarchization of healthcare service levels, associating women's demands and healthcare needs at different healthcare levels. This should benefit the fields of health promotion and medical and interdisciplinary education, highlighting current issues in the daily life of climacteric women and in public health according to healthcare complexity levels.

CONCLUSIONS

Climacteric women aged over 55 years, postmenopausal women, and smokers were most frequently referred to medium- and high-complexity surgical facilities.

Author's Contribution

Isabel Cristina Esposito Sorpreso: concept, data curatorship, formal analysis, research, methodology, project management, software, supervision, validation, visualization, writing (original draft, review, and editing).

Francisco Winter dos Santos Figueiredo: formal analysis, methodology, software, Writing (original draft, review, and editing).

Adna Thaysa Marcial da Silva: data curatorship, methodology, software, writing (original draft, review, and editing).

Juliana Zangirolami-Raimundo: formal analysis, methodology, writing (original draft, review, and editing).

Bhárbara Karolline Rodrigues Silva: formal analysis, methodology, software, writing original draft.

Fernando Adami: formal analysis, methodology, software.

Lea Tami Suzuki Zuchelo: concept, data curatorship, formal analysis, methodology, software, writing (original draft, review, and editing).

Edmund Chada Baracat: concept, data curatorship, project management, supervision, writing (original draft, review, and editing).

José Maria Soares Júnior: formal analysis, methodology, project management, software, supervision, writing (original draft, –review, and editing).

Luiz Carlos de Abreu: project management, supervision, visualization.

RESUMO

INTRODUÇÃO: *A associação entre diagnósticos ginecológicos e sua distribuição nos setores de saúde proporciona benefícios no campo da promoção de saúde e na identificação de temas para educação continuada na assistência.*

OBJETIVO: *Identificar os diagnósticos em saúde e o fluxo de encaminhamento de mulheres no climatério.*

MÉTODO: *Trata-se de estudo transversal realizado no Ambulatório de Saúde da Mulher do Hospital Universitário da Universidade de São Paulo, de referência em ginecologia e de treinamento para residentes de Medicina de Família e Comunidade, entre 2017-2018. A casuística foi realizada a partir de 274 prontuários de mulheres atendidas e foram processados informações sociodemográficas e clínicas, diagnósticos ginecológicos e distribuição dos serviços de saúde (primário, secundário e terciário). O teste qui-quadrado e razão de chance foram utilizados para estatística.*

RESULTADOS: *O tabagismo (OR=2,27, IC95% 1,05;4,89, p=0,035) foi associado ao encaminhamento de mulheres no climatério para a maior complexidade. Em relação aos tipos de diagnóstico, a chance de serem encaminhadas para a média e alta complexidade foi de 135% (OR=1,69, IC95% 0,93;3,08) nos transtornos não inflamatórios do trato genital feminino, 200% (OR=0,98, IC95% 0,23;4,02) nas doenças da mama, 300% (OR=1,51, IC95% 0,47;4,83) nos transtornos inflamatórios do trato genital feminino, sem predomínio entre os diagnósticos.*

CONCLUSÃO: *As mulheres climatéricas e na pós-menopausa acima de 50 anos e tabagistas com diagnósticos de transtornos não inflamatórios do trato genital feminino e inflamatórios, bem como doenças da mama, foram as mais direcionadas para ambulatório cirúrgico na média e alta complexidade.*

PALAVRAS-CHAVE: *Serviços de saúde. Ginecologia. Saúde da mulher. Níveis de atenção à saúde. Sistemas de saúde.*

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ORIGINAL ARTICLE

The role of monocyte to HDL ratio in predicting clinically significant carotid stenosis in patients with asymptomatic carotid artery disease

Mustafa Yurtdaş[1]
Yalin Tolga Yaylali[2]
Mahmut Özdemir[3]

1. Balıkesir Sevgi Hospital, Department of Cardiology, Balıkesir, Turkey
2. Pamukkale University, Faculty of Medicine, Department of Cardiology, Denizli, Turkey
3. İstanbul Aydın University, Department of Cardiology, İstanbul, Turkey

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SUMMARY

OBJECTIVE: *Monocyte count to HDL-C Ratio (MHR) and Fibrinogen to Albumin Ratio (FAR) have recently emerged as markers of inflammation in atherosclerotic diseases. Our goal was to investigate the relationships of MHR and FAR with the severity of carotid artery stenosis (CAS) in patients with asymptomatic carotid artery disease.*

METHODS: *This retrospective study consisted of 300 patients with asymptomatic CAS. Pre-angiographic MHR, FAR, and high-sensitive C-reactive protein (hsCRP) were measured. Carotid angiography was performed in patients with ≥50% stenosis on carotid ultrasonography. Patients were first split into 2 groups based on the degree of CAS and then tertiles (T) of MHR.*

RESULTS: *96 patients had clinically insignificant CAS (<50%) (Group-1), and 204 patients had clinically significant CAS (≥50%) (Group-2). Group-2 had higher MHR, FAR, and hsCRP than group-1. Patients in T3 had higher MHR, FAR, and hsCRP than in T1 and T2. MHR, FAR, and hsCRP were correlated with each other (p<0.001, for all). MHR, FAR, and hsCRP were independent predictors of significant CAS. MHR better predicted a significant CAS than FAR and hsCRP (p<0.05).*

CONCLUSION: *Pre-angiographic MHR may be a better predictor than FAR and hsCRP in identifying a clinically significant carotid stenosis in patients with asymptomatic CAS. Patients with asymptomatic CAS and a high level of MHR should be followed-up closely to supervise risk-factor control and intensify treatment.*

KEYWORDS: *Inflammation. Carotid stenosis. Monocyte to HDL ratio. Fibrinogen to albumin ratio. Angiography.*

INTRODUCTION

Carotid atherosclerotic stenosis is an important risk indicator for the development of cerebro-cardiovascular events. As the severity of carotid stenosis increases, the risk of stroke increases[1]. Inflammation has an important role in the development, advancement, and destabilization of carotid atherosclerosis[2,3]. Atherosclerotic lesions in the carotid arteries have been found to be related to inflammatory markers[3,4].

Monocytes, one type of inflammatory marker, or

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CORRESPONDING AUTHOR: Mustafa Yurtdaş
Balıkesir Sevgi Hospital, Department of Cardiology, Paşaalanı Mahallesi, 10020, Balıkesir, Turkey
Tel: 090 266 246 33 10, Fax: 090 266 246 33 70
E-mail: mustafayurtdas21@gmail.com

their subunits have an independent role in predicting subclinical atherosclerosis and cardiovascular disease[5,6]. High-density lipoprotein-cholesterol (HDL-C) has anti-inflammatory and anti-oxidant effects by inhibiting the adhesion of monocytes to the endothelium. Low HDL-C levels lead to the progression of atherosclerosis[5,7,8]. Fibrinogen, a component of the coagulation cascade, has pro-inflammatory effects and has been demonstrated to have an essential role in atherogenesis[4]. Serum albumin, responsible for the vast majority of total osmotic pressure, plays a crucial role in inhibiting platelet activation and aggregation[9]. A low albumin level is associated with the increased viscosity and concentration of free lysophosphatidylcholine, which may give rise to endothelial dysfunction[10]. Monocyte to HDL-C ratio (MHR) and fibrinogen to albumin ratio (FAR) have been reported to be more suitable and efficient as a marker of cardiovascular disease than their individual measurements[11,12].

In several studies, MHR and FAR have been demonstrated to be related to adverse outcomes in patients with risk factors or cardiovascular disease[5,8,11-17]. MHR and FAR may be used as cost-effective predictors of adverse events. To our knowledge, there has been no research assessing the pre-angiographic value of MHR and FAR to determine the severity of carotid artery stenosis (CAS). The hypothesis of the present research was that the relationship of MHR and FAR with carotid stenosis might help better classify the risk of those patients at a higher risk for carotid events. Therefore, we aimed to investigate MHR and FAR before carotid angiography and determine their relationships with the severity of CAS obtained during carotid angiography in patients with asymptomatic carotid artery disease.

METHODS

The study design was retrospective. A total of 300 asymptomatic patients with multiple cardiovascular risk factors who presented to cardiology and neurology outpatient clinics were included. Patients had undergone bilateral carotid angiograms due to ≥ 50% stenosis in at least 1 internal carotid artery on the duplex carotid ultrasonography between January 2018 and May 2019. Subjects with left ventricular hypertrophy, systolic dysfunction (ejection fraction less than 50%), moderate to severe diastolic dysfunction, moderate to severe valvular disease, arrhythmias, left bundle branch block, heart enlargement or failure, acute coronary syndromes, acute cerebrovascular diseases, connective tissue diseases, thyroid disorders, pulmonary diseases, kidney diseases, recent operation (<3 months), and trauma were excluded. Other systemic disorders such as inflammatory and infectious diseases were also excluded from the trial. All participants gave written informed consent before being enrolled. The study was regulated in compliance with the Declaration of Helsinki. The local ethical committee approved our study.

All blood samples were taken, following 12h of fasting, in the morning of the day in which carotid angiography was performed. A complete blood count with automated differential counts and lipid profile was assessed. MHR was calculated as the ratio of the monocyte count to HDL-C. Fibrinogen and serum albumin levels were evaluated. The FAR was measured as the ratio of fibrinogen to albumin. Clinically significant CAS was defined as a stenosis diameter of ≥50%[18].

Continuous variables were given as means ± standard deviation (SD), whereas categorical ones were given as percentages (%). Kolmogorov-Smirnov test was used to test the normal distribution of measurements. When comparing more than 2 groups, ANOVA or the Kruskal Wallis test was used. For comparison of the continuous data between the two groups, an independent t-test or a Mann-Whitney U test was used according to whether the data had a normal distribution or not, respectively. For categorical data, the $\chi 2$ test was used for further analysis. The Pearson or Spearmen test was used to assess correlations between the variables. In order to identify independent predictors of significant CAS in patients with asymptomatic carotid artery disease, univariate and then multivariate logistic analyses were carried out. To identify the cutoff values of MHR, FAR, and hsCRP in predicting a significant CAS, a receiver operating characteristic (ROC) curve was produced. To compare the AUCs from the ROC curves of these 3 inflammatory parameters, the deLong test was used. A 2-sided p-value of less than 5% was accepted as statistically significant. SPSS software was used for all analyses (version 22.0; SPSS Inc).

RESULTS

A total of 300 patients with asymptomatic CAS were included into the study. Patients were split into 2 groups based on the severity (or clinical significance) of CAS obtained during the carotid angiography. 96

TABLE 1. COMPARISON OF CLINICAL AND LABORATORY DATA ACCORDING TO THE SEVERITY OF CAROTID STENOSIS IN PATIENTS WITH ASYMPTOMATIC CAROTID ARTERY DISEASE.

Variables	(Group-1) Insignificant CAS (< 50%) (n = 96)	(Group-2) Significant CAS (≥ 50%) (n = 204)	P value
Clinical data			
Age, (years)	70.0 ± 7.2	72.5 ± 7.2	0.008
Female gender, n (%)	43 (44.7)	97 (47.5)	0.710
Hypertension, n (%)	47 (48.9)	116 (56.9)	0.216
Hyperlipidemia, n (%)	42 (43.8)	96 (47.1)	0.621
Diabetes mellitus, n (%)	24 (25.0)	79 (38.7)	0.020
Current smoker, n (%)	32 (33.3)	70 (34.3)	0.867
BMI, kg/m^2	26.1 ± 2.5	26.8 ± 2.9	0.031
History of CAD	32 (33.3)	94 (46.1)	0.045
LVEF (%)	62 ± 5.1	61 ± 5.5	0.319
Statin, n (%)	32 (33.3)	89 (43.6)	0.102
ACE-I/ARB, n (%)	43 (44.8)	109 (53.4)	0.175
Beta blocker, n (%)	41 (42.7)	99 (48.5)	0.386
Pre-angiographic laboratory data			
Serum creatinine (mg/dL)	0.90 ± 0.15	0.91 ± 0.16	0.723
hsCRP (mg/dL)	2.68 ± 1.95	3.42 ± 1.76	0.002
Fibrinogen (mg/dL)	289 ± 68	312 ± 63	0.004
Albumin (mg/dL)	3.94 ± 0.48	3.76 ± 0.52	0.006
Fibrinogen to albumin ratio	74 ± 19	84 ± 21	< 0.001
LDL-C (mg/dL)	126 ± 30	127 ± 27	0.698
HDL-C (mg/dL)	41 ± 12	34 ± 8	< 0.001
Hemoglobin (g/dL)	13.6 ± 1.5	13.7 ± 1.6	0.543
WBC (x10^3/mm^3)	6.9 ± 1.3	7.2 ± 1.7	0.081
Platelet (x10^3/mm^3)	242 ± 97	246 ± 88	0.691
Monocyte (x10^6/mm^3)	417 ± 164	495 ± 143	< 0.001
Monocyte to HDL ratio	12.1 ± 8.4	15.7 ± 6.2	< 0.001
Bilateral CAS, n (%)	19 (19.8)	64 (31.3)	0.039
The degree of CAS, (%)	23 ± 11	70 ± 14	< 0.001

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CAS, carotid artery stenosis; hsCRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; LDL-C, low-density lipoprotein-cholesterol; WBC, white blood cell.

patients (32%) had clinically insignificant CAS (<%50) (group-1), 204 patients (68%) had clinically significant CAS (≥50%) (group-2). Table 1 shows the comparison of clinical and laboratory data of the two groups. Group-2 patients had older age, a greater body mass index, higher rates of history of coronary artery disease (CAD), diabetes mellitus, and 2-sided carotid disease, lower albumin and HDL-C, greater hsCRP, monocyte, and fibrinogen, and greater MHR and FAR than group-1 patients. Then, the study population was split into tertiles (T) based on pre-angiographic MHR (T1:<10.33; T2:10.33–16.60; T3:>16.60). Table 2 shows the comparison of clinical and laboratory parameters of patients by tertiles (T) of MHR. Patients in T3 showed higher hsCRP, fibrinogen, monocyte counts, and lower HDL-C, and also higher FAR than in T1 and T2. Patients in T3 and T2 also showed a greater degree of CAS than in T1.

According to univariate analysis, age, diabetes mellitus, BMI, history of CAD, the presence of 2-sided carotid disease, MHR, FAR, and hsCRP were significantly associated with significant CAS. Multivariate analysis showed that diabetes mellitus, BMI, MHR, FAR, and hsCRP were independent predictors of significant CAS.

In the correlation analysis, MHR, FAR and hsCRP were significantly correlated to each other (MHR-hsCRP: r=0.706, p<0.001; FAR-hsCRP: r=0.622, p<0.001; MHR-FAR: 0.637, p<0.001). While the degree of CAS was associated with MHR (r=0.195, p=0.001) and FAR (r=0.166, p=0.004), it was not associated with hsCRP (r=0.093, p=0.106).

Finally, the ROC curve analysis showed the cutoff value of ≥11 for MHR with a sensitivity of 75% and a specificity of 70% (AUC: 0.732; CI, 0.660-0.804;

FIGURE 1. RECEIVER OPERATING CHARACTERISTIC (ROC) CURVES OF THE PRE-ANGIOGRAPHIC MONOCYTE TO HDL RATIO (MHR), FIBRINOGEN TO ALBUMIN RATIO (FAR,) AND HIGH-SENSITIVE C-REACTIVE PROTEIN (HSCRP) FOR PREDICTING CLINICALLY SIGNIFICANT CAROTID STENOSIS IN PATIENTS WITH ASYMPTOMATIC CAROTID ARTERY DISEASE. (AUC, AREA UNDER THE CURVE; CI, CONFIDENCE INTERVAL)

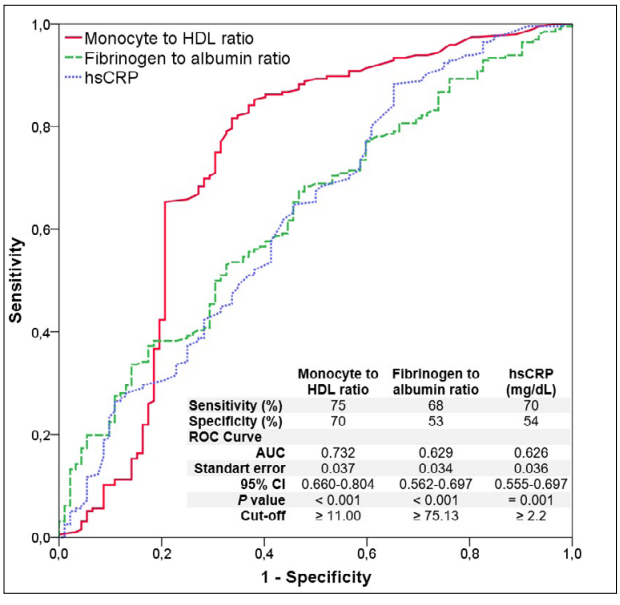

TABLE 2. COMPARISON OF CLINICAL AND LABORATORY DATA BY TERTILES OF MHR IN PATIENTS WITH ASYMPTOMATIC CAROTID ARTERY DISEASE.

Variables	Tertile 1 (<10.33) (n = 100)	Tertile 2 (10.33-16.60) (n = 100)	Tertile 3 (>16.60) (n = 100)	P value
Clinical data				
Age (years)	71.1 ± 7.2	71.4 ± 7.1	72.8 ± 7.5	0.219
Female gender, (n, %)	42 (42)	47 (47)	51 (51)	0.443
Hypertension, (n, %)	49 (49)	59 (59)	55 (55)	0.361
Hyperlipidemia, (n, %)	41 (41)	46 (46)	51 (51)	0.366
Current smoker, (n, %)	31 (31)	38 (38)	33 (33)	0.560
BMI (kg/m2)	26.4 ± 2.5	26.5 ± 2.9	26.8 ± 2.9	0.586
History of CAD, (n, %)	36 (36)	40 (40)	52 (52)	0.059
LVEF (%)	60.9 ± 5.1	61.1 ± 5.3	62.1 ± 5.6	0.240
Statin, (n, %)	40 (40)	39 (39)	42 (42)	0.903
ACE-I/ARB, (n, %)	44 (44)	50 (50)	58 (58)	0.118
Beta blocker, (n, %)	44 (44)	47 (47)	49 (49)	0.760
Pre-angiographic laboratory data				
Serum creatinine (mg/dL)	0.90 ± 0.15	0.91 ± 0.16	0.90 ± 0.16	0.907
hsCRP (mg/L)	2.45 ± 1.7	3.22 ± 1.9*	3.85 ± 1.7**	< 0.001
Fibrinogen (mg/dL)	283 ± 63	305 ± 59*	326 ± 67**	< 0.001
Albumin (mg/dL)	3.87 ± 0.52	3.82 ± 0.42	3.79 ± 0.58	0.522
Fibrinogen to albumin ratio	74 ± 19	81 ± 18*	88 ± 23**	< 0.001
LDL-C (mg/dL)	126 ± 30	127 ± 27	127 ± 26	0.977
HDL-C (mg/dL)	46 ± 9	34 ± 5*	29 ± 5**	< 0.001
Hemoglobin (g/dL)	13.8 ± 1.7	13.7 ± 1.4	13.6 ± 1.6	0.816
WBC (x103/mm3)	6.9 ± 1.6	7.1 ± 1.5	7.2 ± 1.7	0.346
Platelet (x103/mm3)	250 ± 102	253 ± 88	259 ± 84	0.787
Monocyte (x106/mm3)	314 ± 31	447 ± 88*	647 ± 80**	< 0.001
Monocyte to HDL ratio	7.2 ± 1.7	13.3 ± 1.9*	23 ± 4.3**	< 0.001
Bilateral CAS, n (%)	26 (26)	23 (23)	34 (34)	0.199
The degree of CAS, (%)	45 ± 24	59 ± 26*	62 ± 21¶§	<0.05

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CAS, carotid artery stenosis; hsCRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; LDL-C, low-density lipoprotein-cholesterol; WBC, white blood cell. *Tertile-2 vs. Tertile-1; p<0.05. **Tertile-3 vs. Tertile-1 and Tertile-3 vs. Tertile-2; p<0.05. ¶Tertile-3 vs. Tertile-1; p<0.05. §Tertile-3 vs. Tertile-2; p>0.05

p<0.001), and the cutoff value of ≥75 for FAR with a sensitivity of 68% and a specificity of 53% (AUC: 0.629; CI, 0.562-0.697; p<0.001), and the cut-off value ≥2.2 for hsCRP with a sensitivity of 70% and a specificity of 54% (AUC: 0.626; CI, 0.555-0.697; p<0.001) to predict significant CAS. The predictive values of pre-angiographic MHR, FAR, and hsCRP were compared by their AUCs. Pre-angiographic MHR better predicted a significant CAS than FAR and hsCRP (p<0.05) (Figure 1).

DISCUSSION

In our study, we demonstrated that pre-angiographic levels of MHR, FAR, and hsCRP in patients with significant CAS were higher than in those with insignificant CAS. Pre-angiographic MHR was found to be a better predictor than FAR and hsCRP in determining the presence of a clinically significant CAS in patients with asymptomatic carotid artery disease.

Monocytes act as a crucial source of pre-inflammatory species during atherosclerosis, and they change into foam cells to release pro-inflammatory and pro-oxidant cytokines, resulting in the collection of more monocytes and building up of cholesterol ester-loaded plaque at the inflammation area[5]. Several studies have shown that greater monocyte counts are a crucial indicator of adverse events in atherosclerotic diseases such as CAD and ischemic stroke[5,6]. HDL-C has anti-inflammatory and anti-oxidant effects by hindering LDL-C oxidation and monocytes entering into the vascular wall, resulting in endothelial or

vascular protection from inflammation and oxidative stress[5,7]. Ultimately, while monocytes play an active role through their pro-oxidant and pro-inflammatory effects, HDL-C serves as a neutralizer by suppressing inflammatory markers such as monocytes in atherosclerotic events. For this reason, the integration of monocytes and HDL-C into MHR produces an organized formula to assess atherosclerosis by examining the association between inflammation and dyslipidemia[5,8]. Investigations have shown the usability of MHR to predict cardiovascular outcomes of metabolic syndrome and several atherosclerotic diseases, such as in-stent restenosis, and CAD[14-16]. Recently, Cetin et al.[16] found that MHR was an independent predictor of the severity of CAD and had a significantly positive correlation with CRP levels. In our study, MHR was a better predictor than FAR and hsCRP in determining significant CAS and had a significant association with FAR and hsCRP, indicating that inflammation could play a pivotal role in the pathogenesis of carotid atherosclerosis. In line with our findings, Wang et al.[8] evaluated the association between MHR and ischemic stroke and found a linear relation of MHR with ischemic stroke in a large cohort.

Patients with CAS have high fibrinogen levels[3,4]. Little is known about the relationship between albumin level and CAS, and data from those studies have been inconsistent[19,20]. Because fibrinogen and albumin a positive and negative correlations with the inflammatory reaction, respectively, their proportion may be associated with inflammation. Therefore, fibrinogen and albumin levels have been recommended to be assessed together, when their effects on cardiovascular disease are explored[11,12]. FAR was found to be significantly higher in patients with cardiovascular events than in those without any cardiovascular event[11]. In a study by Karahan et al.[17] FAR was reported to be a highly specific indicator for predicting the severity of CAD in ST-elevation myocardial infarction. Moreover, He et al.[12] have recently shown that FAR might serve as a prognostic marker in non-ST-elevation myocardial infarction. In line with these data, we found that FAR was one of the indicators in predicting significant CAS in our study. Additionally, we observed that FAR was associated with other inflammatory parameters such as hsCRP and MHR. Another finding was that FAR and MHR were moderately associated with the degree of CAS. However, we could not find any relationship between hsCRP and the degree of CAS. To our knowledge, there is no information in the literature about the association of MHR and FAR with the degree of stenosis. Puz et al.[2] showed that higher hsCRP values were able to predict the presence of carotid artery plaque, but they were not associated with the degree of carotid stenosis, strongly supporting our data.

Our study has several limitations. First, we could not observe time-dependent changes in MHR and FAR levels or the degree of CAS because of the cross-sectional study design. Second, single computations may not entirely show the true trend of the analyzed variables. Therefore, we could not assess causal relationships between MHR and FAR levels and the development and/or progression of carotid atherosclerosis. Third, we did not investigate other inflammatory markers and whether carotid lesions are stable or unstable. Fourth, we did not include symptomatic patients. Lastly, this study was not designed to report on long-term clinical outcomes.

CONCLUSION

Our results suggested that the ability of pre-angiographic MHR level to predict a clinically significant CAS was superior to that of FAR and hsCRP and could help us to early detect patients with asymptomatic CAS at high risk. Therefore, patients with asymptomatic CAS and high levels of MHR may require a close medical follow-up and an intensive treatment of risk factors. Large-scale clinical studies are needed to prove our findings.

Conflict of Interest

None.

Source of Funding

None.

Author's Contribution

Mustafa Yurtdaş: data curation, formal analysis, investigation methodology, project administration, resources, software, supervision, validation, writing of the original draft, review, and editing.

Yalin Tolga Yaylali: Formal analysis, ınvestigation, methodology, supervision, validation, visualization, writing of the original draft, review, and editing.

Mahmut Özdemir: Formal analysis, ınvestigation methodology, software, supervision, validation, visualization.

RESUMO

OBJETIVO: *Recentemente, a contagem de monócitos para a proporção HDL-C (MHR) e a relação fibrinogênio para albumina (FAR) emergiram como marcadores de inflamação em doenças ateroscleróticas. Nosso objetivo é investigar a relação da MHR e FAR com a gravidade da estenose da artéria carótida (CAS) em pacientes com doença assintomática da artéria carótida.*

MÉTODOS: *Este estudo retrospectivo incluiu 300 pacientes com CAS assintomática. MHR pré-angiográfica, FAR e proteína C reativa de alta sensibilidade (hsCRP) foram medidas. A angiografia carotídea foi realizada em pacientes com estenose ≥50% na ultrassonografia carotídea. Os pacientes foram primeiramente divididos em dois grupos com base no grau de CAS e depois nos tercis (T) da MHR.*

RESULTADOS: *Noventa e seis pacientes apresentaram um CAS clinicamente insignificante (<50%) (grupo 1) e 204 pacientes apresentaram CAS clinicamente significativo (≥50%) (grupo 2). O grupo 2 apresentou MHR, FAR e hsCRP superior ao grupo 1. Pacientes em T3 apresentaram maior MHR, FAR e hsCRP do que em T1 e T2. MHR, FAR e hsCRP foram correlacionados entre si (p<0,001, para todos). MHR, FAR e hsCRP foram preditores independentes de CAS significativa. MHR predisse melhor uma CAS significativa que FAR e hsCRP (p<0,05).*

CONCLUSÕES: *A MHR pré-angiográfica pode ser um melhor preditor que a FAR e a hsCRP na identificação de estenose carotídea clinicamente significativa em pacientes com CAS assintomática. Pacientes com CAS assintomática e alto nível de MHR devem ser acompanhados de perto para supervisionar o controle dos fatores de risco e intensificar o tratamento.*

PALAVRAS-CHAVE: *Inflamação. Estenose das carótidas. Monócitos. HDL-Colesterol. Fibrinogênio. Albuminas. Angiografia.*

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ORIGINAL ARTICLE

Predictive values of C-reactive protein/albumin ratio in new-onset atrial fibrillation after coronary artery bypass grafting

Fatih Aksoy[1]
Dinçer Uysal[2]
Erdogan Ibrişim[3]

1. Associate Professor from the Department of Cardiology, Suleyman Demirel University, Medical School, Isparta, Turkey.
2. Associate Professor from the Department of Cardiovascular Surgery, Suleyman Demirel University, Medical School, Isparta, Turkey.
3. Professor from the Department of Cardiovascular surgery, Suleyman Demirel University, Medical School, Isparta, Turkey.

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SUMMARY

OBJECTIVE: *This study aimed to investigate the predictive value of the newly defined C-Reactive Protein (CRP)/Albumin Ratio (CAR) in determining the development of atrial fibrillation (AF) in comparison with other inflammatory markers, such as Neutrophil/Lymphocyte (N/L) Ratio and Platelet/Lymphocyte (P/L) Ratio, in patients undergoing Coronary Artery Bypass Grafting (CABG) surgery.*

METHODS: *The population of this observational study consisted of 415 patients undergoing CABG. The study cohort was subdivided into two groups based on the development of AF. Complete blood counts, serum CRP, and serum albumin levels were evaluated before the CABG. The CAR, N/L, and P/L ratios of all the patients were calculated. Predictors of postoperative AF were determined by multiple logistic regression analysis (MLRA).*

RESULTS: *During follow-up, 136 patients (32.8%) developed postoperative AF. With MLRA, independent risk factors for postoperative AF were determined as follows: fasting glucose level (OR: 1.01; 95 % CI: 1.00-1.01, P <0.001), age (OR: 1.12; 95 % CI: 1.07-1.17, P <0.001), left ventricle ejection fraction (OR: 0.90; 95 % CI: 0.87-0.94, P <0.001), male gender (OR: 3.32; 95 % CI: 1.39-7.90, P = 0.007), 24-hour drainage amount (OR: 1.004; 95 % CI: 1.002-1.005, P <0.001), and CAR (OR: 1.82; 95 % CI: 1.53-2.16, P <0.001). Receiver Operating Characteristic curve analysis showed that CAR (C-statistic: 0.75; 95% CI: 0.71-0.79, p< 0.001) was a significant predictor of AF.*

CONCLUSION: *Novel inflammatory marker CAR can be used as a reliable marker to predict the development of AF following CABG.*

KEYWORDS: *Protein C. Albumins. Atrial fibrillation. Myocardial revascularization. Coronary artery bypass.*

INTRODUCTION

Inflammation plays an important role both in the onset of Atrial Fibrillation (AF) and its maintenance through myocyte necrosis, fibrosis, and infiltration of inflammatory markers.[1-3] Several inflammatory markers, including the High-Sensitive C-Reactive Protein (Hs-CRP) and Interleukin-6 (IL-6), levels were reported to be elevated in patients with different AF subtypes compared to those with sinus rhythm.[2-5]

Postoperative atrial fibrillation (POAF) is a major and fatal complication of Coronary Artery Bypass Grafting (CABG) surgery. Patients developing Atrial Fibrillation (AF) after cardiac surgery have a higher

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CORRESPONDING AUTHOR: Fatıh Aksoy
Suleyman Demirel Univesitesi Tip Fakultesi, Isparta, Turkey
Tel: +90 505 231-3661 / Fax: +90 246 232-4510
E-mail: dr.aksoy@hotmail.com

risk of morbidities such as cerebrovascular events, pulmonary edema, longer hospital stays, and mortality compared to those who do not develop AF.[6-8] Identifying the patients who may develop POAF before the surgery and taking the necessary precautions may decrease the mortality and morbidity rates. An association between POAF and inflammation has been shown in previous studies[5,6]. C-Reactive Protein (CRP)/Albumin Ratio (CAR), a novel parameter of inflammation, has been shown to be superior to CRP or albumin levels alone in determining inflammatory status in several cardiovascular diseases.[9,10] However there are no published studies on the association between CAR and POAF in the literature. In this study, we aimed to investigate the predictive value of CAR in the development of POAF.

METHODS

Study population

The study included patients who underwent isolated Coronary Artery Bypass Grafting (CABG) surgery at the Suleyman Demirel University, Education and Research Hospital between March 2017 and June 2019. The study population was retrospectively and consecutively analyzed by using a database that collated patient data as a part of routine clinical practice. The overall study population included 475 patients undergoing CABG. The exclusion criteria included hyperthyroidism, age <18 years, prior cardiac surgery, class III or IV heart failure, previous atrial fibrillation, left atrial diameter >55 mm, left ventricular ejection fraction <0.25, sepsis, heart rate <60 bpm, systolic blood pressure <90 mm Hg, inflammatory disease, pericarditis, patients undergoing off-pump surgery, and being on antiarrhythmic treatment. According to these criteria, 35 patients were excluded due to previous atrial fibrillation (n = 15), heart rate <60 bpm (n = 5), hyperthyroidism (n = 5), left atrial diameter> 55 mm (n= 5), ejection fraction <0.25 (n=5), and patients undergoing off-pump surgery (n=25). Therefore, 415 patients were included in the final study cohort. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee (Date: 28.05.2019, Decision no: 181). Similar operative techniques were used for all patients. A transthoracic echocardiogram was recorded for each patient before and after the surgery.

Blood collection and laboratory analysis

Blood samples were drawn from the antecubital vein by careful vein puncture using a 21-G sterile syringe without stasis at 08:00–10:00 h after a fasting period of 12h. Hematologic and biochemical measurements including liver enzymes were carried out. An automatic blood counter (LH 780 Hematology Analyzer, Beckman Coulter Inc., Miami, FL) was used for whole blood counts including total White Blood Cells (WBCs), hemoglobin, platelets, neutrophils, lymphocyte, and monocytes. Serum C-reactive Protein (CRP) levels were measured using BN2 Nephelometry Analyzer II (Dade Behring, Kalletal, Germany). The normal value for CRP is in the range of 0–6 mg/L.

Rhythm follow-up

The rhythms were followed-up by continuous electrocardiogram monitoring during the patients' stay at the intensive care unit and by 24-hour Holter during the rest of hospitalization. A 12-lead electrocardiogram was used for recording routinely every morning and whenever the patients had symptoms suggestive of dysrhythmia. Atrial fibrillation was defined as an irregular rhythm with the absence of discrete P waves in the 12-lead electrocardiogram. An atrial fibrillation episode lasting for at least 5 minutes during hospitalization was defined as POAF.[11]

Statistical analysis

Statistical Package Social Sciences (SPSS) software version 16.0 package and Medcalc version 15.2 were used for statistical analyses in this study. Categorical variables were expressed as frequency (%) and compared using the $\chi 2$ test. The Kolmogorov-Smirnov test was used to test the distribution of numeric variables; those with normal distribution were expressed as mean ± standard deviation and were compared with the Student's t-test. Data without normal distribution were expressed as median (Inter-quartile range (IQR) of 25%-75% percentiles) and were compared with the Mann-Whitney U test. In all statistical analyses, a P-value <0.05 was considered statistically significant. The correlations between CAR, presence of AF, and other clinical, laboratory, and echocardiographic parameters were measured by Pearson or Spearman correlation analysis when appropriate. Univariate analysis of binary logistic regression was performed to identify which factors are associated with incident AF. After including each of these potential confounding factors, backward conditional binary logistic

regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (95% CI) for incident AF. We used a receiver operating characteristic (ROC) analysis with area under the curve and CAR, CRP, and albumin cut-off points for prediction of AF. All ROC comparisons were performed using the DeLong test. Predictors of AF were determined by logistic regression analysis.

RESULTS

A total of 415 patients (mean age: 62.86±11.86 years; range, 28–84 years) were included in this study. During the follow-up period, 136 patients (32.8%) developed POAF. The demographic and clinical characteristics of the patients with and without POAF are listed in Table 1. The patients with POAF were significantly older and there were more males when

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH AND WITHOUT AF.

	Without AF (n = 279)	With AF (n = 136)	P-value
Age (years)	60.5 ± 12.4	67.5 ± 8.9	< 0.001
Body mass index	29.0 ± 5.2	28.0 ± 4.4	0.05
Female gender (n, %)	90 (32.3)	26 (19.1)	0.003
Diabetes mellitus (n, %)	114 (40.9)	73 (53.7)	0.009
Hypertension (n, %)	202 (72.4)	121 (89.0)	< 0.001
Congestive heart failure (n, %)	14 (5.1)	27 (20.8)	< 0.001
Peripheral vascular disease (n, %)	72 (25.8)	50 (36.8)	0.015
History of CVA (n, %)	36 (13.8)	24 (21.8)	0.04
Ejection fraction (%)	56.6 ± 8.4	50.3 ± 10.0	< 0.0001
Left atrial diameter (mm)	39.1 ± 6.1	38.3 ± 7.7	0.289
LVEDD (mm)	47.3 ± 5.5	46.9 ± 4.9	0.379
LVESD (mm)	29.8 ± 6.9	31.7 ± 5.6	0.007
IVSD (mm)	12.0 ± 3.1	12.6 ± 3.4	0.007
PWD (mm)	11.1 ± 2.2	11.1 ± 0.9	0.988
AoD (mm)	26.9 ± 3.5	26.8 ± 3.3	0.832
Total cholesterol (mg/dl)	201.2 ± 41.3	196.9 ± 39.4	0.412
HDL cholesterol (mg/dl)	41.8 ± 10.2	41.2 ± 9.0	0.632
LDL cholesterol (mg/dl)	126.0 ± 40.4	124.3 ± 41.5	0.760
Triglycerides (mg/dl)	158.2 ± 104.1	141.9 ± 48.6	0.187
Creatinine(mg/dl)	1.0 ± 0.3	11.1 ± 0.3	0.103
Glucose(mg/dl)	142.5 ± 67	198.6 ± 119.9	< 0.001
Lymphocyte (10^3/µl)	2556 ± 0.978	2576 ± 1.063	0.849
Platelet (10^3/µl)	230 ± 63	232 ± 78	0.773
CAR, median (IQR)	0.96 (0.5-2.0)	4.3 (1.1-8.6)	< 0.001
N/L ratio	3.3 ± 3.1	4.3 ± 6.8	0.05
P/L Ratio	11.3 ± 9.4	140 ± 17.2	0.04
CRP (mg/l), median (IQR)	4.00(2.00-8.00)	16.00 (4.25-33.0)	<0.001
Albumin (g/l)	3.8± 0.4	3.7±0.3	0.002
WBC (10^3/µl)	8952±4227	8751±2240	0.513
Operative and postoperative parameters			
Cardiopulmonary bypass time (min)	78.8 ± 27.3	86.8 ± 39.6	0,02
X Clamp time (min)	47.5 ± 18.8	46.7 ± 16.2	0,686
24-hour drainage (ml)	28.2 ± 14.5	388.7 ± 232.3	0,003
48-hour drainage (ml)	147.8 ± 83.9	159.9 ± 69.8	0,148
Duration of the hospitalization at the intensive care unit (days)	2.1 ± 0.4	2.6 ± 1.4	< 0.001
Bypass number (n)	2.4±1.7	2.6±2.1	0.158
Reoperation due to hemorrhage (n, %)	6 (2.2)	2 (1.5)	0.985
İntraoperative mortality (n, %)	-	-	
In-hospital mortality (n, %)	8 (2.9)	6 (4.4)	0.786

Data presented as mean ± standard deviation, median (IQR) or number (%) of the patients. CVA = cerebrovascular accident; HDL = high-density lipoprotein; LVEDD= Left Ventricle end diastolic diameter; LVESD= Left Ventricle end systolic diameter; IVSD: interventricular septum diameter; PWD: Posterior wall diameter; AoD: aortic diameter; CAR: C reactive protein to albumin ratio; N: neutrophil; L: Lymphocyte; P: Platelet; WBC: White blood cell

compared to patients without POAF (p< 0.001 and p= 0.003, respectively). The presence of diabetes mellitus, hypertension, congestive heart failure, peripheral vascular disease, and stroke/transient ischemic events was higher in patients with POAF compared to patients without POAF. There were no statistically significant differences in cholesterol parameters (p > 0.05 for all parameters) between patients with and without POAF. Left ventricle ejection fraction was significantly lower in patients with POAF compared to patients without POAF (p< 0.001). Preoperative fasting glucose levels were higher in patients with POAF compared to patients without POAF (p< 0.001). There were no statistically significant differences in patients with and without POAF in whole blood parameters, including platelet (P) count, white blood cell, neutrophil (N), and lymphocyte (L) level; however, the N/L and P/L ratios were significantly higher in patients with POAF compared to patients without POAF (p= 0.05 and p= 0.04 respectively.)

The postoperative drainage amounts in the first 24 and 48 hours were higher in patients with POAF than in patients without POAF (P= 0.003 for 24 hours and P= 0.148 for 48 hours) (Table 1). Cardiopulmonary bypass time was longer in patients with POAF compared to patients without POAF (P= 0.02) but there was no statistically significant difference in the clamp time between patients with and without POAF. The duration of hospitalization at the intensive care unit was longer in patients with POAF than in patients without POAF (P< 0.001). There were no statistically significant differences in events such as reoperation due to hemorrhage, and intraoperative and in-hospital mortality (for all parameters p> 0.05) between patients with and without POAF (Table 1).

The median CAR and CRP levels were significantly lower in patients without POAF compared to patients with POAF [0.96 (0.5-2.0) versus 4.3 (1.1-8.6), p < 0.001 for CAR; 4.00 mg/l (2.00-8.00) versus 16.00 mg/l (4.25-33.0), p<0.001 for CRP]. The mean albumin level was lower in patients with POAF compared to patients without POAF (3.8± 0.4 versus 3.7±0.3, P= 0.002)

Prediction of postoperative atrial fibrillation

Univariate analyses showed that body mass index, CRP/Albumin ratio, N/L ratio, P/L ratio, CRP, albumin, diabetes mellitus, hypertension, congestive heart failure, peripheral vascular disease, low left ventricle ejection fraction, advanced age, fasting glucose level, 24-hour drainage amount, cardiopulmonary bypass time and male gender were significantly associated with a higher risk of development of POAF (Table 2). The correlation analysis revealed that CAR exhibited a weak correlation between CAR and N/L ratio (r= 0.147, P< 0.001) and P/L ratio (r= 0.226, P= 0.001). To determine the independent predictors for

TABLE 2. FACTORS THAT WERE FOUND TO BE INDEPENDENTLY ASSOCIATED WITH THE DEVELOPMENT OF POSTOPERATIVE ATRIAL FIBRILLATION IN UNIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION ANALYSIS MODELS.

	Unadjusted Odds Ratio	Confidence Interval	P-value	Adjusted Odds Ratio	Confidence Interval	P value
Diabetes mellitus	1.67	1.11-2.53	0.014			
Hypertension	3.075	1.69-5.58	< 0.001			
Congestive heart failure	4.85	2.44-9.61	< 0.001			
Peripheral vascular disease	1.67	1.07-2.59	0.02			
BMI	0.96	0.92-1.00	0.06			
Fasting glucose level	1.006	1.004-1.009	< 0.001	1.01	1.00-1.01	< 0.001
Age	1.06	1.04-1.08	< 0.001	1.12	1.07-1.17	< 0.001
Male gender	2.015	1.22-3.33	0.006	3.32	1.39-7.90	0.007
24-hour drainage	1.002	1.001-1.003	0.004	1.004	1.002-1.005	< 0.001
Left ventricle ejection fraction	0.93	0.91-0.95	< 0.001	0.90	0.87-0.94	< 0.001
Cardiopulmonary bypass time	1.007	1.00-1.01	0.04			
CRP to Albumin Ratio	1.575	1.40-1.76	< 0.001	1.82	1.53-2.16	< 0.001
N to L ratio	1.040	0.89-1.08	0.06			
P to L ratio	1.01	1.00-1.03	0.04			
CRP	1.12	1.09-1.15	< 0.001			
Albumin	0.395	0.21-0.71	0.002			

Abbreviations: BMI: body mass ındex, CRP: C reactive protein, N: neutrophil, L: Lymphocyte, P: Platelet

the development POAF, a multivariate binary logistic regression analysis was carried out. The univariate analysis of all parameters associated with new-onset AF except NLR, PLR, CRP and albumin showed that fasting glucose level (OR: 1.01; 95% CI: 1.00-1.01, P <0.001), age (OR: 1.12; 95% CI: 1.07-1.17, P <0.001), left ventricle ejection fraction (OR: 0.90; 95% CI: 0.87-0.94, P <0.001), male gender (OR: 3.32; 95% CI: 1.39-7.90, P = 0.007), 24-hour drainage amount (OR: 1.004; 95% CI: 1.002-1.005, P <0.001), CAR (OR: 1.82; 95% CI: 1.53-2.16, P <0.001) remained as independent factors for incident AF (Table 2). ROC curve analysis showed that CAR (C-statistic: 0.75; 95% CI: 0.71-0.79, p< 0.001) was a significant predictor of POAF (Figure 1). We calculated that a cut-off point of 3.5 for CAR could estimate the presence of POAF with a sensitivity of 58% and 92%. We performed a pairwise comparison of the ROC curves and observed that the predictive value of the CAR with regard to POAF development was superior to that of albumin and CRP (DeLong method, AUC_{CAR} versus AUC_{CRP} z test= 3.592, p= 0.0003; AUC_{CAR} versus $AUC_{Albumin}$ z test= 3.927, p= 0.0001; $AUC_{albumin}$ versus AUC_{CRP} z test= 3.594, p= 0.0003)

DISCUSSION

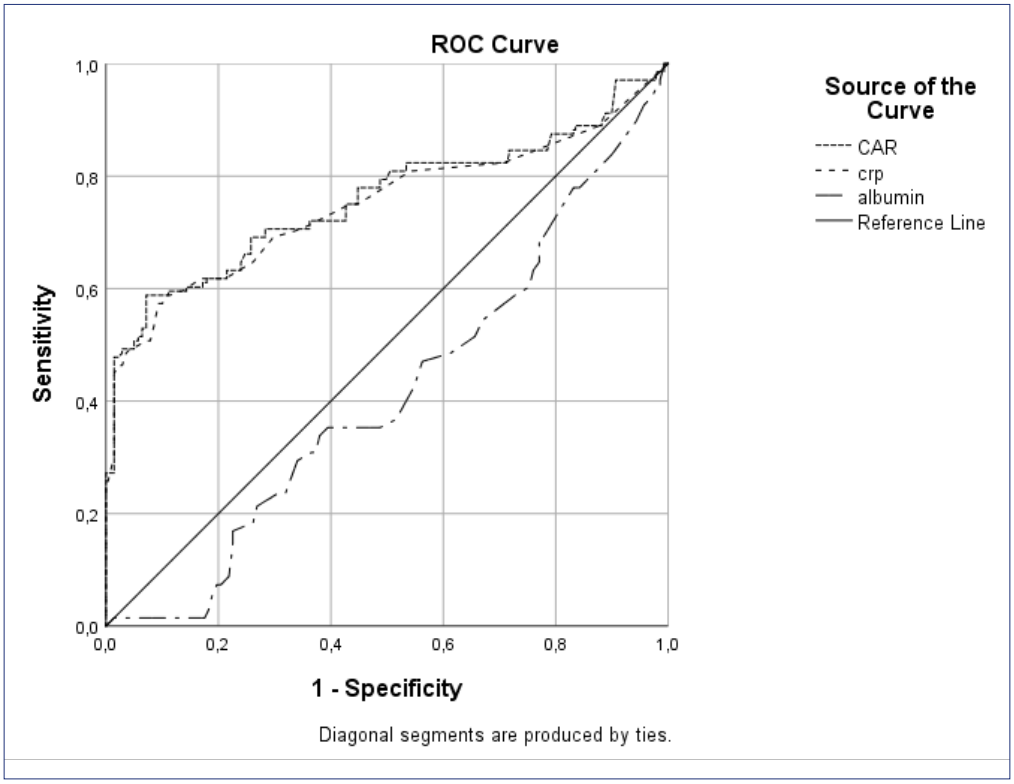
Main findings

The main findings of this observational study are as follows: (i) elevated CAR, high fasting glucose level, advanced age, male gender, first 24-hour drainage amount, and lower LVEF values are significantly associated with POAF; (ii) a CAR value of more than 3.5 and was found to be a predictor of POAF; and (iii) NLR and PLR were higher in patients with POAF compared to without POAF.

Mechanism of postoperative atrial fibrillation

In the postoperative period, AF is thought to be triggered by electrophysiological abnormalities, which

FIGURE 1. RECEIVER OPERATING CHARACTERISTICS (ROC) CURVE ANALYSIS OF THE VARIABLES.



	C-statistic	95 % Confidence İnterval	P-value	Cut-off value	Sensitivity	Specificity
CAR	0,758	0,713 -0,798	<0.001	>3.5	58	92
albumin	0,587	0,538- 0,635	0.0031	≤4.31	98	17
crp	0,748	0,704 -0,789	<0.001	>12.7	58	89

CAR: C reactive protein to albumin ratio; CRP: C reactive protein; pairwise comparision of variables: (by DeLong method, AUC CAR versus AUC CRP z test= 3.592, p= 0.0003; AUC CAR versus AUC Albumin z test= 3.927, p= 0.0001; AUC albumin versus AUC CRP z test= 3.594, p= 0.0003).

frequently happen in patients who have an abnormal atrial substrate.[6] High catecholamine state[7] and postoperative inflammation[6] are thought to have lay a key role.

Inflammation and postoperative atrial fibrillation

As bypass surgery may induce oxidative stress and inflammation, these processes may be responsible for complications after cardiac surgery, including POAF.[6] Several studies have suggested a strong link between inflammation and atrial fibrillation.[12] Increased inflammatory processes are also suggested to be associated with new-onset AF after coronary artery bypass grafting. Lo et al.[13] showed that CRP level >3 mg/L was associated with an increased risk of AF in patients undergoing CABG. Supporting this, high CRP levels were associated with the development of new-onset AF following CABG in the present study. Serum CRP, an acute-phase protein, is released from the liver in response to inflammation and has been associated with poor prognosis for patients with coronary artery disease (CAD).[14] De Lorenzo et al.[15] showed that high preoperative CRP levels were associated with in-hospital mortality after CABG. Kinoshita et al.[16] reported that preoperative CRP levels were independently associated with the development of AF after isolated off-pump CABG. Although the relationship between CRP and CAD is not fully understood, multiple mechanisms may be suggested. CRP has been shown to disturb the endothelial progenitor cells, increase the prothrombotic status, activate the complement system, and play a role in the uptake of low-density lipoprotein-C by macrophages and convert them into foam cells.[17] Additionally, a strong association between AF and inflammation has been reported.[1,3] The most important pathophysiological changes described in previous studies include the presence of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies and the presence of circulating autoantibodies against myosin heavy chain.[1,6] Serum albumin, a negative acute-phase protein, is released from the liver in response to inflammation, and decreased albumin levels have been associated with adverse cardiovascular events.[18] Additionally, albumin is not solely associated with inflammation but also with blood viscosity and endothelial functions. Decreased albumin levels increase blood viscosity and platelet activation and worsen endothelial functions.[19] These factors may explain the association between CRP, albumin levels, and POAF. In the present study, we showed that increased CRP levels, decreased albumin levels, and increased CAR were associated with POAF.

The N/L ratio is a widely available marker of inflammation that is cheap and easy to obtain and can be used in the risk classification of patients with several cardiovascular diseases in addition to the traditionally used markers. It is also considered to be a good and powerful predictor of mortality and morbidity in patients undergoing CABG surgery.[20] In this setting, Gibson et al.[21] showed that high pre- and postoperative N/L ratios were associated with the development of AF after CABG. Corroborating this, preoperatively the N/L ratio was found to be higher in patients with new-onset AF following CABG in the present study. Additionally, platelets are a source of inflammatory mediators. Increased platelet levels were reported to be associated with adverse cardiovascular outcomes.[22] A high P/L ratio, defined as a biomarker of inflammation, was reported to be associated with POAF in patients undergoing CABG.[23] In the current study, the preoperative P/L ratio was also higher in patients with new-onset AF following CABG. Another important finding of the present study is the association of advanced age with the development of POAF. The inflammatory response can increase with advanced age via the activity of the mitochondrial adaptor protein p66 (Shc) and sirtuins, which are a family of deacetylase enzymes.[24] Previous studies have shown that hypertension, diabetes mellitus, obesity, valvular disease, increased age, and left atrial characteristics such as size, volume, and scarring can contribute to the development of POAF.[25] Supporting this, the incidence of hypertension, diabetes mellitus, congestive heart failure, and peripheral arterial disease history was higher in patients with POAF compared to patients without POAF in the present study.

In recent studies, several factors have been mentioned, including predisposing ones, such as obesity, diabetes mellitus, hypertension, advanced age, metabolic syndrome, intraoperative ones, such as surgical methods, off-pump surgery, and acute volume changes, and postoperative ones, such as hypotension and volume overload.[5-8] Our data showed that advanced age, obesity, hypertension, male gender, diabetes mellitus, congestive heart failure, and peripheral vascular disease were more frequently present in patients with POAF than in those without POAF.

Additionally, patients with POAF had a longer hospitalization period, cardiopulmonary bypass time, and 24-hour drainage amount than patients without POAF. Multivariate analysis showed that fasting glucose level, advanced age, male gender, 24-hour drainage, and left ventricle ejection fraction were independent predictors of POAF.

Combining albumin and CRP into a single index was demonstrated to be associated with adverse events in coronary artery disease.[9,10] We showed here that an increased CRP/albumin ratio indicates a higher inflammatory state and may be superior to CRP and albumin alone in determining POAF. To our knowledge, this is the first study to evaluate the relationship between CAR and POAF. Our results suggest that elevated CAR in patients undergoing CABG was an independent predictor of new-onset AF after CABG, and the predictive accuracy of CAR was better than that of CRP and albumin level, as per the comparison of the ROC curves.

Study limitations

Importantly, this study has some limitations. First, it has a retrospective design, a relatively small sample size, and engaged in a single-center experience. Second, we evaluated only baseline CRP and albumin levels before CABG and the changes that would be observed by consecutive measurements, such as in the postoperative period, that may have an additional predictive value. Third, the data acquired did not let us appraise the prognostic value of CAR on adverse cardiovascular outcomes, since we recorded only limited volume and event rate values.

CONCLUSIONS

This study demonstrated that decreased LVEF, elevated CAR, male gender, advanced age, 24-hour drainage, and increased fasting glucose level were independent predictors for the development of POAF. We also observed that the predictive accuracy of CAR for the development of POAF was better than that of NLR or PLR.

This study revealed that CAR was statistically significantly associated with POAF. CAR is an easy to obtain, easily measurable, and cheap parameter that can predict the development of POAF. This parameter can be part of the preoperative evaluation to identify patients who may develop AF.

Sources of funding

None.

Conflict of interest

None.

The study was conducted in the Suleyman Demirel University Education and Research Hospital in Isparta, Turkey

RESUMO

OBJETIVO: *Este estudo teve como objetivo investigar o valor preditivo da recém-definida relação entre Proteína C-Reativa (PCR) e Albumina (CAR) na determinação do desenvolvimento de Fibrilação Atrial (FA) em comparação com outros marcadores inflamatórios, como proporção de Neutrófilos para Linfócitos (N/L) e relação Plaquetas/Linfócitos (P/L) em pacientes submetidos à Cirurgia de Revascularização do Miocárdio (CRM).*

MÉTODOS: *A população deste estudo observacional foi composta por 415 pacientes submetidos à cirurgia de revascularização do miocárdio. A coorte do estudo foi subdividida em dois grupos de acordo com o desenvolvimento da FA. Contagens sanguíneas completas, PCR sérica e albumina sérica foram obtidas antes da CRM. Os valores de CAR, relação N/L e relação P/L foram calculados. Os preditores de FA pós-operatória foram determinados por análise de regressão logística múltipla.*

RESULTADOS: *Durante o acompanhamento, 136 pacientes (32,8%) desenvolveram FA pós-operatória. Com análise de regressão logística múltipla, foram determinados os fatores de risco para FA pós-operatória: glicemia de jejum (OR: 1,01; IC 95%: 1,00-1,01, p<0,001), idade (OR: 1,12; IC 95%: 1,07-1,17, p<0,001), fração de ejeção do ventrículo esquerdo (OR: 0,90; IC 95%: 0,87-0,94, p<0,001), sexo masculino (OR: 3,32; IC 95%: 1,39-7,90, p=0,007), quantidade de drenagem de 24 horas (OR: 1,004; IC 95%: 1,002-1,005, p<0,001), CAR (OR: 1,82; IC 95%: 1,53-2,16, p<0,001). A análise da curva de características operacionais do receptor mostrou que o CAR (estatística C: 0,75; IC 95%: 0,71-0,79, p<0,001) foi um preditor significativo de FA.*

CONCLUSÃO: *O novo marcador inflamatório CAR é confiável para prever o desenvolvimento de FA após a operação de revascularização miocárdica.*

PALAVRAS-CHAVE: *Proteína C. Albuminas. Fibrilação atrial. Revascularização miocárdica. Ponte de artéria coronária.*

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ORIGINAL ARTICLE

Adhesion molecules before and after propylthiouracil in patients with subclinical hyperthyroidism

Ferda Bilgir[1]
Oktay Bilgir[2]
Ozden Yildirim Akan[2]
Ismail Demir[2]

1. Katip Celebi University Ataturk Training and Research Hospital, Department of Allergy and Immunology, Izmir, Turkey.
2. Health Sciences University Bozyaka Training and Research Hospital, Department of Internal Medicine, Bozyaka, Izmir, Turkey.

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SUMMARY

OBJECTIVE: *This study aimed to investigate the effect of propylthiouracil treatment on adhesion molecules in patients with subclinical hyperthyroidism.*

METHODS: *In this study, a total of 168 patients diagnosed with subclinical hyperthyroidism were treated with propylthiouracil for one year. The levels of adhesion molecules, consisting of sICAM-1, sVCAM-1, and sE-Selectin, before and after the treatment were measured and compared. These results were compared with the levels of 148 healthy controls who received a placebo.*

RESULTS: *sICAM-1 levels were significantly higher in subclinical hyperthyroidism patients than in healthy controls (*pa=0.000). sICAM-1 levels were significantly decreased after the treatment (**pb=0.000). Despite this decrease in patients with subclinical hyperthyroidism, it did not decrease to the level of the control group. sVCAM-1 did not change before and after propylthiouracil treatment. The level of sE-selectin was similar to that of the pretreatment control group, but it did not have statistical significance, although it increased after the treatment (**pb=0.004).*

CONCLUSION: *The sICAM level was significantly higher than the pretreatment values and decreased after the propylthiouracil treatment. However, further studies are needed to reduce the risk of atherosclerosis and cancer in patients with subclinical hyperthyroidism.*

KEYWORDS: *Hyperthyroidism. Propylthiouracil. E-Selectin. Intercellular adhesion molecule-1. Vascular cell adhesion molecule-1.*

INTRODUCTION

Subclinical hyperthyroidism is the standard reference mean for free T3 and free T4 values and low or suppressed TSH. It is more common in women and increases with advanced age[1]. While clinical hyperthyroidism increases the risk of cardiovascular disease, heart rhythm, and fracture risk associated with osteoporosis, there are places where subclinical hyperthyroidism presents the same risks. Reports on cardiovascular risk indicate increased coronary artery disease[2]. It increases the risk of atrial fibrillation and ischemic heart disease, and it has been more successful in elderly SHE patients receiving

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CORRESPONDING AUTHOR: Oktay Bilgir
Bozyaka Eğitim ve Araştırma Hastanesi – Saim Çıkrıkçı Caddesi Numara 59 – 35360 Karabağlar, – Izmir
Tel: +902322505050/5242; fax: +902322614444
E-mail: oktaybilgir@gmail.com

l-thyroxine than in the normal population[3]. On the other hand, a five-population-based cohort study found a relative risk of coronary artery disease in patients with subclinical hyperthyroidism (SHE), 1.21 (95% CI = 0.88-1.68) and 1.19 (95% CI = 0.81-1.76) for cardiovascular mortality[4].

The adhesion process and transendothelial migration of leukocytes are provided by cellular adhesion molecules. Cellular adhesion molecules (CAM) are expressed on the surface of the endothelium as a response to various inflammatory cytokines, such as interleukin-1, tumor necrosis factor-α, and interferon-γ[5]. Intracellular CAM-1 and vascular CAM-1 (VCAM-1) are two prototype members of the CAM immunoglobulin superfamily and play a primary role in focal leukocyte accumulation in the subendothelial area. An increase in the plasma levels of ICAM-1 and VCAM-1 can lead to inflammation, and there is also a risk of vascular occlusion in the coronary arteries[6]. E-Selectin, another member of the adhesion molecule family, allows the inclusion of inflammatory leukocytes, which are expressed on the surface of the endothelium.

Adhesion molecules play an important role in atherosclerosis and cancer[6]. TNF-α has an important role at the onset of inflammation, and a signaling pathway through TNF-α, NF-β is responsible for distant centers and the expression of co-ordination lines in the structure. Increased ICAM-1 expression may play an important role in the development of cancer[7].

Subclinical hyperthyroidism may trigger the development of cancer with molecule adhesion, inflammation, and atherosclerotic lesion formation. As a result, we planned to confirm adhesion molecule levels before and after propylthiouracil therapy in patients with subclinical hyperthyroidism.

METHODS

A total of 168 patients diagnosed with subclinical hyperthyroidism were given propylthiouracil treatment for one year. The levels of adhesion molecules before and after the treatment were compared. The cases were compared with 148 control patients receiving a placebo tablet 3 times per day. Subclinical hyperthyroidism was diagnosed in patients with normal free T3 and free T4 levels but below TSH levels. Patients received propylthiouracil 50 mg. The tablets were given 3 times per day. Placebo tablets were given to 148 normal healthy control groups three times a day for control purposes. The sICAM, sVCAM, and sE-Selectin levels of the subclinical hyperthyroid patients were measured before the treatment was started, and the same tests were repeated after 1 year of treatment.

All blood samples were collected under minimal tourniquet pressure. Blood samples were allowed to clot for 15 to 30 minutes and centrifuged at 1500g for 10 minutes. The serum was then separated and stored at -20°C until the analysis. Samples were thawed only once.

Serum sICAM-1, sVCAM-1, and sE-selectin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) kit from Biosource (Bender MedSystems GmbH, Vienna Austria), according to the manufacturer's instructions. The sensitivity of the sICAM-1, sVCAM-1, and sE-selectin assays were 2.2 ng/mL, 0.6 ng/mL, and 0.3 ng/mL respectively. The intra-assay coefficients of variation (CV) for sICAM-1, sVCAM-1, and sE-selectin were 4.1, 3.1, and 5.4 %, respectively, and the inter-assay coefficients of variation (CV) for them were 7.7, 5.2 and 6.0 %, respectively, according to the manufacturer.

STATISTICAL ANALYSIS

All the results were presented as mean ± standard deviation (SD). The Kolmogorov–Smirnov normality test was used to determine the distribution pattern of the variables. To compare the normally distributed data, we used parametric independent-samples t-test (Student's t-test). Pre- and post-treatment sICAM-1, sVCAM-1, and sE-selectin values in patients with SHE were compared using the Wilcoxon Signed Ranks Test which is a nonparametric test.

The statistical analysis was carried out by using Statistical Package for the Social Science (SPSS), version 13.0 (SPSS Inc., Chicago, IL, US). A p-value of <0.05 was considered statistically significant.

RESULTS

The sICAM-1, sVCAM-1, and sE-Selectin levels of 168 SHE patients and 148 healthy controls were compared before and after the propylthiouracil treatment. In the SHE group, sICAM-1 levels were found to be significantly high in comparison with the healthy controls (* p^a=0.000). sICAM-1 levels were significantly decreased after treatment. Despite this decrease in patients with subclinical hyperthyroidism, it did not decrease to the level of the control group. (**

p^b=0.000) (Table, Figure 1). No significant difference was found between healthy controls and patients with SHE in terms of sVCAM-1 levels (p^a=0.632). Although sVCAM-1 levels were slightly increased after the treatment, no significant difference was found (p^b=0.089) (Table, Figure 2).

Although in patients with SHE sE-selectin levels were found to be slightly lower compared to those of healthy controls, that did not reach a statistically significant level (* p^a=0.293). sE-selectin levels did not show a significant change after the treatment (** p^b=0.114) (Table, Figure 3).

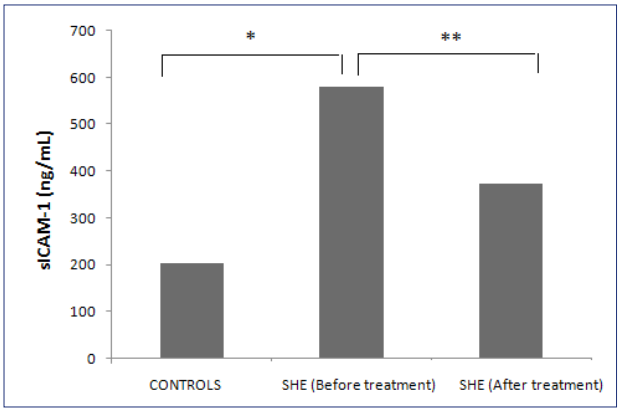

FIGURE 1. sICAM-1 LEVELS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM BEFORE AND AFTER PROPYLTHIOURACIL TREATMENT

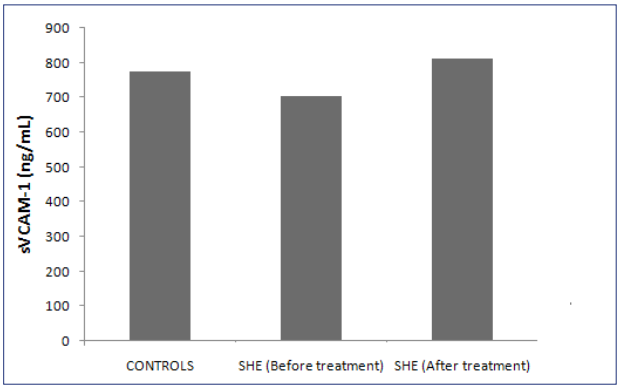

FIGURE 2. sVCAM-1 LEVELS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM BEFORE AND AFTER PROPYLTHIOURACIL TREATMENT

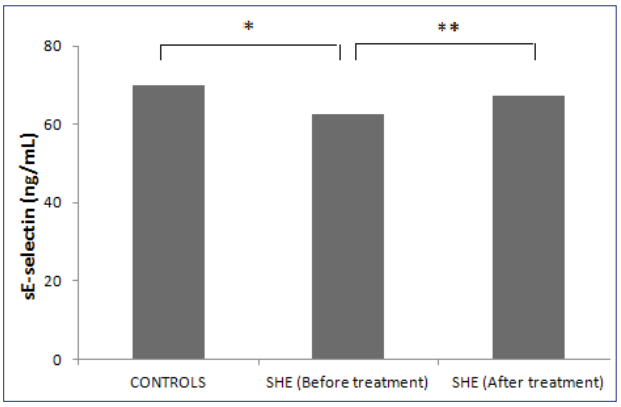

FIGURE 3. sE-SELECTIN LEVELS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM BEFORE AND AFTER PROPYLTHIOURACIL TREATMENT

DISCUSSION

Atherosclerosis is a chronic process involving cellular and humoral responses. In atherogenesis, leukocytes are involved in the early period and this continues during plaque formation. sICAM-1 and sVCAM-1 coordinate leukocyte adhesion and transendothelial migration to generate this process[8]. Both sICAM-1 and sVCAM-1 are transmembrane glycoproteins linked to β-integrins in white blood cells. ICAM-1 endothelial cells are up-regulated with inflammatory cytokines in fibroblasts, epithelial cells, and multiple hemopoietic cells. In the absence of inflammatory conditions, ICAM-1 is expressed in small amounts on the cell surface, and the induction of this adhesion molecule may be important for regulating the intercellular interaction that constitutes the host immune response. VCAM-1 is specific to mononuclear cells and acts as a counter ligand for β1 integrins in lymphocytes and monocytes. Although VCAM-1 expression modulates cytokines similar to ICAM-1, the distribution of VCAM-1 is limited to endothelial cells that are activated in lesion sites and are mostly observed in intimal neovascular structures. Despite the structural and functional similarities between ICAM-1 and VCAM-1, differences between tissue distributions, opposing receptor specificity, and response to hemodynamic forces play an important role in the

TABLE. ADHESION MOLECULE LEVELS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM BEFORE AND AFTER PROPYLTHIOURACIL TREATMENT

	Controls (n=148)	SHE (Before treatment) (n=168)	SHE (After treatment) (n=168)	p^a values	p^b values
sICAM-1 (ng/mL)#	205.42±57.07	581.80±137.12	374.38±121.08	0.000	0.000
sVCAM-1 (ng/mL)#	773.06±817.41	703.97±215.13	811.78±506.77	0.632	0.089
sE-selectin (ng/mL)*	70.08±28.96	62.53±29.25	67.17±29.95	0.293	0.114

p^a values are calculated by statistically evaluating the control group and patients with SHE, and p^b values are calculated by statistically evaluating pre- and post-treatment values of patients with SHE.

formation of systemic atherosclerosis[9]. Studies have shown that the increase in sICAM-1 level may be predictive of myocardial infarction and stroke[10,11] and the same assessment is not valid for sVCAM-1[12]. Many researchers recognize that endothelial activation and inflammation are important precursors for the onset and progression of systemic atherosclerosis and play an independent role in the progression of peripheral atherosclerosis[13].

Adhesion is important for cancer invasion between endothelial cells and cancer cells. ICAM-1 and E-selectin mediate this process. In general, the host's defense mechanisms of immune cells play an important role in the suppression of cancer metastases. It binds to ICAM-1 lymphocytes to suppress the functions of immune cells, i.e., they act as immunosuppressive agents[14,15]. Some studies suggest that there is a relationship between the ICAM-1 molecule and cancer cells based on the increase in ICAM-1 expression, which is released by cancer cells and is related to the progression and metastasis of cancer[16]. In addition, many researchers have shown a significant association between forms of soluble adhesion molecules with gastric cancer, colorectal cancer, and breast cancer metastasis[17-19].

Selectins are adhesion molecules of the leucocytes with microvascular epithelium due to the lectin type interaction[20]. The cytokines on the endothelial cell surface of E-selectin are activated there and adhere to the surface of the target molecules[21]. It has also been reported that cancer cells bind to the endothelial cell surface by E-selectin[22]. E-selectin is thought to be a metastasis tool with microvascular tumor cells[23]. Many studies support the hypothesis that the heterogeneous phase of metastases may provide metastatic distribution in circulating options in selective circulation[24,25].

In this study, we observed that sICAM levels were higher in the subclinical hyperthyroid patients compared to the control group before treatment, and this value decreased after 1 year of treatment. On the other hand, the fact that the ICAM level did not decrease to the values in the control group shows that the risk of atherosclerosis and cancer is not completely eliminated. By varying the dose or duration of the propylthiouracil treatment or by using another therapeutic agent, sICAM levels can be reduced to acceptable levels and the risks can be eliminated. Further studies are needed to prevent cancer risks with atherosclerosis.

Author's Contribution

Ferda Bilgir: Conceptualization, validation; Oktay Bilgir: Methodology, project management; Ozden Yildirim Akan: Software, validation; Ismail Demir: Data curation, investigation.

Funding

None.

Conflict of interest

None.

RESUMO

ANTECEDENTES: *O objetivo deste estudo foi investigar o efeito do tratamento com propiltiouracil nas moléculas de adesão em pacientes com hipertireoidismo subclínico.*

MÉTODOS: *Neste estudo, 168 pacientes diagnosticados com hipertireoidismo subclínico foram tratados com propiltiouracil por um ano. Os níveis de moléculas de adesão, especificamente sICAM-1, sVCAM-1 e sE-Selectina, antes e após o tratamento foram medidos e comparados. Esses resultados foram comparados com os níveis de 148 indivíduos saudáveis no grupo de controle que receberam um placebo.*

RESULTADOS: *Os níveis de sICAM-1 foram significativamente maiores em pacientes com hipertireoidismo subclínico do que nos controles saudáveis (*pa=0,000). Os níveis de sICAM-1 diminuíram significativamente após o tratamento (**pb=0,000). Apesar dessa diminuição em pacientes com hipertireoidismo subclínico, ela não diminuiu para o nível do grupo controle. O sVCAM-1 não se alterou antes e após o tratamento com propiltiouracil. O nível de sE-Selectina foi semelhante ao do grupo de controle pré-tratamento, mas não apresentou significância estatística, embora tenha aumentado após o tratamento (** pb = 0,004).*

CONCLUSÃO: *O nível de sICAM foi significativamente superior aos valores pré-tratamento e diminuiu após o tratamento com propilciliouracil. No entanto, mais estudos são necessários para reduzir o risco de aterosclerose e câncer em pacientes com hipertireoidismo subclínico.*

PALAVRAS-CHAVE: *Hipertireoidismo. Propiltiouracila. Selectina E. Molécula 1 de adesão intercelular. Molécula 1 de adesão de célula vascular.*

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ORIGINAL ARTICLE

Knowledge and acceptability of HPV vaccine among HPV-vaccinated and unvaccinated adolescents at Western Amazon

Maria Sulenir Ferreira de Oliveira[1]
Isabel Cristina Esposito Sorpreso[2]
Lea Tami Suzuki Zuchelo[2]
Adna Thaysa Marcial da Silva[1,2]
Jéssica de Menezes Gomes[1,2]
Bhárbara Karolline Rodrigues Silva[1,3]
Luiz Carlos de Abreu[1,4]
Rubens Wajnsztejn[1]

1. Laboratório de Delineamento de Estudos e Escrita Científica da Faculdade de Medicina do ABC, Santo André, SP, Brasil.
2. Disciplina de Ginecologia, Departamento de Obstetrícia e Ginecologia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil.
3. Laboratório de Epidemiologia e Análise de Dados, Faculdade de Medicina do ABC (FMABC), Santo André, SP, Brasil.
4. Faculdade de Ciências da Santa Casa de Misericórdia de Vitória (Emescam), Vitória, ES, Brasil.

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SUMMARY

PURPOSE: *To analyze the level of knowledge about and the acceptability of the HPV vaccine among vaccinated and unvaccinated adolescents in the Western Amazon.*

METHODS: *A cross-sectional study on adolescents aged 10 to 19 years. The instrument used to collect data contains demographic and socioeconomic information and 27 questions that assess the knowledge and acceptability of the HPV vaccine. To compare the prevalence of vaccinated adolescents with the correct answers to questions about HPV and acceptability and vaccination, the robust variance Poisson regression model was used in the Stata 13.0 software.*

FINDINGS: *A total of 190 adolescents participated in the study, 60.5% in the age group of vaccination recommended by the Brazilian government, among them, 53.9% reported not having been vaccinated (p <0.001). A total of 150 (78.9%) adolescents correctly recognized HPV as a virus; 121 (63.7%) recognized HPV as a cause of cervical cancer. Participants who know HPV is a causative factor for cervical cancer are 1.94 times more likely to have been vaccinated than those who do not. Among the interviewees, the main sources of knowledge about the vaccine were schools (51.6%) and health professionals (22.6%).*

CONCLUSION: *Unvaccinated adolescents have knowledge gaps about HPV and its vaccine when compared to those vaccinated. Our results emphasize the need for effective campaigns to deliver adequate information about HPV and its vaccine to adolescents, their parents, and health professionals.*

KEYWORDS: *Perception. Vaccines. Papillomaviridae. Adolescent.*

INTRODUCTION

The human papillomavirus (HPV) infection is considered a frequent viral infection among sexually active individuals. In adolescence, multi-partner behavior without the use of condoms may increase the risk of infection[1,2].

In 2014, the Ministry of Health introduced in the National Immunization Program (NIP) the quadrivalent human papillomavirus vaccine for girls aged 9 to 14 and, since 2017, for boys aged 11 to 14. The inclusion of male adolescents strengthens health actions targeted at this group[2].

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CORRESPONDING AUTHOR: Isabel Cristina Esposito Sorpreso
Dr. Enéas de Carvalho Aguiar Avenue, n 255, 10. floor, room 10166 - 05403-000, São Paulo, SP, Brasil. Tel.: +55 (11) 2661 7621.
E-mail: icesorpreso@usp.br

The World Health Organization (WHO) considers vaccine coverage adequate when there are 80% inoculations in the priority population at the second dose[2]. In Brasil and in the Federative Unit of Acre there were 64.48% and 49.84% of inoculations in the second dose, respectively[3]. In Acre in 2017, 17.02% of the female adolescent population and 26.75% of males were vaccinated for HPV[3].

Adolescent's parents present a barrier due to myths that the vaccine is applied against sexual initiation and regarding its safe[ty4]. Sousa et al.[5] demonstrated a low level of knowledge about the vaccine safety and efficacy among adolescents.

The population for vaccination is in a period considered ideal for primary prevention because it has safe and effective immunological results to reduce the incidence of HPV-related diseases[2]. Sorpreso and Kelly[6] argue that encouraging information, counseling, and continuing education is an important way to increase the acceptability of the vaccine and ensure that new cases of cervical cancer are reduced in the future.

Elucidating the panorama of the adolescent population and target of the HPV vaccine in the Western Amazon is important to women's and public health because of the low HPV vaccine coverage, low socioeconomic income, high prevalence of HPV infections, as well as its repercussions, such as cervical cancer. Thus, the objective of this study is to analyze the level of knowledge about and acceptability of the HPV vaccine among vaccinated and unvaccinated adolescents in the Western Amazon.

METHODS

Study design

This is a cross-sectional study presented according to STROBE guidelines[7] performed in the municipality of Cruzeiro do Sul, in the state of Acre, between May and August 2017.

The research project was approved by the Research Ethics Committee of the União Educacional do Norte - UNINORTE (2.158.359) and authorized by the Municipal Health Department of Cruzeiro do Sul (AC).

Setting

The study was conducted in a Basic Health Unit (BHU) located in Cruzeiro do Sul that provides health services to low- and middle-income families. The health unit has a multi-professional approach, with doctors, nurses, nursing technicians, and community health agents, all of whom were trained to apply the instrument for data collection.

All adolescents and their legal guardians/parents were informed about the voluntary participation in the research and read the informed consent form. In addition, for adolescents under 14 years of age, we required parental consent and assent form. After signing the informed consent form, the adolescents were interviewed.

The interviews were conducted individually with the adolescents, away from their parents, before the adolescent's medical consultation and during the activities of the group of adolescents, without prejudice to their demands.

Participants

The sample was composed of 190 adolescents aged 10 to 19 years 11 months and 29 days according to the stratification of the World Health Organization[8].

Inclusion criteria: adolescents of both sexes, attending the reference BHU UBS. The criteria for non-inclusion were: participants who did not respond to all domains of the questionnaires, adolescent or legal guardian refusal, and impossibility to contact the adolescent (Figure 1).

— insert Figure 1 here—

The adolescents were informed on HPV, repercussions of the vaccine by HPV, and vaccine for HPV after the interview. The adolescents had their medical care preserved without any prejudice to the treatment or follow-up in the basic health unit. Still, the confidentiality of the responses was maintained and any uncomfortable situations were avoided.

VARIABLES AND DATA SOURCE

Characterization of the sample

The socioeconomic and demographic characteristics were obtained through a socioeconomic and demographic identification form[5]. The form consisted of the following variables: gender, age, partner, children, schooling, family income, and paid work.

Knowledge and Acceptability of HPV Vaccine

The collection instrument for assessing the knowledge about and acceptability of the HPV vaccine was adapted from a validated instrument with a sample of adolescents, parents or guardians, and health professionals[5]. We used 27 questions addressing the knowledge about HPV, the vaccine, barriers to vaccination,

vaccine acceptability, and personal history related to HPV infection. Each question had three possible answers: "Yes," "No," and "Not sure".

Data sources and measurement

In order to score the answers, the value (0) was attributed to non-correct answers and the "Not sure" option, and (1) to correct answers in each question. The instrument was adapted from the original since these are specific questions for health professionals[5].

The questionnaire was tested on similar populations in a previous study[5]. To measure the internal consistency of the instrument, the Cronbach's alpha equation was used, obtaining an alpha (α) value of 0.61, considered a substantial value[9].

Bias

To minimize the selection bias, a validated collection instrument was used and applied in a previous study on adolescents. The responses of the adolescents without intervention during the collection and standardization of the collection by an interviewer previously trained were considered. The registration of the data was standardized in an Excel worksheet and the analysis carried out by two researchers.

Statistical methods

The study was performed with a non-probabilistic sample and for convenience. Considering a power of 80%, a significance level of 5%, and 64% of vaccinated individuals based on the proportion of vaccination by NIP-Acre, 201727, the minimum number of interviewees necessary was 105.

The data were tabulated in a Microsoft Excel spreadsheet and analyzed by the Statistics Data Analysis software for Windows® (Stata®, StataCorp, LLC, 13.0, College Station, TX, USA).

The homogeneity test among the vaccinated and non-vaccinated adolescents, in relation to the socioeconomic variables, was performed by the chi-square test.

For the prevalence of vaccinated adolescents and correct answers, the Poisson regression model with robust variance was used, with a Prevalence Ratio (PR) and Confidence Intervals of 95% (95% CI). All analyses were two-tailed, and $p < 0.05$ were considered statistically significant.

The proportion and respective 95% confidence intervals of the correct answers were used to describe the proportion of correct answers for each question. Information not answered by the participants were considered missing, thus were not considered and not analyzed.

RESULTS

We interviewed 190 adolescents living in Cruzeiro do Sul-AC, Northern region of Brasil, with a mean age of 14.1 years (SD ±2.80 years), of which 115 (60.5%) were in the age group for HPV vaccination recommended by the Health Immunization Program, 98 (51.6%) were female and 92 (48.4%) were males. A total of 179 (94.2%) adolescents reported having no partner, 24 (12.6%) had one child or more, 29 (15.3%) had paid work, and 127 (66.8%) reported lower income than two minimum wages, as seen in Table 1.

TABLE 1. SOCIOECONOMIC CHARACTERISTICS OF ADOLESCENTS ACCORDING TO THE ACCOMPLISHMENT OF VACCINATION AGAINST HPV OF ADOLESCENTS OF THE WESTERN AMAZON, 2017.

Variable	Total population n (%)	Vaccinated against HPV		p*
		NOT n (%)	YES n (%)	
Target Population**				
Not	75 (39.5)	62 (82.7)	13 (17.3)	<0.001
Yes	115 (60.5)	62 (53.9)	53 (46.1)	
Sex				
Female	98 (51.6)	45 (45.9)	53 (54.1)	<0.001
Male	92 (48.4)	79 (85.9)	13 (14.1)	
Partner				
Not	179 (94.2)	115 (64.2)	64 (35.7)	0.235
Yes	11 (5.8)	9 (81.8)	2 (18.2)	
Work				
Not	161 (84.5)	102 (63.4)	59 (36.6)	0.193
Yes	29 (15.3)	22 (75.9)	7 (24.1)	
Have children				
Not	166 (87.4)	105 (63.3)	61 (36.7)	0.126
Yes	24 (12.6)	19 (79.2)	5 (20.8)	
Schooling***				
Suitable	18 (9.5)	16 (88.9)	2 (11.1)	0.027
Inappropriate	172 (90.5)	108 (62.8)	64 (37.2)	
Income				
Up to 2 Min. Wages	127 (66.8)	82 (64.6)	45 (35.4)	0.111
2-4 Min. Wages	22 (11.6)	11 (50)	11 (50)	
4-10 Min. Wages	1 (0.5)	0	1 (100)	
> 10 Min. Wages	2 (1.0)	1 (50	1 (50)	
Do not know	38 (20)	30 (78.9)	8 (21.1)	
	Mean (SD)	Mean (SD)	Mean (SD)	p*
Age (in years)	14.1 (±2.80)	14.50 (±3.01)	13.71 (±2.39)	0.013

* chi-square test in the comparison between vaccinated and non-vaccinated.** Adolescents in the age group for HPV vaccination recommended by the Health Immunization Program.. *** School age according to guidelines at the Brazilian Ministry of Education. SD – Standard Deviation

Table 1 also shows that 53 (54.1%) of the female adolescents reported having been vaccinated, and 62 (53.9%) of the adolescents interviewed in the vaccination target group reported not having been vaccinated (p <0.001). There was no statistical relevance among the vaccinated ones regarding the marital status, employment status, number of children, schooling, and income. The group of unvaccinated adolescents included 79 (85.9%) males, and 62 (82.7%) were outside the age range of free vaccination (p <0.001). The vaccinated group had a lower mean age (13.71 years old ±2.39) than the unvaccinated (14.50 years old ±3.01) adolescents (p=0.013).

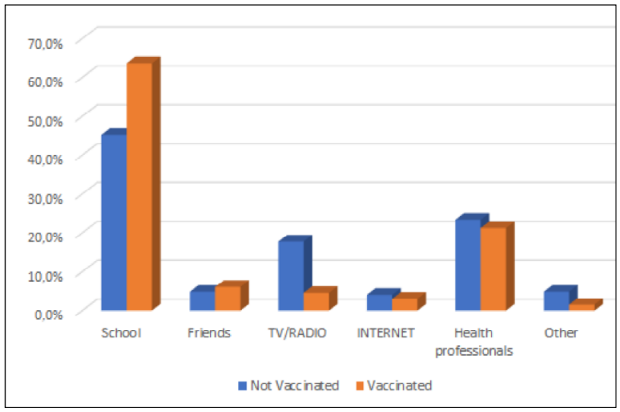
Table 2 shows the proportion of correct answers (knowledge) among adolescents interviewed. Of a total of 190 respondents, 150 (78.9%) recognized HPV as a virus; 121 (63.7%) described HPV as a cause of cervical cancer; 148 (77.9%) knew that the HPV vaccine is part of the immunization program for female adolescents; 115 (60.5%) knew someone who had already received a vaccine.

TABLE 2. COMPARISON OF CORRECT ANSWERS ON HPV KNOWLEDGE AND ITS VACCINE AND ACCEPTABILITY AMONG ADOLESCENTS VACCINATED AND UNVACCINATED IN THE WESTERN AMAZON, 2017.

Question	Total population n (%)	Vaccinated		Gross model	
		Not n (%)	Yes n (%)	RP (CI95%)*	p
Do you know what HPV is?	79 (41.6)	50 (63.3)	29 (36.7)	1.10 (0.74 to 1.63)	0.630
Is HPV a virus?	150 (78.9)	103 (68.7)	47 (31.3)	0.66 (0.44 to 0.99)	0.043
Is HPV a sexually transmitted disease?	130 (68.4)	88 (67.7)	42 (32.3)	0.81 (0.54 to 1.20)	0.293
Can HPV cause cervical cancer?	121 (63.7)	70 (57.9)	51 (42.1)	1.94 (1.18 to 3.18)	0.009
Can HPV cause changes in the Papanicolaou?	93 (49)	65 (699)	28 (30.1)	0.77 (0.52 to 1.14)	0.195
Is cervical cancer a major cause of cancer in women?	149 (78.4)	93 (62.4)	56 (37.6)	1.54 (0.86 to 2.75)	0.143
Can smoking increase the risk of cervical cancer?	101 (53.2)	70 (69.3)	31 (30.7)	0.78 (0.53 to 1.15)	0.215
Does the HPV vaccine prevent cervical cancer?	141 (74.2)	93 (66)	48 (34)	0.93 (0.60 to 1.43)	0.731
Should the HPV vaccine be given before the first sexual intercourse?	116 (61)	74 (63.8)	42 (36.2)	1.12 (0.74 to 1.68)	0.598
Can the HPV vaccine be given to people who have had sex?	86 (45.3)	57 (66.3)	29 (33.7)	0.95 (0.64 to 1.40)	0.790
Can the HPV vaccine be harmful to health?	121 (63.7)	75 (62)	46 (38)	1.31 (0.85 to 2.03)	0.222
Can the HPV vaccine cause HPV infection?	75 (39.5)	51 (68)	24 (32)	0.88 (0.58 to 1.32)	0.527
Is the HPV vaccine provided by the Government?	155 (81.6)	101 (65.2)	54 (34.8)	1.02 (0.61 to 1.69)	0.951
Is the HPV vaccine part of the girls' immunization record?	148 (77.9)	88 (59.5)	60 (40.5)	2.84 (1.32 to 6.12)	0.008
Are 3 doses required for complete vaccination?	109 (57.4)	66 (60.6)	43 (39.4)	1.39 (0.91 to 2.11)	0.123
Does the HPV vaccine lessen the chance of having genital warts?	89 (46.8)	61 (68.5)	28 (31.5)	0.84 (0.56 to 1.24)	0.378
Does the HPV vaccine decrease the chance of having Pap test changes?	85 (44.7)	59 (69.4)	26 (30.6)	0.80 (0.54 to 120)	0.286
Acceptability					
Do you think that the HPV vaccine would stimulate the onset of sexual life earlier?					
Yes	89(46.8)	64 (71.9)	25 (28.1)		
No	101 (53.2)	60 (59.4)	41 (406)	1.44 (0.96 to 2.17)	0.077
Do you think that after the HPV vaccine you still need to use a condom?					
No	32 (16.8)	21 (65.6)	11 (34.4)		
Yes	158 (83.2)	103 (65.2)	55 (34.8)	1.01 (0.60 to 1.71)	0.963
Do you think that after the HPV vaccine you still need to have the Pap test?					
No	38 (20)	24 (63.2)	14 (36.8)		
Yes	152 (80)	100 (65.8)	52 (34.2)	0.93 (0.58 to 1.49)	0.758
Do you know anyone who has already had the HPV vaccine?					
No	75 (39.5)	62 (82.7)	13 (17.3)		
Yes	115 (60.5)	62 (53.9)	53 (46.1)	2.66 (1.56 to 4.53)	<0.001
Would you recommend the HPV vaccine for a child, friend or relative to take?					
No	20 (10.5)	16 (80)	4 (20)		
Yes	170 (89.5)	108 (63.5)	62 (36.5)	1.82 (0.74 to 4.49)	0.191

* Poisson regression with robust variance

GRAPH 1



Participants who knew HPV was a causative factor in cervical cancer were 1.94 (CI 1.18 a 3.18; p=0.009) times more likely to have been vaccinated than those who did not. Moreover, participants who knew that the HPV vaccine is part of the immunization program for female adolescents were 2.84 (CI 1.32 – 6.12; p=0.008) times more likely to have been vaccinated than those who did not.

Graph 1 shows the main sources of information for adolescents in Acre. Schools (51,6%) and health professionals (22,6%) are the main sources of information on HPV vaccination in both populations.

DISCUSSION

The assessment of acceptability and knowledge about HPV and its vaccine among adolescents is an important outcome for the health of this population and public health since they are in a period of experimentation and initiation of sexual activity, thus vulnerable to Sexually Transmitted Infections (STI)[5,10,11]. In the State of Acre, vaccination rates for HPV have not yet reached the goals of the National Immunization Program or the goals expected by WHO[2,3].

The results show that non-vaccinated adolescents present knowledge gaps compared to those vaccinated. In addition, they reported barriers to vaccine acceptability, such as the belief that this stimulates the onset of sexual activity.

The prevalence of high-risk HPV cervical cancer in the territory of Acre is 71%, which reinforces the importance of identifying knowledge gaps and acceptance barriers for the HPV vaccine[12]. In our study, we describe the knowledge of adolescents from Acre about HPV being a virus and its relation to cervical cancer. A convergent study[13] conducted with adolescents in Santa Catarina, Brasil, with the fourth-highest income[14], showed the respondents presented basic notions about the subject.

Gualano et al.[15] corroborate that adolescents vaccinated for HPV have a better level of knowledge about the virus and its relation to cancer than those not vaccinated. Studies have shown divergent results with adolescents in Greece[16] and the US[17], pointing out that the HPV vaccine is present in the vaccination program for adolescents in Greece; however, in the US, the vaccine for HPV is not provided without cost by the Government.

Knowledge gaps among the unvaccinated include: HPV being a virus and not being related to cervical cancer; no knowledge about the HPV vaccine being part of the girls' official immunization calendar, and not knowing individuals who have already been vaccinated for HPV. Studies conducted in England, where the vaccine is available for adolescents aged 12 to 18 years, corroborate our findings and state that vaccinated girls were more likely to have heard of HPV than unvaccinated ones[18].

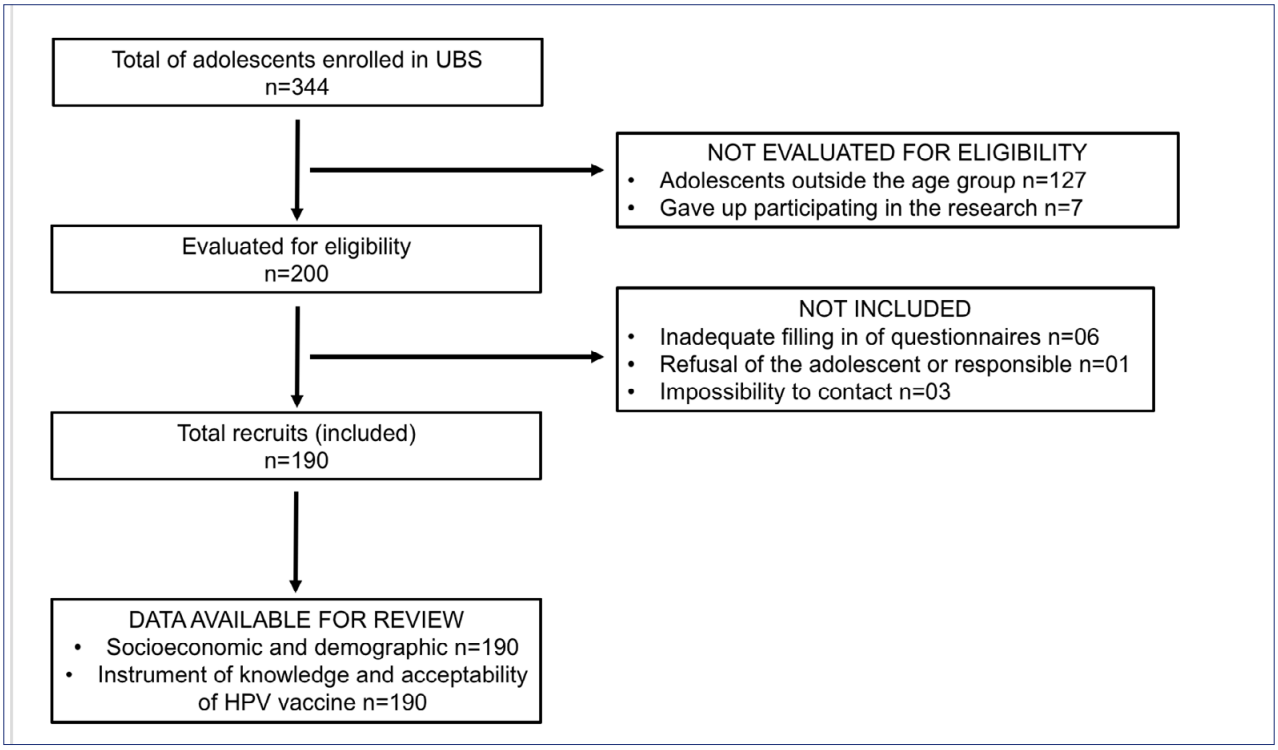
Respondents showed they did not know that the HPV vaccine can be applied to those who have had sex. It is important to emphasize that vaccination will be more effective if applied early, especially before the first sexual contact. However, even if they are already sexually active, they will benefit because they will be protected against other types of HPV contained in the vaccine. And the vaccinated population will present an effective and timely immune response[2].

In addition, adolescents may be reinfected with different types of HPV viruses during their lifetime if there is no double protection (association with a condom). In this study, the adolescents demonstrated knowledge about preventive measures, the use of condoms, and cervical cancer screening exams after vaccination[2]. Chiang et al.[19] found in their results that adolescents of both sexes are interested in being vaccinated or recommending the vaccine to relatives.

The main sources of information for HPV vaccination among adolescents in Acre were schools and health professionals. It is worth mentioning the importance of the school environment in the sexual, reproductive, and preventive education of adolescents. Investments in sex education and the development of educational campaigns on Papanicolaou with an appropriate approach and appropriate language applied in the prevention of cervical cancer are necessary[15,20].

Our results reinforce the importance of intersectionality between health and education, with

FIGURE 1.



joint informative campaigns expanded and making it possible to demystify the concepts for better knowledge and acceptability of the HPV vaccine. The first HPV vaccination campaign in Brasil took place in partnership with public and private schools[21], which may have contributed to the school environment being the source of information most cited among adolescents.

The literature shows that health professionals advise on Pap smears and cervical cancer, but have gaps in knowledge about the effects of HPV on adolescent health and the benefits of the vaccine for men[5,22,23]. In the study by Lorenzi et al.[24], they analyzed whether age is a barrier against the acceptability of cervicovaginal self-sampling in screening for cervical cancer, and they concluded that younger women indicated more fear and discomfort in self-sampling, which points to the need for attraction strategies that are more appealing to the younger generations. Moreover, the self-collection method is well-accepted and may, therefore, encourage greater participation in cervical cancer screening programs[25].

Misconceptions about the HPV vaccine are barriers to the vaccination of adolescents, especially in the age group that depends on the parents or guardians to get vaccinated. The stimulation of early sexual life should be demystified among the population, and counseling on the subject should be encouraged by health professionals[5,6,26].

Papillomavirus infection is a viral STI that most affects the sexually active world population. Knowledge about HPV strengthens the prevention of STIs and the long-term incidence of new cases of cervical cancer[2].

In a cross-sectional study, a descriptive approach can be considered a limitation, as well as the impossibility of measuring causal relationships between knowledge and acceptability barriers in the interviewed population. Still, there is a bias in the variable of knowledge and perception about the HPV vaccine, since the interviewees were ill with easy access to health, not expressing the reality of other adolescents.

The novelty of the study is considering the perceptions of adolescents of both sexes about HPV, its clinical repercussions, and its vaccine in a territory of low adherence to vaccination and high rates of cervical cancer. Adolescents showed good acceptance and are recommended for HPV vaccination.

Thus, health team counseling for family members and adolescents about the HPV vaccine is necessary, as well as intersectoral health promotion actions to resolve doubts and myths about the vaccine and raise awareness among the population (adolescents and guardians) on the prevention of HPV to extend the recommended vaccination coverage.

CONCLUSION

Unvaccinated adolescents have gaps in knowledge about HPV and its vaccine compared to those vaccinated. Moreover, unvaccinated adolescents reported barriers to vaccination, such as the belief that this stimulates the onset of sexual activity. School and friends are sources of exchange of knowledge and information among vaccinated adolescents.

Thus, it is noted that training is necessary for health teams, families, and adolescents in order to increase the population's knowledge about HPV, its clinical repercussions, and its vaccine, as well as to cover the WHO recommended percentage of vaccine coverage.

Implications and Contribution: To address the perceptions of adolescents of both sexes about HPV, its clinical repercussions, and its vaccine in a territory of low adherence to vaccination and high rates of cervical cancer. Adolescents showed good acceptance and are recommended for HPV vaccination.

RESUMO

OBJETIVO: *Analisar o nível de conhecimento e aceitabilidade da vacina contra o HPV entre adolescentes vacinados e não vacinados na Amazônia Ocidental.*

MÉTODOS: *Estudo transversal com adolescentes de 10 a 19 anos. O instrumento usado para coletar dados contém informações demográficas e socioeconômicas e 27 perguntas que avaliam o conhecimento e a aceitabilidade da vacina contra o HPV. Para comparar a prevalência de adolescentes vacinados com as respostas corretas para perguntas sobre conhecimento, aceitabilidade e vacinação contra o HPV, o modelo de regressão de Poisson de variância robusta foi utilizado no software Stata 13.0. Resultados: Participaram do estudo 190 adolescentes, 60,5% (n=115) na faixa etária de vacinação recomendada pelo governo brasileiro; dentre eles, 53,9% (n=62) relataram não ter sido vacinados (p<0,001). A proporção de resultados corretos entre os adolescentes foi de 78,9% (n=150), que reconheceram o HPV como vírus; 63,7% (n=121) relataram o HPV como causa de câncer do colo do útero. Os participantes que sabem que o HPV é um fator causal no câncer do colo do útero têm 1,94 (IC 1,18-3,18; p=0,009) vez mais chances de terem sido vacinados do que aqueles que não sabem. Entre os entrevistados, as principais fontes de conhecimento sobre a vacina foram escolas (51,6%) e profissionais de saúde (22,6%).*

CONCLUSÃO: *Adolescentes não vacinados apresentam lacunas de conhecimento sobre o HPV e sua vacina quando comparados aos vacinados. Nossos resultados enfatizam a necessidade de campanhas eficazes para fornecer informações adequadas sobre o HPV e sua vacina a adolescentes, pais e profissionais de saúde.*

PALAVRAS-CHAVE: *Percepção. Vacinas. Papillomaviridae. Adolescente.*

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ORIGINAL ARTICLE

Relationship between c-reactive protein/albumin ratio and new-onset atrial fibrillation after coronary artery bypass grafting

Fatih Aksoy [1]
Dinçer Uysal [2]
Erdogan Ibrişim [3]

1. Associate Professor from the Department of Cardiology, Suleyman Demirel University, Medical School, Isparta, Turkey
2. Associate Professor from the Department of Cardiovascular Surgery, Suleyman Demirel University, Medical School, Isparta, Turkey
3. Professor from the Department of Cardiovascular surgery, Suleyman Demirel University, Medical School, Isparta, Turkey

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SUMMARY

OBJECTIVE: *This study aimed to investigate the predictive value of the newly defined C-Reactive Protein (CRP)/Albumin Ratio (CAR) in determining the development of atrial fibrillation (AF) in comparison with other inflammatory markers, such as Neutrophil/Lymphocyte (N/L) Ratio and Platelet/Lymphocyte (P/L) Ratio, in patients undergoing Coronary Artery Bypass Grafting (CABG) surgery.*

METHODS: *The population of this observational study consisted of 415 patients undergoing CABG. The study cohort was subdivided into two groups based on the development of AF. Complete blood counts, serum CRP, and serum albumin levels were evaluated before the CABG. The CAR, N/L, and P/L ratios of all the patients were calculated. Predictors of postoperative AF were determined by multiple logistic regression analysis (MLRA).*

RESULTS: *During follow-up, 136 patients (32.8%) developed postoperative AF. With MLRA, independent risk factors for postoperative AF were determined as follows: fasting glucose level (OR: 1.01; 95 % CI: 1.00-1.01, P <0.001), age (OR: 1.12; 95 % CI: 1.07-1.17, P <0.001), left ventricle ejection fraction (OR: 0.90; 95 % CI: 0.87-0.94, P <0.001), male gender (OR: 3.32; 95 % CI: 1.39-7.90, P = 0.007), 24-hour drainage amount (OR: 1.004; 95 % CI: 1.002-1.005, P <0.001), and CAR (OR: 1.82; 95 % CI: 1.53-2.16, P <0.001). Receiver Operating Characteristic curve analysis showed that CAR (C-statistic: 0.75; 95% CI: 0.71-0.79, p< 0.001) was a significant predictor of AF.*

CONCLUSION: *Novel inflammatory marker CAR can be used as a reliable marker to predict the development of AF following CABG.*

KEYWORDS: *Protein C. Albumins. Atrial fibrillation. Myocardial revascularization. Coronary artery bypass.*

INTRODUCTION

Inflammation plays an important role both in the onset of Atrial Fibrillation (AF) and its maintenance through myocyte necrosis, fibrosis, and infiltration of inflammatory markers.[1-3] Several inflammatory markers, including the High-Sensitive C-Reactive Protein (Hs-CRP) and Interleukin-6 (IL-6), levels were reported to be elevated in patients with different AF subtypes compared to those with sinus rhythm.[2-5]

Postoperative atrial fibrillation (POAF) is a major and fatal complication of Coronary Artery Bypass Grafting (CABG) surgery. Patients developing Atrial Fibrillation (AF) after cardiac surgery have a higher risk of morbidities such as cerebrovascular events, pulmonary edema, longer hospital stays, and mortality compared to those who do not develop AF.[6-8] Identifying the patients who may develop POAF before

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CORRESPONDING AUTHOR: Fatih Aksoy
Suleyman Demirel Unşversity – Faculty of Medicine, Cardiology Department – Isparta, Turkey – 32040
Tel.: +90505 2313661, Fax: +90 2462324510
E-mail: dr.aksoy@hotmail.com

the surgery and taking the necessary precautions may decrease the mortality and morbidity rates. An association between POAF and inflammation has been shown in previous studies[5,6]. C-Reactive Protein (CRP)/Albumin Ratio (CAR), a novel parameter of inflammation, has been shown to be superior to CRP or albumin levels alone in determining inflammatory status in several cardiovascular diseases.[9,10] However there are no published studies on the association between CAR and POAF in the literature. In this study, we aimed to investigate the predictive value of CAR in the development of POAF.

METHODS

Study population

The study included patients who underwent isolated Coronary Artery Bypass Grafting (CABG) surgery at the Suleyman Demirel University, Education and Research Hospital between March 2017 and June 2019. The study population was retrospectively and consecutively analyzed by using a database that collated patient data as a part of routine clinical practice. The overall study population included 475 patients undergoing CABG. The exclusion criteria included hyperthyroidism, age <18 years, prior cardiac surgery, class III or IV heart failure, previous atrial fibrillation, left atrial diameter >55 mm, left ventricular ejection fraction <0.25, sepsis, heart rate <60 bpm, systolic blood pressure <90 mm Hg, inflammatory disease, pericarditis, patients undergoing off-pump surgery, and being on antiarrhythmic treatment. According to these criteria, 35 patients were excluded due to previous atrial fibrillation (n = 15), heart rate <60 bpm (n = 5), hyperthyroidism (n = 5), left atrial diameter> 55 mm (n= 5), ejection fraction <0.25 (n=5), and patients undergoing off-pump surgery (n=25). Therefore, 415 patients were included in the final study cohort. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee (Date: 28.05.2019, Decision no: 181). Similar operative techniques were used for all patients. A transthoracic echocardiogram was recorded for each patient before and after the surgery.

Blood Collection and Laboratory Analysis

Blood samples were drawn from the antecubital vein by careful vein puncture using a 21-G sterile syringe without stasis at 08:00–10:00 h after a fasting period of 12h. Hematologic and biochemical measurements including liver enzymes were carried out. An automatic blood counter (LH 780 Hematology Analyzer, Beckman Coulter Inc., Miami, FL) was used for whole blood counts including total White Blood Cells (WBCs), hemoglobin, platelets, neutrophils, lymphocyte, and monocytes. Serum C-reactive Protein (CRP) levels were measured using BN2 Nephelometry Analyzer II (Dade Behring, Kalletal, Germany). The normal value for CRP is in the range of 0–6 mg/L.

Rhythm Follow-up

The rhythms were followed-up by continuous electrocardiogram monitoring during the patients' stay at the intensive care unit and by 24-hour Holter during the rest of hospitalization. A 12-lead electrocardiogram was used for recording routinely every morning and whenever the patients had symptoms suggestive of dysrhythmia. Atrial fibrillation was defined as an irregular rhythm with the absence of discrete P waves in the 12-lead electrocardiogram. An atrial fibrillation episode lasting for at least 5 minutes during hospitalization was defined as POAF.[11]

Statistical analysis

Statistical Package Social Sciences (SPSS) software version 16.0 package and Medcalc version 15.2 were used for statistical analyses in this study. Categorical variables were expressed as frequency (%) and compared using the $\chi 2$ test. The Kolmogorov-Smirnov test was used to test the distribution of numeric variables; those with normal distribution were expressed as mean ± standard deviation and were compared with the Student's t-test. Data without normal distribution were expressed as median (Inter-quartile range (IQR) of 25%-75% percentiles) and were compared with the Mann-Whitney U test. In all statistical analyses, a P-value <0.05 was considered statistically significant. The correlations between CAR, presence of AF, and other clinical, laboratory, and echocardiographic parameters were measured by Pearson or Spearman correlation analysis when appropriate. Univariate analysis of binary logistic regression was performed to identify which factors are associated with incident AF. After including each of these potential confounding factors, backward conditional binary logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (95% CI) for incident AF. We used a receiver operating characteristic (ROC)

analysis with area under the curve and CAR, CRP, and albumin cut-off points for prediction of AF. All ROC comparisons were performed using the DeLong test. Predictors of AF were determined by logistic regression analysis.

RESULTS

A total of 415 patients (mean age: 62.86±11.86 years; range, 28–84 years) were included in this study. During the follow-up period, 136 patients (32.8%) developed POAF. The demographic and clinical characteristics of the patients with and without POAF are listed in Table 1. The patients with POAF were significantly older and there were more males when compared to patients without POAF (p< 0.001 and p= 0.003, respectively). The presence of diabetes mellitus, hypertension, congestive heart failure, peripheral vascular disease, and stroke/transient ischemic events was higher in patients with POAF compared to patients without POAF. There were no statistically significant differences in cholesterol parameters (p > 0.05 for all parameters) between patients with and without POAF. Left ventricle ejection fraction was significantly lower in patients with POAF compared to patients without POAF (p< 0.001). Preoperative fasting glucose levels were higher in patients with POAF compared to patients without POAF (p< 0.001). There were no statistically significant differences in patients with and without POAF in whole blood parameters, including platelet (P) count, white blood cell, neutrophil (N), and lymphocyte (L) level; however, the N/L and P/L ratios were significantly higher in patients with POAF compared to patients without POAF (p= 0.05 and p= 0.04 respectively.)

The postoperative drainage amounts in the first 24 and 48 hours were higher in patients with POAF than in patients without POAF (P= 0.003 for 24 hours and P= 0.148 for 48 hours) (Table 1). Cardiopulmonary bypass time was longer in patients with POAF compared to patients without POAF (P= 0.02) but there was no statistically significant difference in the clamp time between patients with and without POAF. The duration of hospitalization at the intensive care unit was longer in patients with POAF than in patients without POAF (P< 0.001). There were no statistically significant differences in events such as reoperation due to hemorrhage, and intraoperative and in-hospital mortality (for all parameters p> 0.05) between patients with and without POAF (Table 1).

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH AND WITHOUT AF

	Without AF (n = 279)	With AF (n= 136)	P-value
Age (years)	60.5 ± 12.4	67.5 ± 8.9	< 0.001
Body mass index	29.0 ± 5.2	28.0 ± 4.4	0.05
Female gender (n, %)	90 (32.3)	26 (19.1)	0.003
Diabetes mellitus (n, %)	114 (40.9)	73 (53.7)	0.009
Hypertension (n, %)	202 (72.4)	121 (89.0)	< 0.001
Congestive heart failure (n, %)	14 (5.1)	27 (20.8)	< 0.001
Peripheral vascular disease (n, %)	72 (25.8)	50 (36.8)	0.015
History of CVA (n, %)	36 (13.8)	24 (21.8)	0.04
Ejection fraction (%)	56.6 ± 8.4	50.3 ± 10.0	< 0.0001
Left atrial diameter (mm)	39.1 ± 6.1	38.3 ± 7.7	0.289
LVEDD (mm)	47.3 ± 5.5	46.9 ± 4.9	0.379
LVESD (mm)	29.8 ± 6.9	31.7 ± 5.6	0.007
IVSD (mm)	12.0 ± 3.1	12.6 ± 3.4	0.007
PWD (mm)	11.1 ± 2.2	11.1 ± 0.9	0.988
AoD (mm)	26.9 ± 3.5	26.8 ± 3.3	0.832
Total cholesterol (mg/dl)	201.2 ± 41.3	196.9 ± 39.4	0.412
HDL cholesterol (mg/dl)	41.8 ± 10.2	41.2 ± 9.0	0.632
LDL cholesterol (mg/dl)	126.0 ± 40.4	124.3 ± 41.5	0.760
Triglycerides (mg/dl)	158.2 ± 104.1	141.9 ± 48.6	0.187
Creatinine(mg/dl)	1.0 ± 0.3	11.1 ± 0.3	0.103
Glucose(mg/dl)	142.5 ± 67	198.6 ± 119.9	< 0.001
Lymphocyte (103/µl)	2556 ± 0.978	2576 ± 1.063	0.849
Platelet (103/µl)	230 ± 63	232 ± 78	0.773
CAR, median (IQR)	0.96 (0.5-2.0)	4.3 (1.1-8.6)	< 0.001
N/L ratio	3.3 ± 3.1	4.3 ± 6.8	0.05
P/L Ratio	11.3 ± 9.4	140 ± 17.2	0.04
CRP (mg/l) , median (IQR)	4.00(2.00-8.00)	16.00 (4.25-33.0)	<0.001
Albumin (g/l)	3.8± 0.4	3.7±0.3	0.002
WBC (103/µl)	8952±4227	8751±2240	0.513
Operative and postoperative parameters			
Cardiopulmonary bypass time (min)	78.8 ± 27.3	86.8 ± 39.6	0,02
X Clamp time (min)	47.5 ± 18.8	46.7 ± 16.2	0,686
24-hour drainage (ml)	28.2 ± 14.5	388.7 ± 232.3	0,003
48-hour drainage (ml)	147.8 ± 83.9	159.9 ± 69.8	0,148
Duration of the hospitalization at the intensive care unit (days)	2.1 ± 0.4	2.6 ± 1.4	< 0.001
Bypass number (n)	2.4±1.7	2.6±2.1	0.158
Reoperation due to hemorrhage (n, %)	6 (2.2)	2 (1.5)	0.985
İntraoperative mortality (n, %)	-	-	
In-hospital mortality (n, %)	8 (2.9)	6 (4.4)	0.786

Data presented as mean ± standard deviation, median (IQR) or number (%) of the patients. CVA = cerebrovascular accident; HDL = high-density lipoprotein; LVEDD= Left Ventricle end diastolic diameter; LVESD= Left Ventricle end systolic diameter; IVSD: interventricular septum diameter; PWD: Posterior wall diameter; AoD: aortic diameter; CAR: C reactive protein to albumin ratio; N: neutrophil; L: Lymphocyte; P: Platelet; WBC: White blood cell

The median CAR and CRP levels were significantly lower in patients without POAF compared to patients with POAF [0.96 (0.5-2.0) versus 4.3 (1.1-8.6), p < 0.001 for CAR; 4.00 mg/l (2.00-8.00) versus 16.00 mg/l (4.25-33.0), p<0.001 for CRP]. The mean albumin level was lower in patients with POAF compared to patients without POAF (3.8± 0.4 versus 3.7±0.3, P= 0.002)

Prediction of postoperative atrial fibrillation

Univariate analyses showed that body mass index, CRP/Albumin ratio, N/L ratio, P/L ratio, CRP, albumin, diabetes mellitus, hypertension, congestive heart failure, peripheral vascular disease, low left ventricle ejection fraction, advanced age, fasting glucose level, 24-hour drainage amount, cardiopulmonary bypass time and male gender were significantly associated with a higher risk of development of POAF (Table 2). The correlation analysis revealed that CAR exhibited a weak correlation between CAR and N/L ratio (r= 0.147, P< 0.001) and P/L ratio (r= 0.226, P= 0.001). To determine the independent predictors for the development POAF, a multivariate binary logistic regression analysis was carried out. The univariate analysis of all parameters associated with new-onset AF except NLR, PLR, CRP and albumin showed that fasting glucose level (OR: 1.01; 95% CI: 1.00-1.01, P <0.001), age (OR: 1.12; 95% CI: 1.07-1.17, P <0.001), left ventricle ejection fraction (OR: 0.90; 95% CI: 0.87-0.94, P <0.001), male gender (OR: 3.32; 95% CI: 1.39-7.90, P = 0.007), 24-hour drainage amount (OR: 1.004; 95% CI: 1.002-1.005, P <0.001), CAR (OR: 1.82; 95% CI: 1.53-2.16, P <0.001) remained as independent factors for incident AF (Table 2). ROC curve analysis showed that CAR (C-statistic: 0.75; 95% CI: 0.71-0.79, p< 0.001) was a significant predictor of POAF (Figure 1). We calculated that a cut-off point of 3.5 for CAR could estimate the presence of POAF with a sensitivity of 58% and 92%. We performed a pair-wise comparison of the ROC curves and observed that the predictive value of the CAR with regard to POAF development was superior to that of albumin and CRP (DeLong method, AUC_{CAR} versus AUC_{CRP} z test= 3.592, p= 0.0003; AUC_{CAR} versus $AUC_{Albumin}$ z test= 3.927, p= 0.0001; $AUC_{albumin}$ versus AUC_{CRP} z test= 3.594, p= 0.0003)

DISCUSSION

Main Findings

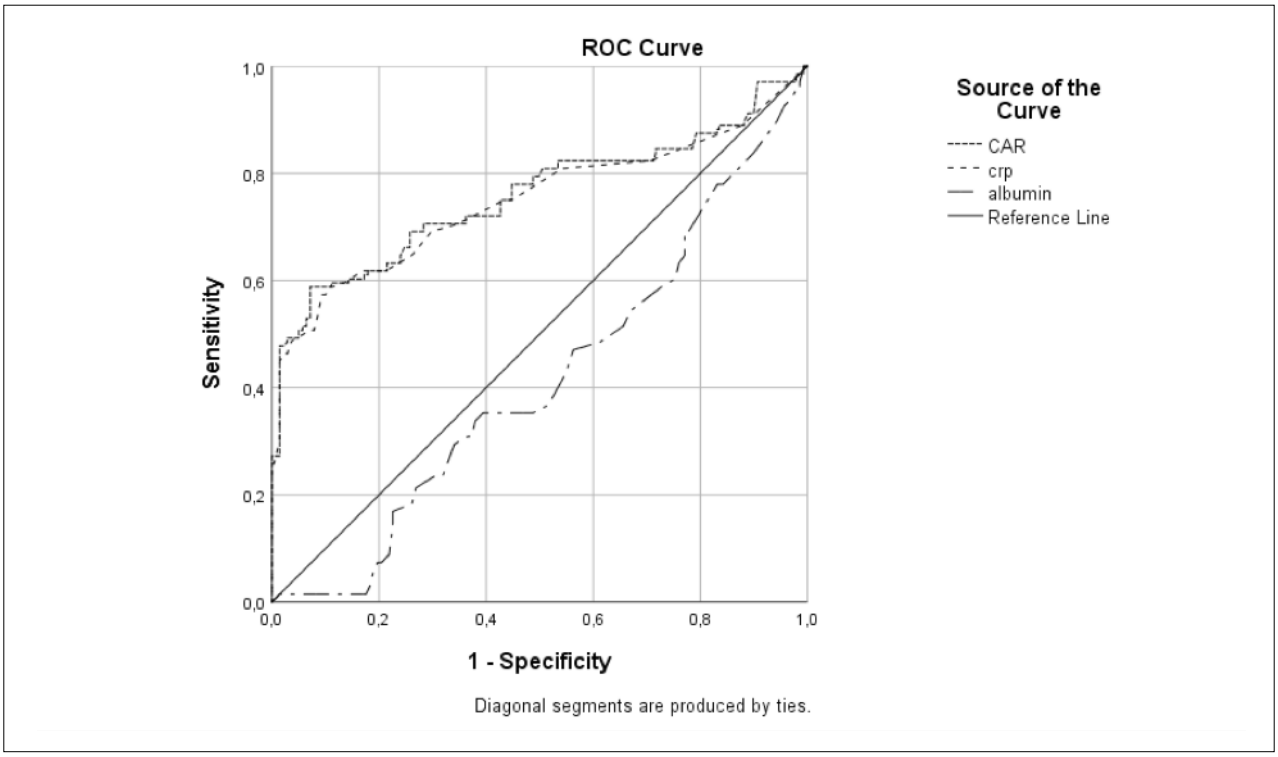
The main findings of this observational study are as follows: (i) elevated CAR, high fasting glucose level, advanced age, male gender, first 24-hour drainage amount, and lower LVEF values are significantly associated with POAF; (ii) a CAR value of more than 3.5 and was found to be a predictor of POAF; and (iii) NLR and PLR were higher in patients with POAF compared to without POAF.

TABLE 2. FACTORS THAT WERE FOUND TO BE INDEPENDENTLY ASSOCIATED WITH THE DEVELOPMENT OF POSTOPERATIVE ATRIAL FIBRILLATION IN UNIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION ANALYSIS MODELS

	Unadjusted Odds Ratio	Confidence Interval	P-value	Adjusted Odds Ratio	Confidence interval	P value
Diabetes mellitus	1.67	1.11-2.53	0.014			
Hypertension	3.075	1.69-5.58	< 0.001			
Congestive heart failure	4.85	2.44-9.61	< 0.001			
Peripheral vascular disease	1.67	1.07-2.59	0.02			
BMI	0.96	0.92-1.00	0.06			
Fasting glucose level	1.006	1.004-1.009	< 0.001	1.01	1.00-1.01	< 0.001
Age	1.06	1.04-1.08	< 0.001	1.12	1.07-1.17	< 0.001
Male gender	2.015	1.22-3.33	0.006	3.32	1.39-7.90	0.007
24-hour drainage	1.002	1.001-1.003	0.004	1.004	1.002-1.005	< 0.001
Left ventricle ejection fraction	0.93	0.91-0.95	< 0.001	0.90	0.87-0.94	< 0.001
Cardiopulmonary bypass time	1.007	1.00-1.01	0.04			
CRP to Albumin Ratio	1.575	1.40-1.76	< 0.001	1.82	1.53-2.16	< 0.001
N to L ratio	1.040	0.89-1.08	0.06			
P to L ratio	1.01	1.00-1.03	0.04			
CRP	1.12	1.09-1.15	< 0.001			
Albumin	0.395	0.21-0.71	0.002			

Abbreviations: BMI: body mass index, CRP: C reactive protein, N: neutrophil, L: Lymphocyte, P: Platelet

FIGURE 1. RECEIVER OPERATING CHARACTERISTICS (ROC) CURVE ANALYSIS OF THE VARIABLES



Mechanism of Postoperative Atrial Fibrillation

In the postoperative period, AF is thought to be triggered by electrophysiological abnormalities, which frequently happen in patients who have an abnormal atrial substrate.[6] High catecholamine state[7] and postoperative inflammation[6] are thought to have lay a key role.

Inflammation and Postoperative Atrial Fibrillation

As bypass surgery may induce oxidative stress and inflammation, these processes may be responsible for complications after cardiac surgery, including POAF.[6] Several studies have suggested a strong link between inflammation and atrial fibrillation.[12] Increased inflammatory processes are also suggested to be associated with new-onset AF after coronary artery bypass grafting. Lo et al.[13] showed that CRP level >3 mg/L was associated with an increased risk of AF in patients undergoing CABG. Supporting this, high CRP levels were associated with the development of new-onset AF following CABG in the present study. Serum CRP, an acute-phase protein, is released from the liver in response to inflammation and has been associated with poor prognosis for patients with coronary artery disease (CAD).[14] De Lorenzo et al.[15] showed that high preoperative CRP levels were associated with in-hospital mortality after CABG. Kinoshita et al.[16] reported that preoperative CRP levels were independently associated with the development of AF after isolated off-pump CABG. Although the relationship between CRP and CAD is not fully understood, multiple mechanisms may be suggested. CRP has been shown to disturb the endothelial progenitor cells, increase the prothrombotic status, activate the complement system, and play a role in the uptake of low-density lipoprotein-C by macrophages and convert them into foam cells.[17] Additionally, a strong association between AF and inflammation has been reported.[1,3] The most important pathophysiological changes described in previous studies include the presence of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies and the presence of circulating autoantibodies against myosin heavy chain.[1,6] Serum albumin, a negative acute-phase protein, is released from the liver in response to inflammation, and decreased albumin levels have been associated with adverse cardiovascular events.[18] Additionally, albumin is not solely associated with inflammation but also with blood viscosity and endothelial functions. Decreased albumin levels increase blood viscosity and platelet activation and worsen endothelial functions.[19] These factors may explain the association between CRP, albumin levels, and POAF. In the present study, we showed that increased CRP levels, decreased albumin levels, and increased CAR were associated with POAF.

The N/L ratio is a widely available marker of inflammation that is cheap and easy to obtain and can be used in the risk classification of patients with several cardiovascular diseases in addition to the traditionally used markers. It is also considered to be a good and powerful predictor of mortality and morbidity in patients undergoing CABG surgery.[20] In this setting, Gibson et al.[21] showed that high pre- and postoperative N/L ratios were associated with the development of AF after CABG. Corroborating this, preoperatively the N/L ratio was found to be higher in patients with new-onset AF following CABG in the present study. Additionally, platelets are a source of inflammatory mediators. Increased platelet levels were reported to be associated with adverse cardiovascular outcomes.[22] A high P/L ratio, defined as a biomarker of inflammation, was reported to be associated with POAF in patients undergoing CABG.[23] In the current study, the preoperative P/L ratio was also higher in patients with new-onset AF following CABG. Another important finding of the present study is the association of advanced age with the development of POAF. The inflammatory response can increase with advanced age via the activity of the mitochondrial adaptor protein p66 (Shc) and sirtuins, which are a family of deacetylase enzymes.[24] Previous studies have shown that hypertension, diabetes mellitus, obesity, valvular disease, increased age, and left atrial characteristics such as size, volume, and scarring can contribute to the development of POAF.[25] Supporting this, the incidence of hypertension, diabetes mellitus, congestive heart failure, and peripheral arterial disease history was higher in patients with POAF compared to patients without POAF in the present study.

In recent studies, several factors have been mentioned, including predisposing ones, such as obesity, diabetes mellitus, hypertension, advanced age, metabolic syndrome, intraoperative ones, such as surgical methods, off-pump surgery, and acute volume changes, and postoperative ones, such as hypotension and volume overload.[5-8] Our data showed that advanced age, obesity, hypertension, male gender, diabetes mellitus, congestive heart failure, and peripheral vascular disease were more frequently present in patients with POAF than in those without POAF. Additionally, patients with POAF had a longer hospitalization period, cardiopulmonary bypass time, and 24-hour drainage amount than patients without POAF. Multivariate analysis showed that fasting glucose level, advanced age, male gender, 24-hour drainage, and left ventricle ejection fraction were independent predictors of POAF.

Combining albumin and CRP into a single index was demonstrated to be associated with adverse events in coronary artery disease.[9,10] We showed here that an increased CRP/albumin ratio indicates a higher inflammatory state and may be superior to CRP and albumin alone in determining POAF. To our knowledge, this is the first study to evaluate the relationship between CAR and POAF. Our results suggest that elevated CAR in patients undergoing CABG was an independent predictor of new-onset AF after CABG, and the predictive accuracy of CAR was better than that of CRP and albumin level, as per the comparison of the ROC curves.

Study limitations

Importantly, this study has some limitations. First, it has a retrospective design, a relatively small sample size, and engaged in a single-center experience. Second, we evaluated only baseline CRP and albumin levels before CABG and the changes that would be observed by consecutive measurements, such as in the postoperative period, that may have an additional predictive value. Third, the data acquired did not let us appraise the prognostic value of CAR on adverse cardiovascular outcomes, since we recorded only limited volume and event rate values.

CONCLUSIONS

This study demonstrated that decreased LVEF, elevated CAR, male gender, advanced age, 24-hour drainage, and increased fasting glucose level were independent predictors for the development of POAF. We also observed that the predictive accuracy of CAR for the development of POAF was better than that of NLR or PLR.

This study revealed that CAR was statistically significantly associated with POAF. CAR is an easy to obtain, easily measurable, and cheap parameter that can predict the development of POAF. This parameter can be part of the preoperative evaluation to identify patients who may develop AF.

Sources of funding

None

Conflict of interest

None

RESUMO

OBJETIVO: *Este estudo teve como objetivo investigar o valor preditivo da recém-definida relação entre Proteína C-Reativa (PCR) e Albumina (CAR) na determinação do desenvolvimento de Fibrilação Atrial (FA) em comparação com outros marcadores inflamatórios, como proporção de Neutrófilos para Linfócitos (N/L) e relação Plaquetas/Linfócitos (P/L) em pacientes submetidos à Cirurgia de Revascularização do Miocárdio (CRM).*

MÉTODOS: *A população deste estudo observacional foi composta por 415 pacientes submetidos à cirurgia de revascularização do miocárdio. A coorte do estudo foi subdividida em dois grupos de acordo com o desenvolvimento da FA. Contagens sanguíneas completas, PCR sérica e albumina sérica foram obtidas antes da CRM. Os valores de CAR, relação N/L e relação P/L foram calculados. Os preditores de FA pós-operatória foram determinados por análise de regressão logística múltipla.*

RESULTADOS: *Durante o acompanhamento, 136 pacientes (32,8%) desenvolveram FA pós-operatória. Com análise de regressão logística múltipla, foram determinados os fatores de risco para FA pós-operatória: glicemia de jejum (OR: 1,01; IC 95%: 1,00-1,01, p<0,001), idade (OR: 1,12; IC 95%: 1,07-1,17, p<0,001), fração de ejeção do ventrículo esquerdo (OR: 0,90; IC 95%: 0,87-0,94, p<0,001), sexo masculino (OR: 3,32; IC 95%: 1,39-7,90, p=0,007), quantidade de drenagem de 24 horas (OR: 1,004; IC 95%: 1,002-1,005, p<0,001), CAR (OR: 1,82; IC 95%: 1,53-2,16, p<0,001). A análise da curva de características operacionais do receptor mostrou que o CAR (estatística C: 0,75; IC 95%: 0,71-0,79, p<0,001) foi um preditor significativo de FA.*

CONCLUSÃO: *O novo marcador inflamatório CAR é confiável para prever o desenvolvimento de FA após a operação de revascularização miocárdica.*

PALAVRAS-CHAVE: *Proteína C. Albuminas. Fibrilação atrial. Revascularização miocárdica. Ponte de artéria coronária.*

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ORIGINAL ARTICLE

Evaluation of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, and lymphocyte to monocyte ratio in patients with cellulitis

Nevin Ince[1]
Ertuğrul Güçlü[2]
Mehmet Ali Sungur[3]
Oğuz Karabay[2]

1. Department of Infectious Diseases and Clinical Microbiology, Duzce University Faculty of Medicine, Duzce, Turkey
2. Sakarya University Medical Faculty Teaching and Research Hospital Infectious Diseases and Clinical Microbiology Department, Sakarya, Turkey
3. Department of Biostatistics, Duzce University Faculty of Medicine, Duzce, Turkey

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SUMMARY

OBJECTIVE: *Cellulite infection is a non-necrotizing inflammation of the skin and subcutaneous tissue and is one of the most common reasons for admission to hospital. This retrospective study aimed to investigate the Neutrophil to Lymphocyte Ratio (NLR), Platelet to Lymphocyte Ratio (PLR), and Lymphocyte to Monocyte Ratio (LMR) in patients with cellulitis.*

METHODS: *In our study, we retrospectively analyzed 96 patients with cellulitis and 98 age- and sex-matched healthy controls. The study and control groups were compared regarding NLR, PLR, and LMR.*

RESULTS: *The PLR and NLR of the cellulitis group were significantly higher than those of the control group ($p < 0.001$). When patients with cellulitis were divided into two groups, i.e., ≥65 years and <65 years, a statistically significant difference was noted in the NLR and LMR values ($p < 0.05$). In the ROC curve analysis, NLR had the highest discriminative power in distinguishing between cellulitis and healthy controls (AUC = 0.950, 95% CI: 0.920–0.979, $p < 0.001$; 91.6% sensitivity and 89.8% specificity).*

CONCLUSION: *NLR was significantly higher in differentiating cellulite and in patients older than 65 years. Larger, prospective studies are required to determine its usefulness in assessing differential diagnosis and prognosis in cellulitis patients.*

KEYWORDS: *Cellulitis. Lymphocytes. Neutrophils. Blood platelets. Monocytes. Diagnosis.*

INTRODUCTION

Cellulitis is a non-necrotizing inflammation of the skin and subcutaneous tissue that is caused by the Staphylococcus or Streptococcus bacterium[1]. It is estimated that 2.3 million people visit the emergency room annually due to cellulitis[2]. Cellulitis occurs most frequently in the lower extremities and usually unilaterally. Several risk factors such as lymphedema, venous insufficiency, traumatic wounds, leg ulcers, and intertrigo are associated with cellulitis[3,4]. Clinically, erythema, edema, temperature, and sensitivity develop in the patient's skin[5]. There is no definitive or reliable diagnostic method for cellulitis, which is one

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CORRESPONDING AUTHOR: Nevin Ince
Department of Infectious Diseases and Clinical Microbiology, Duzce University Faculty of Medicine, Duzce, Turkey
Tel: +90 505 350-5839, Fax: +90 380 542-1387
E-mail: drnevince@gmail.com

of the most frequently occurring infectious diseases requiring hospitalization. The spectrum of the disease can range from localized erythema in a systemically well patient to rapidly spreading erythema and fulminant sepsis occurring concurrently with necrotizing fasciitis[6]. Clinical findings based on anamnesis and physical examination should be evaluated together for the diagnosis. Routine biochemical and hematological blood tests and blood cultures are not specific for the diagnosis of cellulitis[1]. Recently, it has been pointed out that the changes in peripheral blood leukocyte ratios are a simple, rapid, and novel, promising inflammation parameter for several diseases[7-9]. Some of these markers are Neutrophil/Lymphocyte Ratio (NLR), Platelet/Lymphocyte Ratio (PLR), and Lymphocyte/Monocyte Ratio (LMR).

NLR, PLR, and LMR values have been suggested to be useful in differentiating between infections and predicting prognosis[10,11]. The ready availability and low cost of such parameters would make them particularly helpful in low- and middle-income countries. Hemogram parameters have been used in the viral–bacterial differentiation of infections. In a study with cases in which patients in the hospital had fever were examined retrospectively, it was found that NLR was higher in patients with fever due to bacterial infections than those with viral infection[8].

To the best of our knowledge, there is no study investigating the peripheral blood leukocyte ratios in cellulitis in the literature. Therefore, in the present study, we aimed to evaluate the relationship between the use of hemogram parameters as diagnostic biomarkers and age and the duration of hospital stay in patients with cellulitis.

METHODS

Study population and design

We enrolled 96 patients who were diagnosed with cellulitis at the Department of Infectious Disease of Duzce University Medicine Faculty Hospital and Sakarya University Medical Faculty Teaching and Research Hospital, from January 2017 to March 2019. Patients' medical records were retrospectively reviewed. The control group included 98 age- and sex-matched healthy subjects. Age, sex, involved site(s), infectious route, systemic manifestations, and the duration of hospital stay were evaluated. Patients' demographic characteristics, age, sex, clinical findings, involved site(s), the duration of hospital stay, blood values (white blood cell [WBC], neutrophil count, lymphocyte count, monocyte count, and platelet count [PLT]) were recorded in forms. NLR, PLR, and LMR were calculated as the ratio of neutrophils to lymphocytes, platelets to lymphocytes, and monocytes to lymphocytes, respectively. Venous blood samples were obtained simultaneously to determine the peripheral blood leukocyte ratios on the first day after admission before receiving antimicrobial therapy.

Blood sample analyses

Venous blood samples were collected in sterile standard tubes containing ethylenediaminetetraacetic acid as an anticoagulant. Complete blood count parameters were measured using the Abbott CELL-DYN 3700 automatic analyzer.

Statistical Analysis

The distribution of the data was examined using the Kruskal–Wallis test. Independent samples t-test was used for continuous variables showing normal distribution, and the Mann–Whitney U test was used for continuous variables not showing normal distribution. Cut-off values were calculated using receiver operating characteristic (ROC) curve analysis for parameters that differ significantly in Cellulitis and control group comparisons. The relationships between categorical variables were examined using Pearson chi-square or Fisher-Exact tests. Statistical analysis was performed with SPSS v.22 package program, and the significance level was considered as 0.05.

RESULTS

Our study included 96 patients with cellulitis and 98 healthy, age- and sex-matched control subjects. The male/female ratio of patients with cellulitis was 39/57, while it was 35/63 for the control group. The mean age of the patients with cellulitis was 66.01±12.13 years, and the mean age of the control group was 63.66±9.91. No statistically significant difference was noted between the groups for age and sex; 92.7% of the patients had cellulitis in the lower extremities, 5.2% had cellulitis in the upper extremities, and 2.1% had cellulitis in the body. The WBC, PLR, and NLR of the cellulitis group were significantly higher than those of the control group ($p < 0.001$). In the cellulitis group, the Hb, PLT, and LMR were lower compared with those in the control group ($p < 0.001$). Laboratory

data of the cellulitis and control groups are summarized in Table 1.

The data regarding the evaluation of the cellulitis group by age are presented in Table 2. Accordingly, when patients with cellulitis were divided into two groups as ≥65 years and <65 years, a statistically significant difference was noted among the WBC, NLR, and LMR values ($p < 0.05$), while a difference was noted among the Hb levels, PLT, and PLR ($p > 0.05$). No significant relationship was noted between the duration of hospital stay of patients with cellulitis and their peripheral blood leukocyte ratios ($p > 0.05$).

In the ROC curve analysis, areas under the curve (AUC) of WBC, Hb, PLT, PLR, NLR, and LMR were 0.919, 0.672, 0.645, 0.691, 0.950, and 0.814, respectively. Of these parameters, NLR showed the highest discriminative power in distinguishing between cellulitis and healthy controls (AUC = 0.950, 95% CI: 0.920–0.979, $p < 0.001$; 91.6% sensitivity and 89.8% specificity). The comparison of the ROC curves of parameters is shown in Fig. 1.

DISCUSSION

We know that laboratory parameters and clinical symptoms are unspecific in cellulitis. Presently, no appropriate case definition and definitive diagnostic criteria exist for cellulitis[12]. To the best of our knowledge, this is the first study to investigate the peripheral blood leukocyte ratios in cellulitis infections. The primary findings of our study were that WBC, PLR, and NLR levels were significantly higher in cellulitis than in the control group. In addition, when we divided the patients by age (<65 and ≥65 years), a significant difference was noted in the NLR and LMR values between the groups.

An increase in the NLR and a decrease in the LMR are considered indicators of systemic inflammation. Several studies have indicated that NLR, PLR, and LMR may predict systemic inflammation, and these markers may be useful in many diseases[13-15]. Horne et al.[16] reported that NLR is more effective than leukocyte levels in predicting inflammation. In a study conducted by Kartal et al.[17], a significant difference was found between community-acquired pneumonia and control groups in terms of NLR and PLR values. Similar to other studies, in our study, patients with cellulitis had higher levels of PLR and NLR than those in the control group.

Studies investigating the role of peripheral blood leukocyte rates in predicting complications and prognosis in various diseases reported that PLR and NLR

TABLE 1. DEMOGRAPHIC AND LABORATORY CHARACTERISTICS FOR THE TWO GROUPS.

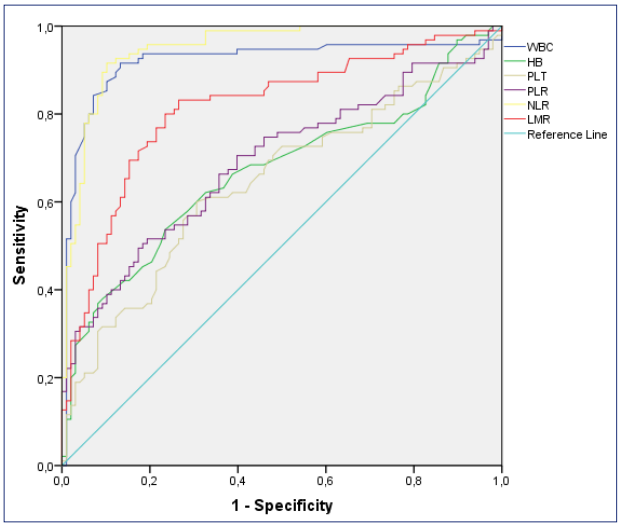
	Cellulitis group (n=96)	Healtyh control group (n=98)	P value
Gender [n (%)] Female Male	 57 (59.4%) 39 (40.6%)	 63 (64.3%) 35 (35.7%)	0.481
Age	66.01±12.13	63.66±9.91	0.141
WBC (10^9/L)	14100 (1000-35800)	6445 (4000-72100)	<0.001
Hb (g/dl)	12.36±1.67	13.32±1.19	<0.001
Plt (10^3/mm^3) Median (min-max)	204500 (67000-438000)	245500 (75000-409000)	0.001
PLR	160.68 (41.22-710.00)	115.39 (0.15-307.50)	<0.001
NLR	8.65 (1.70-34.36)	1.76 (0.71-17.93)	<0.001
LMR	2.15 (0.37-12.00)	4.51 (1.14-10.91)	<0.001

WBC: White blood cell count; Hb: hemoglobin; Plt: platelet count; PLR: platelet-to lymphocyte ratio; NLR; neutrophil-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio.

TABLE 2. COMPARISON OF HEMOGRAM PARAMETERS OF CELLULITE GROUP ACCORDING TO AGE.

	Age <65 (n=38)	Age ≥65 (n=58)	P value
WBC (10^9/L)	12050 (3900-26300)	14600 (1000-35800)	0.026
Hb (g/dl)	12.70±1.74	12.14±1.60	0.104
Plt (10^3/mm^3) Median (min-max)	215000 (67000-413000)	203500 (110000-438000)	0.449
PLR	154.96 (60.45-678.00)	178.89 (41.22-710.00)	0.438
NLR	7.04 (2.06-25.30)	10.06 (1.70-34.36)	0.032
LMR	2.58 (0.67-12.00)	1.85 (0.37-7.20)	0.032

FIGURE 1. RECEIVER OPERATING CHARACTERISTIC (ROC) CURVES OF THE HEMOGRAM PARAMETERS FOR DIFFERENTIATING CELLULITIS PATIENTS FROM HEALTHY ONES.



rates can be used as prognostic markers with diagnoses. For example, two different studies revealed higher NLR rates in patients requiring amputation in diabetic foot infections and in those with osteoarticular involvement in brucella infection[14,18]. Yapici et al.[19] showed in their study that the NLR values of patients who had diabetic foot infection with concurrent osteomyelitis were significantly higher than those without osteomyelitis (p = 0.004). The treatment period for cellulitis infections is recommended as 5–10 days. Longer treatment may be required in cases of clinical unresponsiveness or in cases of lower extremity edema and obesity[20]. In the present study, no significant relationship was noted between the requirement for prolonged hospitalization (≥10 days) and PLR, NLR, and LMR levels in patients with cellulitis.

There are few studies evaluating the peripheral blood leukocyte rates' relationship with disease and age. In a prospective study, it was reported that NLR may reflect systemic inflammatory processes in Alzheimer's disease, and that age is a dominant variable that should be considered[21]. In a study conducted by Cataudella et al.[22], patients with community-acquired pneumonia over 65 years of age were evaluated. In this study, it was found that NLR was more of a prognostic marker for the diagnosis and mortality of patients over 65 years of age in community-acquired pneumonia. In our study, we divided patients with cellulitis into groups over and under 65 years of age and evaluated whether there is a relationship between age and peripheral blood leukocyte rates. No difference was noted in PLR values between age groups, whereas NLR was significantly lower in the group of patients under 65 years (p = 0.032) and LMR was significantly higher in the group of patients over 65 years (p = 0.032). Further studies are required to establish a relationship between age and peripheral blood leukocyte ratios.

This study has some limitations. First, it was retrospectively conducted and the sample size is relatively small. Second, patients were compared using only hemogram parameters and rates. Other inflammatory biomarkers were not evaluated. Third, the complete blood count parameters of patients with cellulitis were compared at the beginning of treatment. Changes in parameters after treatment were not examined.

CONCLUSION

In conclusion, peripheral blood leukocyte rates are fairly simple, inexpensive, and easy for evaluating new inflammatory biomarkers. We found that the PLR, NLR, and LMR values were significantly different in the patients with cellulitis compared with the healthy control group. Our study is the first in the literature showing peripheral blood leukocyte rates in patients with cellulitis. We believe that the use of these parameters together with clinical diagnosis in the planning for further studies will help in differential diagnosis and prognosis.

Source of support

None.

Conflict of interest

None.

Approval was obtained from Sakarya University and Düzce University Medical Faculty Ethics Committee in 2019/244.

Author's Contribution

Nevin Ince: study design, writing, and data collection; Ertuğrul Güçlü: data collection; Mehmet Ali Sungur: statistics; Oğuz Karabay: study design and writing.

RESUMO

OBJETIVO: *A celulite infecciosa é uma inflamação não necrotizante da pele e do tecido subcutâneo e uma das causas mais comuns para internação. O objetivo deste estudo retrospectivo foi investigar as relações Neutrófilo/Linfócito (RNL), Plaqueta/Linfócito (RPL) e Linfócito/Monócito (RLM) em pacientes com celulite.*

MÉTODOS: *Nós analisamos, retrospectivamente, 96 pacientes com celulite e 98 controles saudáveis equivalentes em sexo e idade. Os grupos foram comparados quanto a RNL, RPL e RLM.*

RESULTADOS: *Os valores de RPL e RNL do grupo com celulite foram significativamente mais elevados do que os do grupo de controle (p < 0,001). Após dividir os pacientes com celulite em dois grupos, ≥65 anos e <65 anos, uma diferença estatisticamente significativa foi observada nos valores de RNL e RLM (p < 0,05). Na análise da curva ROC, a RNL apresentou o maior poder de discriminação para distinguir entre pacientes com celulite e controles saudáveis (AUC = 0,950, 95% CI: 0,920 - 0,979; p < 0,001; 91,6% de sensibilidade e 89,8% de especificidade).*

CONCLUSÃO: *O valor de RNL foi significativamente maior para a diferenciação de pacientes com celulite e pacientes com mais de 65 anos. Estudos prospectivos maiores são necessários para determinar a sua utilidade na avaliação de diagnósticos diferenciais e prognósticos em pacientes com celulite.*

PALAVRAS-CHAVE: *Celulite. Linfócitos. Neutrófilos. Plaquetas. Monócitos. Diagnóstico.*

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ORIGINAL ARTICLE

Can the risk of anal fistula development after perianal abscess drainage be reduced?

Feridun Suat Gokce[1]
Aylin Hande Gokce[2]

1. Balıklı Rum Hospital, Department: General Surgery, İstanbul, Turkey.
2. Istanbul Atlas University; Medical Faculty Department: General Surgery, İstanbul, Turkey.

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SUMMARY

OBJECTIVE: *Perianal abscesses are frequently seen in clinical practice, and perianal fistulas develop in 30%–50% of cases after treatment. This study investigated whether the type of dressing applied after abscess drainage is correlated with fistula development. Prevention of fistula formation would reduce both the loss of work and healthcare costs.*

METHODS: *The records of patients who underwent drainage of perianal abscesses between January 2015 and January 2018 were retrospectively reviewed. Patients with postoperative dressings changed with washing of the area in the hospital were included as Group 1. Patients with dressings changed at home and the area bathed in 10% povidone-iodine sitz bath were included as Group 2. The frequency and time of fistula formation, age, sex, cost, and workdays lost in the two groups were compared.*

RESULTS: *Between-group differences in age, sex, body mass index, and type of fistula that developed after months and 1 year of the abscess drainage were not statistically significant ($p > 0.05$). During follow-up, fistula development was significantly lower in Group 1 than in Group 2 ($p < 0.001$). The risk of perianal fistula development was significantly increased in those with a body mass index (BMI) > 30 ($p = 0.004$).*

CONCLUSIONS: *After perianal abscess drainage, in-hospital washing and dressing of the abscess area until abscess closure reduced the risk of perianal fistula, lost work time, and cost. The risk of perianal fistula development appeared to increase with BMI. A large, prospective study is needed for confirmation.*

KEYWORDS: *Abscess. Rectal fistula. Fistula. Drainage.*

INTRODUCTION

Perianal abscesses are frequently seen in clinical practice, and drainage is the standard treatment. Approximately 12,500 cases are surgically treated annually in England, and the annual incidence in Scotland is estimated at 16.1/100.000 population[1,2]. The most generally accepted etiology is cryptoglandular, in which the abscess is caused by inflammation of the anal glands[3]. Perianal abscesses are most common in men in their 30s and 40s[4]. Patients with small superficial abscess can be treated with antibiotics, but the vast majority requires drainage. Untreated perianal abscesses can lead to Fournier's gangrene, which is a life-threatening complication[5]. Perianal abscesses are generally drained surgically in a hospital setting. Abscesses that drain spontaneously usually require additional drainage[1]. Perianal fistulas develop in 30%–50% of patients after drainage and abscess healing. Perianal fistulas may be intersphincteric (simple

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CORRESPONDING AUTHOR: Feridun Gokce
Belgrad kapi yolu, n.2, Zeytinburnu, Istanbul, Turkey – 34010
E-mail: fsgokce70@hotmail.com

fistula), transsphincteric, suprasphincteric (complex fistula), or extrasphincteric (horseshoe fistula), and generally require surgery[6]. The development of a perianal fistula adds to the overall cost of abscess treatment, additional loss of work time, and the risk of surgical complications. The drainage of perianal abscesses involves preoperative and postoperative antibiotics, which are intended for infection management but have no effect on preventing fistulas[7,8]. Perianal abscesses and fistulas are commonly seen in patients with inflammatory bowel diseases including Crohn's disease, and the treatment of perianal fistulas should be delayed during the active period of the disease[9].

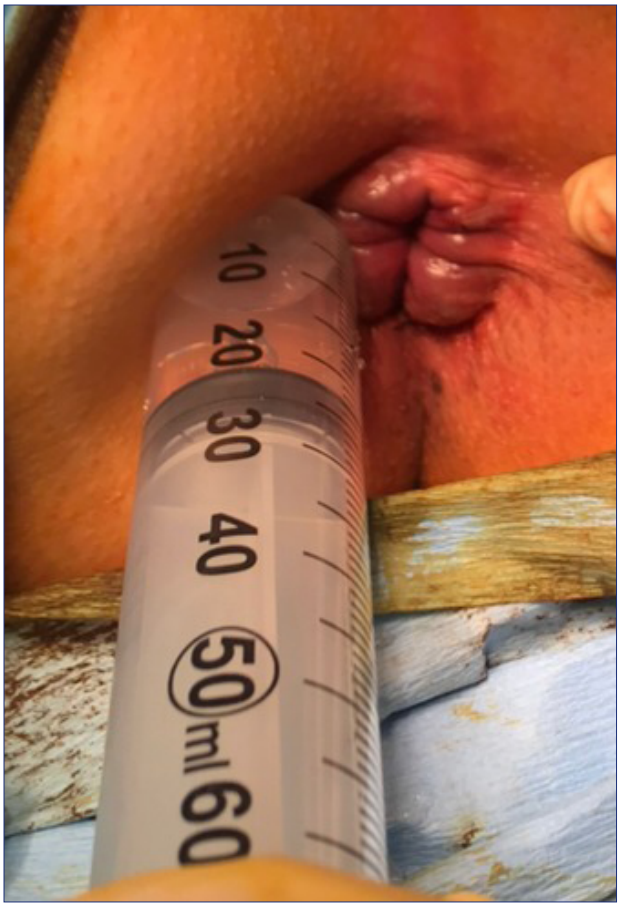
In this study, fistula development after abscess drainage in patients who sat in hot water during dressing performed outside the hospital and in patients with dressing and abscess area washing in the hospital were compared. Fistula development, the type of fistula, differences in simplicity/complexity, cost, and work time lost in the two groups were compared. The relationship between body mass index (BMI) and fistula development regardless of the dressing change procedure was investigated.

METHODS

This retrospective cohort study was approved by the local ethics committee (Verdict Number 2019/35-10) and was conducted following the ethical guidelines of the Declaration of Helsinki for biomedical studies involving humans. Perianal abscess surgeries performed in our clinic between January 2015 and January 2018 were evaluated. Some surgeons preferred dressing changes with sitz baths at home, others preferred dressing changes in the hospital with washing of the inside of the pouch until closure. The study outcome was fistula development following abscess drainage. The frequencies of fistula development, types of fistula, patient characteristics, cost, and work time lost in each group were determined and compared.

The study included patients aged 18–65 years who visited the clinic for follow-up after a first-time diagnosis and drainage of a perianal abscess. Patients with a history of cancer, a prior perianal fistula and/or abscess, tuberculosis, recto-vaginal fistula, diabetes mellitus, inflammatory bowel or Crohn's disease, perianal abscess caused by trauma, or internal hemorrhoid surgery were excluded. Pregnant women and patients who did not visit the clinic for follow-up examinations were also excluded. Group 1 included patients with abscess dressing changes done in the hospital. In the first 3 days after drainage, the abscess pouch of Group 1 patients was filled with 3% hydrogen peroxide followed by 10% povidone-iodine, and finally with 0.9% sodium chloride. After that, the site was washed with 0.9% sodium chloride daily until the abscess pouch was closed (Image 1). Group 2 included patients with dressing changes twice a day after a sitz bath using a 10% povidone-iodine solution until discharge from the area stopped. Anal fistula development was monitored at 3 and 12 months after abscess drainage (Image 2). Age, sex, the type of the fistula that developed, the time until development, the frequency of development, the cost of treatment, and lost work time in each group were compared. Simple, transsphincteric, and suprasphincteric fistulas were considered complex; extrasphincteric types were considered horseshoe fistulas. Patients were stratified by BMI, as up to < 18.4 kg/m², 18.5–24.9 kg/m², 25–29.9 kg/m², and > 30 kg/m². Whether fistula development was correlated with BMI regardless of the type of dressing was determined. The cost of hospitalization included the total

IMAGE 1. DRESSING OF PERIANAL ABSCESSES IN THE HOSPITAL SETTING.

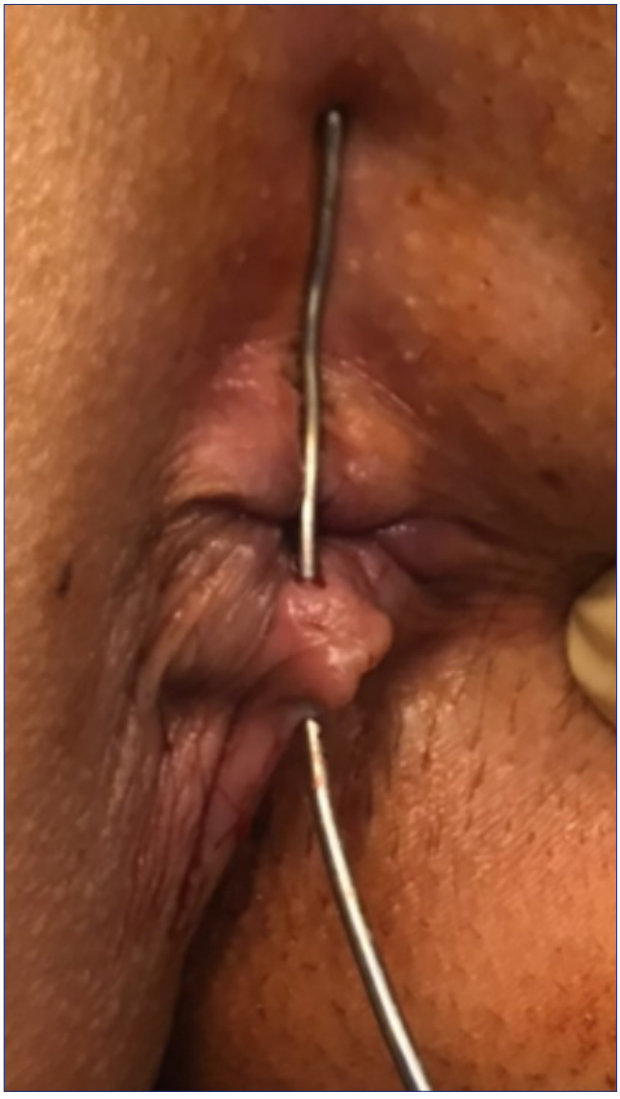


cost of abscess surgery and dressings in United States dollars (USD). Days of home rest and hospital visits for dressings were included; each dressing required after returning to work were calculated as half-days of worktime lost.

Statistical analysis

Statistical analysis was conducted with SPSS for Windows, version 22.0 IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to determine whether continuous variables were normally distributed. Between-group differences in the values of normally distributed continuous variables were compared using Student's *t*-test. Those that were not normally distributed were compared using the Mann–Whitney U test. Continuous variables were reported as means and standard deviation. The chi-square test was used to compare the values of categorical variables. *P*-values < 0.05 were considered statistically significant.

IMAGE 2. DEVELOPMENT OF PERIANAL FISTULAS AFTER PERIANAL ABSCESS DRAINAGE.



RESULTS

Fistula development and fistula type

Of 340 patients with abscess drainage, the 52 had diabetes mellitus, five a history of abscess drainage, and one an inflammatory bowel disease were excluded. Thirty-eight who did not complete the follow-up visits were not included in the analysis. A total of 119 patients, 79 men and 40 women, were included in Group 1; 125 patients, 90 men and 35 women, were included in Group 2. Differences in sex (p = 0.342), mean age (37.6 ± 12 years versus 39 ± 12.3 years), and BMI were not significant (P = 0.778, Table 1). In Group 1, 14 patients (11.8%) had anal fistulas at the 3-month follow-up and eight had anal fistulas at the 1-year follow-up (6.7 %), a total of 22 cases (18.5%). Ninety-seven of the 119 patients with in-hospital dressings (81.5%) did not develop anal fistulas. Forty patients in group 2 had fistulas at the 3-month follow-up (32%) and seven had fistulas at the 1-year follow-up (5.6%), a total of 47 cases (37.6 %). Seventy-eight of the patients with at-home dressings (62,4%) did not develop anal fistulas. Fistula development at 3 months was significantly more frequent in Group 2 patients with at-home dressings than it was in Group 1 with in-hospital dressings (P = 0.001). In Group 1, 11 fistulas (50%) were simple, nine (41%) were complex, and two (9%) were horseshoe. In Group 2, 26 fistulas (55,3%) were simple type, 20 (42,5%) were complex, and one (2,2%) was horseshoe. The differences in fistula type were not significant (P = 0.415).

Workdays lost, cost, and BMI

A mean of 7.4 ± 6.8 work days were lost by Group 1 patients; 6.8 ± 7.7 days were lost by Group 2 patients. The difference was not significant (P = 0.499). Patients who required anal fistula surgery lost a mean of eight more days of work than those who did not require anal

TABLE 1. BODY MASS INDEX IN GROUPS 1 AND 2.

Body mass index	(Group 1) (n:119)	(Group 2) (n:125)	
			χ^2 =1.097; p=0.778
Less than 18.4	1 (%0.9)	3 (%2.4)	
18.5-24.9	43 (%36.1)	47 (%37.6)	
25-29.9	67 (%56.3)	66 (%52.8)	
Over than 30	8 (%6.7)	9 (%7.2)	

fistula surgery. The average total cost of the abscess surgery and dressings was USD 626.9 ± 419.2 on average for Group 1 patients, and USD 579.2 ± 462.2 for Group 2 patients. The difference was not significant (P = 0.843). The increased cost attributable to elective surgery for patients who developed an anal fistula was around USD 1100 per case. Anal fistulas developed in one of the four patients (25%) with BMIs < 18.4 kg/m², 19 of 90 (21,1%) with a BMI from 18.5–24.9 kg/m², 38 of 133 (28,6%) with a BMI from 25–29.9 kg/m², and 11 of 17 (64,7%) with a BMI > 30 kg/m². The risk of anal fistula formation was significantly higher in patients with a BMI > 30 kg/m² than in those with a BMI of < 30 kg/m² (P = 0.004).

DISCUSSION

Anorectal abscesses are cryptoglandular infections including a pus-filled cavity within and deep in the dermis. Perianal abscesses are the most common type[1,2]. The incidence of anorectal abscesses varies among countries and areas. Approximately 29%–70% of patients with anorectal abscesses will develop a fistula, about 33% of which develop in the months or years after drainage[1,6]. Perianal abscesses most often occur in men in their 30s and 40s[4]. The study groups were similar in sex and age, 66.3 % of those in Group 1 and 72% in Group 2 were men, the mean age was 37.6 ± 12 years in Group 1 and 39 ± 12.3 in Group 2. The two groups did not differ in BMI. The majority had a BMI between 18.4 and 29.9 kg/m², which is similar to that seen in the general population. A study by Cyzmek et al.[10] reported that the occurrence of Fournier gangrene in untreated perianal abscess was more frequent in patients with a BMI of 25 or more. Our findings are in line with those of Lu et al.[11] who reported that anorectal abscess relapse and fistula formation were higher in patients with high BMIs. In this study, perianal fistulas developed significantly more frequently in patients with BMIs ≥ 30 than in those with lower BMIs (P = 0.004). A previous study by Pigot et al.[12] described the performance of fistula surgery at the same time as drainage of coexistent abscesses but this study excluded patients with both perianal abscess and fistulas.

Few studies have investigated the influence of post-drainage case management on the development of perianal fistulas. Chen et al.[8] reported that the negative pressure irrigation after abscess drainage decreased fistula development. In this study, perianal fistula development was decreased by washing the abscess area in comparison with the use of a sitz bath (P = 0.001). The fistula types that developed in both study groups did not differ, but washing the abscess area in a clinical setting until pouch closure significantly reduced the risk of fistula development compared to the dressings changed at home following a sitz bath. Closure of the fistula pouch by the dressing materials and the mechanical and chemical effects of the medications might account for the difference. Dressing with routine washing of the abscess area may be the preferable method. Published estimates indicate that 30%–50% of patients develop fistulas after drainage of perianal abscesses[6]. In this study, 32% of the patients in the sitz bath group developed fistulas, which is consistent with previous reports. Only in 18.5% of patients with that underwent washing of the abscess area before dressing developed fistulas, which was much lower than previously reported. The differences in lost work days and dressing cost in the two groups were not statistically significant, but since Group 1 patients experienced fewer fistulas, there was less need for elective fistula surgery. The cost of USD 1,100 for surgery and eight fewer days of lost work cannot be ignored.

In conclusion, dressing with regular washing of the area until the closure of the abscess pouch reduced the risk of perianal fistula development. Therefore, workforce loss and cost will also decrease. The risk of perianal fistula development was increased in patients with BMIs > 30, compared to those with lower BMI's.

Author's Contribution

Planning, data collection, data entry, data analysis, article writing, manuscript analysis and interpretation: Feridun Suat Gokce, Aylin Hande Gokce.

No competing financial interests exist. This research did not receive grants from any funding agency in the public, commercial, or not-for-profit sectors. There are no conflicts of interest present. Ethical approval for this study was obtained from the Ethics Committee (2019/35-10).

RESUMO

OBJETIVO: *Abscessos perianais são vistos com freqüência na clínica e uma fístula perianal se desenvolve em 30% a 50% dos casos após o tratamento. Este estudo investigou se o tipo de curativo aplicado após a drenagem do abscesso está correlacionado com o desenvolvimento da fístula. A prevenção da formação de fístulas reduziria a perda de trabalho e os custos com saúde.*

MÉTODOS: *Os prontuários de pacientes com drenagem de abscessos perianais entre janeiro de 2015 e janeiro de 2018 foram revisados retrospectivamente. Os pacientes com curativos pós-operatórios trocados com a lavagem da área no hospital foram incluídos no grupo 1. Os pacientes com curativos trocados em casa e a área banhada em 10% de banho de povidona com iodo povidona-Sitz foram incluídos no grupo 2. A frequência e o tempo de fístula formação, idade, sexo, custo e dias de trabalho perdidos nos dois grupos foram comparados.*

RESULTADOS: *As diferenças entre os grupos em idade, sexo, índice de massa corporal e o tipo de fístula que se desenvolveu após 3 meses e 1 ano após a drenagem do abscesso não foram estatisticamente significantes (p> 0,05). Durante o acompanhamento, o desenvolvimento da fístula foi significativamente menor no grupo 1 do que no grupo 2 (p <0,001). O risco de desenvolvimento de fístula perianal aumentou significativamente naqueles com índice de massa corporal (IMC)> 30 (p = 0,004).*

CONCLUSÕES: *Após a drenagem do abscesso perianal, a lavagem hospitalar e o curativo da área do abscesso até o fechamento do abscesso reduziram o risco de fístula perianal, perda de tempo de trabalho e custo. O risco de desenvolvimento de fístula perianal pareceu aumentar com o IMC. Um grande estudo prospectivo é necessário para confirmação.*

PALAVRAS-CHAVE: *Abscesso. Fístula retal. Fístula. Drenagem.*

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ORIGINAL ARTICLE

Risk factors associated with potential cardiovascular and cerebrovascular adverse events in elderly individuals assisted at secondary level

Henrique Souza Barros de Oliveira[1]
Ana Claudia Michels[1]
Maysa Nahime[2]
Lorena de Melo Caetano[2]
Maria Luiza Galoro Corradi[1,2]

1. Faculdade de Medicina, Centro Universitário São Camilo (Cusc), São Paulo, SP, Brasil.
2. Departamento de Clínica Médica, Hospital Geral de Carapicuíba (HGC), Carapicuíba, SP, Brasil.

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SUMMARY

OBJECTIVE: *To identify the use of Potentially Inappropriate Medications with imminent risk of Cardiovascular and Cerebrovascular Adverse Events (PIM-CCVAE), in addition to the factors associated with a group of elderly individuals undergoing therapeutic care in a Brazilian public service.*

METHODS: *A cross-sectional retrospective study conducted at a secondary level service located in Carapicuíba, SP, Brasil. Only elderly individuals (≥60 years) who were treated in one of the outpatient departments were included. The use of PIM-CCVAE was defined based on the PIM-CCVAEs list. In this research, we used descriptive statistics and logistic regression to identify and track possible predictors of MPI use. All statistical analyses were performed using Stata software version 15.1 (Stata Corporation).*

RESULTS: *The sample included 233 elderly individuals, with a mean age of 74.9 (± 9.4) years. Of these, 74.2% used at least one PIM-CCVAE, with an average daily intake of 1.3 (± 1) PIM/elderly. The presence of comorbidities, diseases of the circulatory system, polypharmacy, and low to moderate scores in morbidity and mortality were important factors associated with an increased odds ratio for the consumption of PIM-CCVAE. It is also emphasized that the presence of neurological symptoms proved to be a protective factor for this outcome.*

CONCLUSION: *Given the clinical severity and imminent risk of CCVAE in the researched group, preventive measures should be instituted to minimize health problems related to medication in the public network.*

KEYWORDS: *Aged. Treatment outcome. Iatrogenic disease. Potentially inappropriate medication list. Drug-related side effects and adverse reactions. Cardiovascular system.*

INTRODUCTION

Cardiovascular and cerebrovascular events are, in large part, responsible for the high rates of mortality worldwide, especially in the oldest population groups[1]. Due to the changes caused by the aging process, the elderly are more susceptible to acute and subacute events related to underlying causes and iatrogenic outcomes, such as: drug interactions; adverse events; use of medications that aggravate the underlying condition and/or use of drugs without scientific evidence (Potentially Inappropriate Medications - PIM)[2-5].

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CORRESPONDING AUTHOR: Maria Luiza Galoro Corradi
Centro Universitário São Camilo, Avenida Nazaré, 1501, Ipiranga, SP, Brasil – 04262-200
E-mail: malugaloro@gmail.com

Given this scenario, Aguiar et al.[6] developed a list of PIM with a potential risk for cardiovascular and cerebrovascular adverse events in elderly patients based on a systematic review that included 24 lists of PIM indexed into the literature and implemented in various places worldwide. Despite this recent indexation, there is still little evidence related to the use of PIM and the potential risk of cardiovascular events in elderly patients, especially in the oldest populations of South America.

Given that, the objective of this research was to identify the use of PIM with a potential risk of adverse cardiovascular and cerebrovascular events (ADE-CC), in addition to its associated factors in a group of elderly patients undergoing therapeutic follow-up in a public service located in the city of Carapicuíba, SP, Brasil.

METHODS

Research design and scope

A cross-sectional and retrospective study conducted with a group of elderly individuals assisted by a medical team with clinical subspecialties in a secondary health care service, located in the city of Carapicuíba, São Paulo, SP, Brasil.

Inclusion and exclusion criteria

The following criteria were considered for the inclusion of patients in this study: (i) age ≥60 years; (ii) both sexes; (iii) have, at least, one consultation with the staff of the internal medicine clinic and subspecialties (geriatrics, cardiology, pulmonology, nephrology, rheumatology; neurology; general practitioners; hematology; dermatology); (iv) and undergoing a drug regimen (at least, one drug).

Exclusion was considered for those who met the following criteria: (i) absence of a description of the medications in use in electronic records, regardless of the reason.

Analysis variables

For the characterization, we evaluated: age; sex; medical specialties under follow-up or that were previously followed-up by this service; time under outpatient care in this service; the presence of morbidities diagnosed and under treatment; stratification of the clinical severity by the Charlson Comorbidity Index (CCI)[7], Charlson Age-Comorbidity Index (CACI) *online*[8] and Functional Comorbidity Index (FCI)[9]; the chief complaint of the most recent consultation; use of continuous medication; the presence of polypharmacy, determined as the continuous use of ≥5 medications[3]; use of psychotropic drugs and presence of PIM wit a potential risk of ADE-CC, based on the PIM-CCVAEs list[6].

METHODS

Based on a list for patient control containing previously scheduled consultations, we conducted a systematic search of electronic records considering the criteria described above for eligibility of the sample. The researchers conducted filled out the survey forms and, subsequently, this information was transferred to an exclusive database for tabulation, stratification, tracking, and storage of the data. All the data collected were subjected to revision and codification, and when inconsistency was detected, it was corrected based on the electronic medical record.

All medicines with trade names were identified using the dictionary of pharmaceutical specialties and classified according to the Anatomical Therapeutic Chemical Code (ATC) *index on-line* (*version* 2019), which is recommended worldwide by the World Health Organization Collaborating Center for Drug Statistics Methodology, Norwegian Institute of Public Health. This codification is recognized as an international standard for the development of pharmacological research and divides drugs into five levels. For this study, we adopted the 1st level classification (organ or system on which the medicine acts)[10].

The application of the PIM-CCVAEs list for screening PIM with a potential risk of causing: cerebrovascular accident; transitory ischemic accident; acute myocardial infarction; heart failure; systemic arterial hypertension; orthostatic hypotension or postural hypotension, bradycardia; prolongation of the QT interval, and arrhythmias in the elderly was employed, including for screening of factors associated with this outcome[6].

Statistical analysis

We carried out a descriptive analysis of the data obtained, which included a description of the population studied and the distributions of absolute and relative frequency of the categorical variables and measures of central tendency and dispersion of quantitative variables. The screening of factors was done between the variables described above and the potential iatrogenic outcome - use of PIM and risk of

ADE-CC - through the use of multiple logistic regression per Stepwise Forward model.

For the logistic regression, we calculated the respective *odds ratios* (OR), confidence interval of 95% (95% CI), and *p-value* of the variables included in this model. We selected for this model only the variables with a *p-value* <0.20 and that remained in the final model, upon adjustment and statistical significance. All tests were carried out using the *Stata* software *version* 15.1 (Stata Corporation, College Station, United States), and we considered a level of statistical significance of 5% for all tests.

RESULTS

We included in the sample 233 electronic records of elderly patients undergoing therapeutic regimens, whose mean age was 74.9 (±9.4) years. Of these, there was a predominance of males (50.2%), with three or more chronic comorbidities (86.6%), mostly circulatory diseases (73.8%), with an estimated moderate relative risk of death between the indexes measured (CCI: 2.5 ±2.3 – estimated 10-year survival: 77%; CACI: 5.6 ±2.6 - estimated relative risk of death: 6.38 and 95% confidence interval: 3.07-12.2; FCI: 2.1±1.2) (Figure 1); with complaints, mainly, of symptoms related to the respiratory (33%) and neurological (19.3%) systems, undergoing medical assistance from an average of 2.5 (±1.2) prescribing professionals, and for an average period of 3 (±2.8) years under clinical assistance in this service.

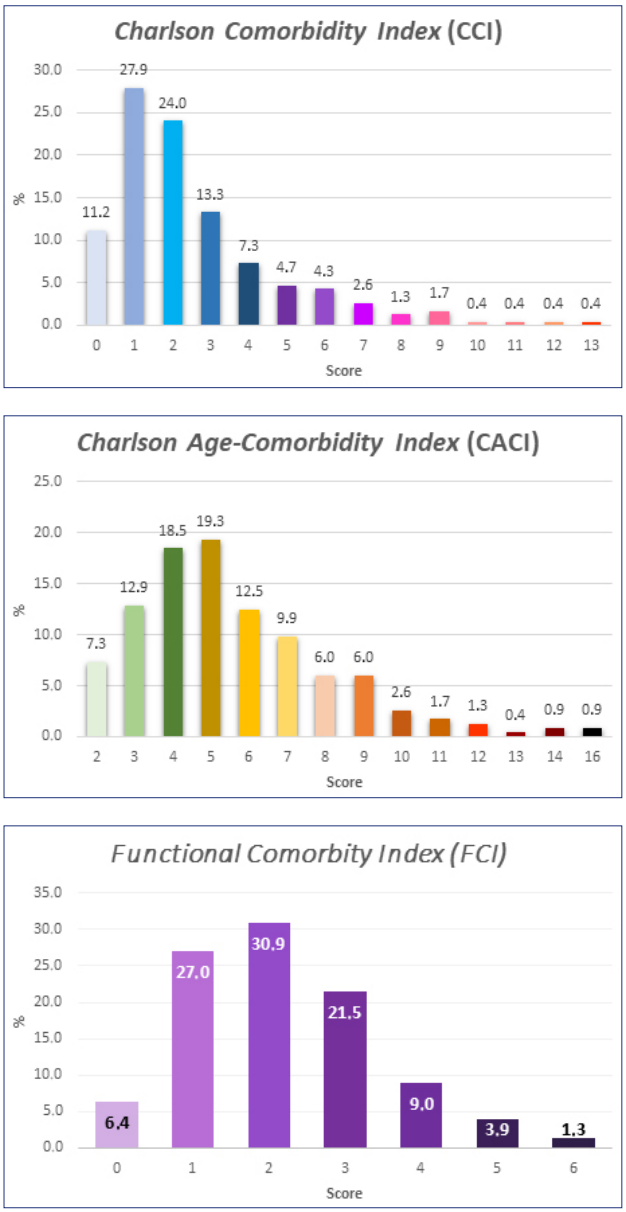
From the systematic review of the medical records, we obtained a total of 1,548 medications prescribed during medical assistance, with the presence of polypharmacy in 72.5% of the sample and an average of 6.6 (± 3.1) drugs per elderly individual. In addition, 39.4% made use of psychotropic substances continuously and concomitant to other medicines.

Based on the ATC classification, we noticed a prevalence of medicines prescribed for the cardiovascular system, followed by the digestive system and metabolism, blood and hematopoietic organs, and respiratory system (Table 1). The main medicines prescribed to this group were acetylsalicylic acid - ASS (7.7%), simvastatin (7.4%), losartan (5.6%), omeprazole (4.6%) and carvedilol (4.3%).

We identified that 74.2% of elderly respondents made use of, at least, one PIM with a potential risk of ADE-CC, reaching an average of daily consumption equal to 1.3 (±1) PIM/elderly. Of these, we must highlight that 78.5% made use of medications with an imminent risk of ADE-CC considered of lesser severity. Systemic arterial hypertension (39.5%) and the prolongation of the QT interval (21.2%) were the main and potential adverse events of lower severity (Table 2). While the potential risk of heart failure/exacerbation (21.2%) was the most prevalent among the potential adverse events of greater severity.

Regarding the analyses of the association between potential ADE-CC and the other variables, we found that the presence of comorbidities equal to or greater than three was the variable most strongly associated

FIGURE 1. USE OF THE CHARLSON COMORBIDITY INDEX (CCI), CHARLSON AGE-COMORBIDITY INDEX (CACI), AND FUNCTIONAL COMORBIDITY INDEX (FCI) TO ANALYZE THE PREVALENCE OF COMORBIDITIES IN ELDERLY INDIVIDUALS UNDERGOING OUTPATIENT CARE. CARAPICUÍBA, SP, BRASIL, 2016-2017.



with them. We further noted that with every one-point increase on the scales of morbidity and mortality, the odds ratio for the possible iatrogenic outcome analyzed was increased significantly. Like it is the case with polypharmacy, the presence of a disease of the circulatory system was also strongly associated with increased risk for PIM with a potential risk of ADE-CC.

It is necessary to highlight that the presence of neurological symptoms was a protective factor of up to 63% in comparison with the others for the outcome analyzed. Table 3 shows the main findings of the logistic regression of the group researched.

DISCUSSION

In addition to being the main cause of mortality worldwide, CADs are also an important cause of morbidity in elderly individuals. As a result of the characterization of the morbidity profile of the group studied, we observed that the prevalence of chronic

TABLE 1. ANATOMICAL CLASSIFICATION OF THE MAIN DRUGS USED BY ELDERLY INDIVIDUALS ASSISTED IN A TEACHING OUTPATIENT CLINIC, ACCORDING TO THE ANATOMICAL THERAPEUTIC CHEMICAL (ATC). CARAPICUÍBA, SP, BRASIL, 2016-2017.

Code	Anatomical Classification	n (%)
A	Digestive System and Metabolism	242 (15.6)
B	Blood and Hematopoietic Organs	189 (12.2)
C	Cardiovascular System	672 (43.4)
D	Dermatological	01 (0.06)
G	Genitourinary System and Sex Hormones	09 (0.5)
H	Hormonal Preparations for Systemic Use, Excluding Sex Hormones and Insulin	34 (2.1)
J	General Anti-Infectives for Systemic Use	06 (0.3)
L	Antineoplastic and Immunomodulating Agents	02 (0.1)
C	Musculoskeletal System	53 (3.4)
N	Central Nervous System	166 (10.7)
P	Antiparasitic Products	0 (0)
R	Respiratory System	169 (10.9)
S	Sensory Organs	03 (0.1)
V	Various or Others	02 (0.1)
Total		1548 (100)

TABLE 2. PREVALENCE OF MAJOR AND POTENTIAL CARDIOVASCULAR AND CEREBROVASCULAR ADVERSE EVENTS (ADE-CC) SECONDARY TO THE USE OF POTENTIALLY INAPPROPRIATE MEDICATIONS FOUND IN THE ELDERLY. CARAPICUÍBA, SP, BRASIL, 2016-2017.

Potential Adverse Events	n (%)
Cardiovascular and cerebrovascular adverse events (Major) - (21.46%)	
Cerebrovascular Accident	11 (03.5)
Acute Myocardial Infarction	09 (02.9)
Sudden Cardiac Death	06 (01.9)
Heart Failure (Exacerbation of Heart Failure)	65 (21.2)
Cardiovascular adverse events (Minor) - (78.53%)	
Systemic Arterial Hypertension	121 (39.5)
Heart Block	13 (04.2)
Postural and Orthostatic Hypotension	60 (19.6)
Heart Arrhythmia	50 (16.3)
Bradycardia	24 (07.8)
Prolonged QT interval	65 (21.2)

TABLE 3. STEPWISE FORWARD LOGISTIC REGRESSION ANALYSIS FOR SCREENING FACTORS ASSOCIATED WITH THE USE OF POTENTIALLY INAPPROPRIATE MEDICATIONS (PIM) AND THE POTENTIAL RISK OF ADVERSE CARDIOVASCULAR AND CEREBROVASCULAR EVENTS IN ELDERLY INDIVIDUALS UNDER THERAPEUTIC REGIMEN. CARAPICUÍBA, SP, BRASIL, 2016-2017.

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p	*OR* (95% CI)	p
Sex				
Male	1		1	
Female	1.32 (0.73-2.39)	0.349	1.13 (0.5-2.4)	0.757
Age				
60-69	1		1	
70-70	1.46 (0.70-3.01)	0.311	1.19 (0.46-3.11)	0.721
≥80	1.31 (0.66-2.64)	0.44	0.71 (0.29-1.75)	0.455
Comorbidities				
<3	1			
≥3	10.55 (4.5-24.7)	<0.001		
DCS:				
No	1		1	
Yes	5.67 (2.98-10.8)	<0.001	3.77 (1.65-8.58)	0.002
Morbidity and Mortality				
CCI	1.44 (1.18-1.76)	<0.001		
CACI	1.29 (1.11-1.49)	0.001		
FCI	2.38 (1.71-3.31)	<0.001		
Respiratory symptoms				
No	1			
Yes	0.73 (0.40-1.35)	0.313		
Neurological symptoms				
No	1		1	
Yes	0.82 (0.40-1.69)	0.592	0.37 (0.14-0.96)	0.041
Polypharmacy				
No	1		1	
Yes	11.0 (5.58-21.7)	<0.001	6.56 (2.84-15.1)	<0.001
Use of psychotropics				
No	1			
Yes	1.43 (0.77-2.64)	0.259		
Period under medical assistance	1.01 (0.89-1.14)	0.903		

OR: Odds Ratio (estimated by Stepwise Forward logistic regression); 95 CI%: Confidence interval of 95%; DCS: Diseases of the circulatory system; CCI: Charlson Comorbidity Index; CACI: Charlson Age-Comorbidity Index; FCI: Functional Comorbidity Index.

degenerative diseases, predominantly CAD, matches the data given in the literature[1,5,11,12]. We further noted that considering the index of comorbidities in addition to senescence, the relative risk of death (estimated by the CCI, CACI, FCI) for this group was categorized as moderate.

It is known that an increased number of professionals attending a single patient predisposes iatrogenic outcomes[13]. However, there was a relatively low average of prescribing physicians per elderly individual in this sample. In contrast, we found a high rate of polypharmacy, particularly of drugs acting on the cardiovascular system. Among these, there was a predominance of drugs for the treatment and prevention of atherothrombotic events in the elderly. In line with these findings, Brasil has an estimated prevalence between 5% and 8% of coronary disease in the adult population, which makes it the main cause of mortality. For this reason, actions for the progressive reduction of the mortality rate from cardiovascular diseases are being implemented since, in most cases, these are measures liable to interventions[11].

It is important to highlight in this group that 74.2% of the elderly individuals made use of, at least, one MPI with an imminent risk of ADE-CC. This is a particular concern for researchers, considering the clinical vulnerability of the group researched. Among the potential risks of ADE-CC of lower severity, systemic arterial hypertension was the most prevalent, whereas, among the ADE-CC of greater severity, heart failure/exacerbation was the main potential risk. These data indicate that a portion of the diseases related to the circulatory system may be influenced by medication. However, this has been little explored by researchers and neglected by professionals in clinical practice.

Brazilian studies have great variability in the prevalence of PIM consumption[14]. However, it should be considered that this wide variation in prevalence is justified in detriment of two major aspects: (i) sample size and (ii) the difference in the application of separate instruments for screening PIM, thus, hindering further analyses.

Among the factors associated with the consumption of PIM with a potential risk of ADE-CC found here, the presence of variables related to clinical conditions were the main ones associated with this outcome, such as: the presence of comorbidities, diseases of the circulatory system, score in addition to the risks of morbidity and mortality, and the use of polypharmacy. It is important to highlight that since this is a group of individuals undergoing an active process of aging, whose changes facilitate the increasing rates of chronic conditions, i.e., three or more comorbidities, particularly cardiovascular conditions, this led to the high consumption of medicines, predisposing iatrogenic outcomes, as evidenced. Studies have shown a significant and increased risk for the consumption of PIM in elderly patients with comorbidities, particularly cardiovascular diseases and in the presence of polypharmacy, in line with the findings of this study[13,15-17].

We should warn that, although these findings are unprecedented, they corroborate their consolidation, in addition to acting as indicators for the pharmacological management of risk groups. These findings corroborate the development of preventive measures targeted at the elderly in health care services, in order to avoid the inappropriate consumption of medicines, emergence of potential interactions and adverse events, increase in the number of hospital admissions, and even the prolongation of hospitalization[13.18].

It is interesting to highlight the occurrence of an unprecedented finding: the presence of neurological symptoms as a 63% protective factor to ADE-CC. The presence of symptoms such as headache, insomnia, amnesia, and mental confusion had an unexpected weigh on this analysis and needs to be further investigated in this scenario.

Limitations

This study has some limitations. The results do not allow for generalization to all elderly individuals because the study included only individuals undergoing clinical follow-up in teaching outpatient clinic of a secondary hospital located in Carapicuíba, SP, Brasil. The analysis of undesirable outcomes, such as functional deterioration, hospitalization, admission in emergency care services, institutionalization, and death was not assessed given the scope of the analysis and the nature of the study design.

It is important to highlight that, due to the limitations of the clinic's digital system, the absolute outlining of prior hospitalizations per elderly individual was impaired. However, it is estimated that nearly two-thirds of the elderly individuals undergoing follow-up in this outpatient clinic of medical specialties have already had at least one hospitalization in this service.

CONCLUSION

The vast majority of the elderly patients included in this study were part of a group of imminent risk for ADE-CC. It should be noted that, in addition to the considerable risk of morbidity and mortality in this group, in most cases, the therapeutic care provided focused on the treatment and prevention of atherothrombotic events.

The screening of risk factors showed that having three or more comorbidities, diseases of the circulatory system, making use of polypharmacy, and having a moderate *score* in indexes of morbidity and mortality were important factors associated with an increased odds ratio for the use of PIM with a risk of ADE-CC. It is also worth mentioning that the presence of neurological symptoms was a protective factor for this outcome.

RESUMO

OBJETIVO: *Identificar o uso de Medicamentos Potencialmente Inapropriados (MPI) com risco iminente de EAM cardiovascular e cerebrovascular (EAM-CC), além dos fatores associados a um grupo de idosos em vigência terapêutica de um serviço público brasileiro.*

MÉTODOS: *Estudo seccional e retrospectivo realizado em um serviço de nível secundário localizado em Carapicuíba, SP, Brasil. Incluíram-se, apenas, idosos (idade ≥60 anos) que foram tratados em um departamento de atendimento ambulatorial. O uso de MPI com risco iminente de EAM-CC foi definido empregando a lista PIM-CCVAEs. Nesta pesquisa, apropriou-se da estatística descritiva e de regressão logística para identificação e rastreio de possíveis preditores de uso de MPI. Todas as análises estatísticas foram realizadas usando o software Stata version 15.1 (Stata Corporation).*

RESULTADOS: *Incluíram-se, na casuística, 233 idosos, com média de idade igual a 74,9 (±9,4) anos. Destes, 74,2% faziam uso de pelo menos um MPI com risco de EAM-CC, atingindo uma média de consumo diário igual a 1,3 (± 1) MPI/idoso. Verificou-se que a presença de comorbidades, doenças do aparelho circulatório, polifarmácia e score baixo a moderado em índices de morbimortalidade foram importantes fatores associados ao aumento da razão de chances para o consumo de MPI com risco de EAM-CC. Ressalta-se ainda que a presença de sintomas neurológicos mostrou-se como fator protetor para este desfecho.*

CONCLUSÃO: *Dada a gravidade clínica e o risco iminente de EAM-CC do grupo pesquisado, medidas de prevenção devem ser instituídas com o intuito de minimizar os problemas de saúde relacionados à medicação na rede pública.*

PALAVRAS-CHAVE: *Idoso. Resultado do tratamento. Doença iatrogênica. Lista de medicamentos potencialmente inapropriados. Efeitos colaterais e reações adversas relacionados a medicamentos. Sistema cardiovascular.*

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ORIGINAL ARTICLE

Lipid profile of pediatric patients with chronic rheumatic diseases - a retrospective analysis

Wellington Douglas Rocha Rodrigues[1]
Roseli Oselka Saccardo Sarn[2]
Thais Tobaruela Ortiz Abad[1]
Simone Guerra Lopes da Silva[1]
Fabiola Isabel Suano de Souza[3]
Claudio Arnaldo Len[4]
Maria Teresa Terreri[4]

1. Nutricionista, Doutor, Departamento de Pediatria, Universidade Federal de São Paulo, São Paulo, SP, Brasil.
2. Médica assistente e pesquisadora associada, Universidade Federal de São Paulo, São Paulo, SP, Brasil.
3. Médica, Professora assistente, Departamento de Pediatria, Universidade Federal de São Paulo, São Paulo, SP, Brasil.
4. Médico, Professor associado, Departamento de Pediatria, Universidade Federal de São Paulo, São Paulo, SP, Brasil.

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SUMMARY

AIM: *To describe the prevalence of dyslipidemia in children and adolescents with autoimmune rheumatic diseases (ARDs), particularly juvenile idiopathic arthritis (JIA), juvenile systemic lupus erythematosus (jSLE), and juvenile dermatomyositis (JDM).*

METHODS: *Retrospective cross-sectional study conducted in the pediatric rheumatology outpatient clinic. We evaluated 186 children and adolescents between the ages of 5 and 19 years. The medical records were reviewed for the following data: demographic and clinical features, disease activity, and lipid profile (triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and very low density lipoprotein (VLDL-C)). In addition, non-HDL cholesterol was calculated as TC minus HDL-C. The cut-off points proposed by the American Academy of Pediatrics were used to classify the lipid profile.*

RESULTS: *Dyslipidemia was observed in 128 patients (68.8%), the most common being decreased HDL-C (74 patients, 39.8%). In the JIA group there was an association between the systemic subtype and altered LDL-C and NHDL-C, which demonstrated a more atherogenic profile in this subtype (p=0.027 and p=0.017, respectively). Among patients with jSLE, the cumulative corticosteroid dose was associated with an increase in LDL-C (p=0.013) and with a decrease in HDL-C (p=0.022).*

CONCLUSION: *Dyslipidemia is common in children and adolescents with ARDs, especially JIA, jSLE, and JDM, and the main alteration in the lipid profile of these patients was decreased HDL-C.*

KEYWORDS: *Juvenile idiopathic arthritis; Juvenile systemic lupus erythematosus; Juvenile dermatomyositis; Dyslipidemia*

INTRODUCTION

Cardiovascular diseases (CVDs) are highly prevalent and a serious public health problem in the general population.[1]

Evidence points to an association between autoimmune rheumatic diseases (ARDs) and increased risk of CVD.[1,2] The prevalence of acute myocardial infarction

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CORRESPONDING AUTHOR: Maria Teresa Terreri
Rua Borges Lagoa, 802, Vila Clementino, SP, Brasil – 04038-001
Tel: +55 11 5574-7627
E-mail: teterreri@terra.com.br

is approximately 50 times higher in women with systemic lupus erythematosus (SLE) than in those without the disease.[2] In relation to the pediatric population, a study evaluating the impact of juvenile SLE (jSLE) on cardiovascular risk demonstrated not only an incidence of infarction similar to that seen in adult onset SLE patients but also that the first event occurred much earlier (at 32 years of age on average).[3]

A recent study showed that adults with juvenile idiopathic arthritis (JIA) were five times more likely to have metabolic syndrome and consequent cardiovascular risk than healthy individuals.[4] A study evaluating adults with juvenile dermatomyositis (JDM) for 29 years showed changes in biomarkers and imaging exams associated with risk of CVD.[5]

Thus, with the increase in survival rates and the advancement in the treatment of ARDs, a new challenge arises: the identification of and early approach to risk factors for CVD.

In addition to the classic risk factors for the development of CVD, there are other triggers for ARD, such as chronic inflammatory processes and adverse events resulting from the therapy.

Our group has previously reported a high prevalence of dyslipidemia in children and adolescents with JIA and jSLE.[6-9] It is well established in the literature that dyslipidemia is a relevant factor for triggering the atherosclerotic process.[1] To our knowledge, this is the first study conducted in the pediatric population that describes the prevalence of dyslipidemia in individuals with JDM.

Therefore, the aim of this study was to describe the prevalence of dyslipidemia in children and adolescents with ARD, in particular JIA, jSLE and JDM.

METHODS

This was a retrospective cross-sectional study conducted in the pediatric rheumatology outpatient clinic. It involved 186 children and adolescents between five and 19 years of age with a diagnoses of JIA, jSLE, and JDM, according to the criteria of the International League of Association for Rheumatology (ILAR), the American College of Rheumatology (ACR), and the criteria of Bohan and Peter, respectively.[10-12]

Patients with any clinical manifestations and degree of disease activity, and having the disease for at least six months were included. Exclusion criteria were as follows: patients taking statins, pregnant women, patients with endocrine disorders, such as hypothyroidism, and with other autoimmune diseases or overlap syndrome. The medical records were reviewed for the following data: demographic and clinical features, disease activity, and lipid profile (triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and very low density lipoprotein (VLDL-C)). Non-HDL cholesterol (NHDL-C) was also calculated as TC minus HDL-C. The cut-off points proposed by the American Academy of Pediatrics were used to classify the lipid profile.[13]

Wallace criteria were used to evaluate the activity of JIA.[14] The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)[15] and the Systemic Lupus International Collaborating/ACR Damage Index (SLICC-ACR/DI)[16] were used to evaluate the activity of jSLE and irreversible cumulative damage. The activity of JDM was assessed considering the presence of skin alterations, muscle weakness, and increased levels of muscle enzymes.

SPSS software, version 20.0, was used to perform the statistical analysis. The categorical variables were expressed as frequencies and percentages and the continuous variables were presented as medians and interquartile intervals. The upper quartile (UQ) was used in the statistical analysis and was different for each disease: JIA: age ≥ 14.8 years, duration of progression ≥ 7.3 years, body mass index (BMI) ≥ 23.0, BMI-for-age Z-score (ZBMI) ≥ 1.6, height-for-age Z-score (ZHAZ) ≥ 0.47, erythrocyte sedimentation rate (ESR) ≥ 23.3, C-reactive protein (CRP ≥ 5.9); jSLE: age ≥ 18.6 years, duration of progression ≥ 6.6 years, BMI ≥ 24.3, ZBMI ≥ 1.08, ZHAZ ≥ -0.41, ESR ≥ 29, cumulative corticosteroid dose (CCD) ≥ 425.3 mg; and JDM: age ≥ 15.2 years, duration of progression ≥ 7.6 years, BMI ≥ 22.0, ZBMI ≥ 0.73, ZHAZ ≥ 0.29, and CCD ≥ 258.2 mg. Pearson's chi-square or Fisher's exact association tests were used for the qualitative variables, and logistic regression and Mann-Whitney test were used for the quantitative variables. The significance level was set at $p < 0.05$.

RESULTS

Table 1 shows the demographic, clinical, and laboratory data of children and adolescents with JIA, jSLE, and JDM, as well as their nutritional status. There was a predominance of patients with the oligoarticular subtype of JIA. According to the nutritional status classification, 63 patients (33.9%) were overweight/

TABLE 1. DEMOGRAPHIC, CLINICAL, AND LABORATORY FEATURES AND NUTRITIONAL STATUS OF CHILDREN AND ADOLESCENTS WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA), JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS (JSLE), AND JUVENILE DERMATOMYOSITIS (JDM).

	JIA (N= 96)	jSLE (N= 62)	JDM (N=28)	Total (N=186)	*p*-value
Female	65 (67.7)	52 (83.9)	16 (57.1)	133 (71.5)	-
Age (years)	11.7 (5.1-19.0)	16.6 (5.2-19.0)	13.1 (5.2-19.0)	-	<0.001
Duration of progression (years)	4.4 (0.6-16.7)	4.6 (0.9-11.0)	5.0 (0.13-13.7)	-	0.691
Disease subtype					
Systemic N (%)	11 (11.4)	-	-	-	-
Polyarticular	38 (39.6)	-	-	-	-
Oligoarticular	47 (49)	-	-	-	-
BMI	19.3 (13.4-39.9)	21.3 (15.1-36.6)	18.2 (14.3-30.4)	-	0.136
≥ Upper quartile	24 (25.7)	16 (25.8)	7 (25)	47 (25.3)	-
ZBMI	0.57 (-3.4-+4.35)	0.28 (-2.48-+3.07)	0.3 (-1.79-+2.46)	-	0.365
ZHAZ	-0.23 (-3.56-+2.73)	-1.08 (-4.59-+1.05)	-0.91 (-2.39-+2.07)	-	0.001
Nutritional status					
Eutrophic	59 (61.5)	42 (67.7)	22 (78.6)	123 (67.2)	-
Overweight	22 (22.9)	11 (17.8)	4 (14.3)	37 (19.9)	-
Obese	15 (15.6)	9 (14.5)	2 (7.1)	26 (14)	-
Status of disease activity					
Active	32 (33.3)	-	8 (28.6)	40 (32.3)	-
SLEDAI ≥4	-	12 (19.4)	-	-	-
SLICC	-	5 (8.1)	-	-	-
us-CRP	1.8 (0.04-138.5)	2.67 (0.06-47.7)	-	-	-
≥ Upper quartile	22 (22.9)	-	-	-	-
ESR	12 (2-93)	14.7 (1-75)	-	-	1
≥ Upper quartile	22 (22.9)	16 (25.8)	-	38 (23.9)	-
Cumulative corticosteroid dose	-	267.8 (75-1212.8)	138.9 (32.3-572.6)	-	1
Glucocorticoids					
Yes	-	33 (53.2)	9 (32.1)	42 (46.7)	-
Biologics					
Yes	22 (22.9)	-	-	-	-
Non-biologics					
Yes	63 (65.6)	62 (100)	23 (82.1)	148 (79.6)	-

BMI: Body mass index; ZBMI: BMI-for-age Z-score; ZHAZ: height-for-age Z-score; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC: Systemic Lupus International Collaborating/ACR Damage Index; us-CRP: ultrasensitive C-reactive protein; ERS: erythrocyte sedimentation rate

TABLE 2. LIPID PROFILE OF CHILDREN AND ADOLESCENTS WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA), JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS (JSLE), AND JUVENILE DERMATOMYOSITIS (JDM).

	JIA (N= 96)	jSLE (N=62)	JDM (N= 28)	Total (N=186)	*p*-value
TC	152.5 (101-313)	153.5 (105-259)	150 (90-277)	-	1
Borderline/High N (%)	27 (28.1)	20 (32.3)	6 (21.4)	53 (28.5)	-
LDL-C	90 (46-218)	85 (44-201)	85 (43.2-165)	-	0.285
Borderline/High N (%)	20 (20.8)	10 (16.1)	7 (25)	37 (19.9)	-
HDL-C	48.5 (17-82)	51 (27-93)	45 (25-90)	-	0.317
Borderline/Low N (%)	39 (40.6)	20 (32.3)	15 (56.3)	74 (39.8)	-
VLDL-C	15 (4.8-82)	17 (5-54)	19.5 (7-56)	-	0.223
NHDL-C	104 (61-243)	102 (57-220)	110 (51-199)	-	0.316
Borderline/High N (%)	28 (29.2)	12 (19.4)	11 (39.3)	51 (27.9)	-
TG	74 (31-271)	86 (45-270)	97 (35-279)	-	0.74
Borderline/High N (%)	33 (34.4)	24 (38.7)	14 (50)	71 (38.2)	-
Dyslipidemia N	65 (67.7)	42 (67.7)	21 (75)	128 (68.8)	-

TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; VLDL-C: Very-low-density lipoprotein cholesterol; NHDL-C: Non-high-density lipoprotein cholesterol; TG: Triglycerides

TABLE 3. DEMOGRAPHIC, CLINICAL, AND LABORATORY FEATURES AND LIPID PROFILE OF CHILDREN AND ADOLESCENTS WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA), JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS (JSLE), AND JUVENILE DERMATOMYOSITIS (JDM).

	JIA										jSLE										JDM								
	TC	P	LDL	P	HDL	P	NHDL	P	TG	P	CT	P	LDL	P	HDL	P	NHDL	P	TG	P	CT	P	LDL	P	HDL	P	NHDL	P	TG	P
Women	19	0.727	13	0.767	24	0.304	19	0.83	24	0.446	18	0.365	9	0.531	17	0.836	11	0.38	21	0.529	5	0.196	4	1	6	0.047	7	1	8	1
Age ≥ UQ	9	1	4	0.186	8	0.003	10	0.346	11	0.74	0	0.053	0	0.176	3	0.702	0	0.13	3	1	3	0.634	2	0.678	5	0.706	4	1	7	0.218
Active disease	11	0.347	7	1	16	0.265	13	0.150	12	0.644	-	-	-	-	-	-	-	-	-	-	3	0.311	2	1	5	0.408	3	1	5	0.180
SLEDAI > 4	-	-	-	-	-	-	-	-	-	-	3	0.735	1	1	5	0.261	1	0.665	7	0.176	-	-	-	-	-	-	-	-	-	-
Duration of progression ≥ UQ	11	1	10	0.611	16	0.832	13	0.817	16	0.663	4	0.595	1	0.367	5	0.666	2	0.718	6	0.784	1	1	2	1	4	1	2	0.662	3	0.056
JIA subtype											-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligoarticular	14	0.06	11	0.027	17	0.379	16	0.017	15	0.052	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyarticular	7		4		18		6		11		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Systemic	7		5		4		6		7		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ESR ≥ UQ	5	0.592	4	0.771	15	0.006	8	0.791	6	0.603	6	0.550	8	0.209	3	0.688	4	0.460	7	0.765	-	-	-	-	-	-	-	-	-	-
CRP ≥ UQ	5	0.434	3	0.378	12	0.204	6	0.602	7	1	7	0.211	2	1,00	5	0.503	3	0.693	8	0.232	-	-	-	-	-	-	-	-	-	-
ZBMI ≥ UQ	16	0.009	14	<0.001	17	0.259	18	<0.001	18	0.013	9	0.157	4	0.438	4	0.359	5	0.279	10	0.146	2	0.423	2	0.633	3	1	8	0.662	3	1
ZHAZ ≥ UQ	2	1	2	0.641	3	1	2	1	3	0.696	2	0.192	1	0.420	6	0.513	1	0.254	6	0.784	1	0.389	1	0.459	0	1	1	1	1	1
CCD ≥ UQ	-	-	-	-	-	-	-	-	-	-	7	0.211	6	0.013	9	0.022	6	0.057	7	0.551	2	0.622	3	0.328	3	0.662	4	0.391	4	0.661
Use of a biologic	7	0.788	5	0.768	9	1	6	1	7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Use of a non-biologic	17	0.812	5	0.116	27	0.515	17	0.474	21	0.822	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

UQ: Upper quartile; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index 2000; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ZBMI: BMI-for-age Z-score; ZHAZ: height-for-age Z-score; CCD - Cumulative corticosteroid dose; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; NHDL-C: Non-high-density lipoprotein cholesterol; TG: Triglycerides

CONTINUE>>

obese. We observed that ZHAZ was lower among the patients with jSLE than among the other two groups of patients.

Table 2 shows the lipid profile of children and adolescents with JIA, jSLE, and JDM. Dyslipidemia was present in 128 patients (68.8%), the most common being decreased HDL-C (74 patients, 39.8%). TG elevation was the most prevalent dyslipidemia in patients with jSLE and decreased HDL-C in patients with JIA and JDM. We did not detect significant differences in lipid profile between the three diseases.

Table 3 shows the associations of demographic, clinical, and laboratory data with the lipid profile of children and adolescents with JIA, jSLE, and JDM. In the JIA group there was an association between the systemic subtype and LDL-C and NHDL-C abnormalities, which demonstrated a more atherogenic profile in this subtype (p=0.027 and 0.017, respectively). Logistic regression analysis showed that altered NHDL-C was associated with the JIA systemic subtype (p=0.002) (data not shown). High levels of ESR were associated with decreased HDL-C (p=0.006). With regard to the nutritional status, patients with JIA who had ZBMI ≥ 1.6 also had higher levels of TC (p=0.009), LDL-c (p<0.001), NHDL-c (p<0.001), and TG (p=0.013). Among the patients with jSLE, the CCD was associated with elevated LDL-C (p=0.013) and decreased HDL-C (p=0.022).

DISCUSSION

This study showed a high frequency of dyslipidemia in children and adolescents with JIA, jSLE, and JDM. The most prevalent dyslipidemia was decreased HDL-C.

Studies evaluating lipid metabolism-related biochemical markers in children and adolescents with ARD are of great relevance[6-9, 17] and have focused on patients with JIA and jSLE. To our knowledge, there are no studies assessing the frequency of dyslipidemia in the population with JDM, and this work is therefore the first on this topic.

The pathophysiological mechanisms involved in the origin of dyslipidemia and risk of CVD in ARD have not yet been fully elucidated. However, some studies[18-20] have shown that these diseases are associated with chronic inflammatory processes with elevated proinflammatory cytokines and also with adverse events related to drug therapy. In addition, classical risk factors for CVD such as unhealthy eating habits and poor lifestyle choices are involved.[6, 21, 22]

It has been previously reported that the prevalence of dyslipidemia in patients with ARD ranges from 46% to 85%.[23, 24] In our sample, the prevalence of dyslipidemia was 67.7% for JIA and jSLE and 75% for JDM.

Low HDL-C was the most frequently observed change among the cholesterol-carrying lipoproteins. Other studies that also evaluated the prevalence of dyslipidemia in childhood ARD have confirmed our findings.[8, 22]

Little is known about the causes of low HDL in ARD. However, it is worth noting that, in the presence of systemic inflammation, protective HDL can be converted into dysfunctional or pro-inflammatory HDL. This means that there is a modification in the protein content of the HDL molecules—a modification that favors the loss of its anti-inflammatory, anti-atherogenic, and anti-thrombotic effects, in addition to the loss of its function in the reverse transport of cholesterol.[25,26]

The most atherogenic lipid profile was observed in patients with the systemic subtype of JIA, with high levels of LDL-C and NHDL-C. Moreover, these patients had lower levels of HDL-C in association with increased ESR. It is known that the systemic subtype is associated with higher intensity of inflammation and manifestations such as anemia and thrombocytosis.

Our findings showed that JIA patients with a higher ZBMI had higher concentrations of TC, LDL-C, NHDL-C, and TG. In a study aiming to determine the prevalence of excess body mass and to investigate the influence of obesity on early subclinical changes in the cardiovascular system, Glowinska-Olszewska *et al.*[27] showed that patients with JIA and obesity had higher values of TC and TG than both non-obese JIA patients and healthy controls. Thus, these findings corroborate our results regarding these changes.

In relation to jSLE, we observed an association between CCD and high levels of LDL-C and low levels of HDL-C. Our findings are in line with those reported by other authors.[8, 28]

With regard to JDM, our results were similar to those obtained in patients with JIA and jSLE. There was a tendency for an association between the duration of disease progression and high concentrations of TG.

This study is the first to describe the prevalence of dyslipidemia in children and adolescents with JDM. However, it had some limitations, including its retrospective design and the absence of a control group.

CONCLUSION

We concluded that dyslipidemia is frequent in children and adolescents with ARD, especially JIA, jSLE, and JDM. The main change in the lipid profile of these patients was decreased HDL-C. Therefore, regular monitoring of lipid metabolism- related biomarkers is of paramount importance in the planning of interventions to reduce the risk of CVD in this population.

Declaration of conflict of interest

Nothing to declare

RESUMO

OBJETIVO: *Descrever a prevalência de dislipidemias em crianças e adolescentes com doenças reumáticas autoimunes (Drai), em particular artrite idiopática juvenil (AIJ), lúpus eritematoso sistêmico juvenil (Lesj) e dermatomiosite juvenil (DMJ).*

MÉTODOS: *Estudo transversal retrospectivo realizado no ambulatório de reumatologia pediátrica. Foram avaliados 186 crianças e adolescentes com idades entre 5 e 19 anos. Foram coletados dos prontuários dados demográficos, clínicos, atividade de doença e perfil lipídico (triglicérides (TG), colesterol total (CT) e frações LDL-c (low density lipoprotein); HDL-c (high density lipoprotein) e VLDL-c (very low density lipoprotein). Foi também calculada a fração não HDL do colesterol (CT-NHDL -c). Para classificação do perfil lipídico, foram adotados os pontos de corte propostos pela American Academy of Pediatrics.*

RESULTADOS: *A dislipidemia foi observada em 128 pacientes (68,8%), sendo a mais comum a diminuição do HDL-c em 74 (39,8%). No grupo AIJ houve uma associação entre o subtipo sistêmico com alteração de LDL-c e NHDL-c, mostrando um perfil mais aterogênico neste subtipo (p=0,027 e 0,017, respectivamente). Em relação aos pacientes com Lesj, podemos observar que a dose cumulativa de CTC teve associação com o aumento do LDL-c (p=0,013) e com a diminuição do HDL-c (p=0,022).*

CONCLUSÃO: *A dislipidemia é frequente em crianças e adolescentes com Drai, em especial, AIJ, Lesj e DMJ, e a principal alteração no perfil lipídico desses pacientes foi a diminuição do HDL-c.*

PALAVRAS-CHAVE: *Artrite idiopática juvenil. Lúpus eritematoso sistêmico juvenil. Dermatomiosite juvenil. Dislipidemia.*

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ORIGINAL ARTICLE

The effect of hepcidin on components of metabolic syndrome in chronic kidney disease: a cross-sectional study

Sibel Gökçay Bek[1]
Berna Üstüner[2]
Necmi Eren[1]
Zeynep Sentürk[2]
Betül Kalender Gönüllü[1]

1. Assistant Professor, Nephrology Department, Kocaeli University Hospital, Internal Medicine, İzmit/Kocaeli, Turkey.
2. Resident in Internal Medicine, Kocaeli University Hospital, Internal Medicine, İzmit/Kocaeli, Turkey.

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SUMMARY

BACKGROUND: *Hepcidin is an important regulator of iron homeostasis.*

OBJECTIVES: *This cross-sectional study was conducted to evaluate the association between hepcidin and components of metabolic syndrome in patients with chronic kidney disease (CKD).*

DESIGN AND SETTING: *103 CKD patients and 59 healthy volunteers were included in the study from the University Hospital.*

METHODS: *Serum hepcidin levels were measured by enyzme-linked immunosorbent assay (ELISA) test. As for the study parameters, age, sex, body mass index, renal diseases, serum biochemistry, complete blood count, iron and total iron-binding capacity, ferritin, high-sensitive C-reactive protein (hsCRP), C- reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were evaluated.*

RESULTS: *The mean age of the patients was 58.63 ± 11.8 years. Hepcidin level was significantly associated with hypertension and higher uric acid levels ($P < 0.05$). There was a positive correlation between hepcidin and urea, uric acid, creatinine, ferritin, CRP, ESR, phosphorus, triglyceride, low-density lipoprotein (LDL), proteinuria and albuminuria in 24-hour urine collection. A negative correlation was found between hepcidin and estimated glomerular filtration rate (eGFR), hemoglobin, hematocrit, calcium, 25 OH vitamin D, pH, and bicarbonate levels.*

CONCLUSION: *Hepcidin, a well-known hormone regulator of iron metabolism, may play an important role in the pathogenesis of metabolic syndrome in patients with CKD, and further studies might delineate in-depth its potential as a promising early marker in these patients.*

KEYWORDS: *Hepcidins. Renal ınsufficiency, chronic. Metabolic syndrome.*

INTRODUCTION

The metabolic syndrome (MetS) is a condition highly prevalent worldwide, involving nearly one-fourth of the adult population and chronic kidney disease (CKD) patients in all stages.[1] MetS is a combination of hyperglycemia, dyslipidemia, hypertension, and obesity,[2] leading to increased risk for

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CORRESPONDING AUTHOR: Sibel Gökçay Bek
İstanbul Yolu Umuttepe Kocaeli University Hospital, Kocaeli, Turkey – 41380
Tel: +90 262 303-7041
E-mail: beksibel@gmail.com

cardiovascular diseases, nonalcoholic fatty liver disease (NAFLD), and diabetes.

Excess body iron stores are frequently detected in subjects with insulin resistance and related metabolic alterations.[3] The complex pathophysiological links between iron and metabolic disorders remain poorly clarified.[3]

Iron stores, expressed as serum ferritin concentration, are a component of insulin-resistance syndrome. Circulating ferritin has been shown to be associated with centrally distributed body fat and other measures of obesity.[4,5] In the healthy population, ferritin levels were positively correlated with the serum glucose level.[4] Ferritin levels were also correlated with diastolic arterial blood pressure after adjustment for BMI.[4] Moirand et al.[6] first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia. Similarly high hepcidin levels were linked to metabolic syndrome.[7]

Over the past ten years, hepcidin has emerged as the key iron-regulatory hormone.[8] This defensin-like 25 amino acid peptide is mainly produced by the liver in response to increased plasma or tissue iron to homeostatically downregulate the absorption and recycling of the metal.[9]

Animal studies have shown that hepcidin is directly induced by insulin[10] and down-regulated in high-fat/high-energy diet-induced insulin resistance.[11] Moreover, hepcidin levels have been found to be increased in obesity because of autonomous production in adipose tissue,[12] independently of insulin resistance, whereas a high-fat diet reduced hepcidin levels because of impaired iron absorption.[13] In severe obesity, serum hepcidin concentrations were found to be elevated without true iron deficiency[12].

Hepcidin levels that were inadequately low in relation to body iron status have recently been reported in subjects with type 2 diabetes[14] and suggest relative hepcidin deficiency as a potential cause of diabetes-related iron overload similar to that observed in genetic hemochromatosis.[15,16] Hepcidin may represent signals from obese adipose tissue that dysregulate not only iron homeostasis but also components of metabolic syndrome.

OBJECTIVES

This study aimed to investigate the relationship between hepcidin and the main parameters of MetS in CKD.

METHODS

Patient characteristics

This is a cross-sectional and observational study that enrolled 103 CKD patients and 59 healthy volunteers. Informed consent of the participants and approval by the local ethics committee were obtained before the study (KAEK/KOU 2012/29). The exclusion criteria were: presence of an acute infection, history of malignancy, pregnancy, age under 18 years or over 80 years, parenteral iron replacement or blood transfusion within six months, history of bleeding within four weeks, history of bleeding disorders, treatment of erythropoietin stimulating agents within the past two months, history of liver disease or Hepatitis B or Hepatitis C infection, corticosteroid treatment, history of hemochromatosis or thalassemia, history of hyperparathyroidism, or renal replacement therapy within the last two months.

Study design

The patients' demographic characteristics, anthropometric measurements, body mass indexes (BMI), history of smoking and medical treatment, modality of renal replacement treatment, and etiology of renal failure were recorded. Blood samples were obtained after at least eight hours of fasting and before the peritoneal or hemodialysis session. Three milliliters of samples for hepcidin measurement were centrifugated at 3000 rpm for ten minutes and frozen at -20 °C until they were analyzed.

For all patients, the tests of complete blood count, plasma albumin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP), high sensitive CRP, alkaline phosphatase (ALP), sodium (Na), potassium (K), calcium (Ca), phosphate (P), parathyroid hormone (PTH) levels, 25-OH vitamin D, ferritin, iron, serum iron-binding capacity (SIBC), vitamin B12, folic acid, uric acid, thyroid hormones, lipid profile, Hepatitis B surface antigen

(HbsAg), antiHbs, antiHCV, venous blood gas analysis, creatinine clearance, microalbuminuria, and daily proteinuria were obtained.

Plasma hepcidin-25 levels were measured using enzyme immunoassay (EIA) method (Competition ELISA; DRG İnternational ınc, USA). Results were expressed in pg/ml.

Statistical methods

Statistical analysis was performed using SPSS for Windows (Version 17.0, Chicago, USA). The

distribution of the dataset was evaluated prior to the analyses. The association between hepcidin level and measured variables was evaluated using Spearman's rank correlation coefficient (rho). Normally distributed categorical variables were described using counts and percentages, with the mean value (±standard deviation) used to describe continuous variables. Non-normally distributed variables were described by the median and range (minimum-maximum). Between-group differences (CKD *versus* control group) were evaluated using the Mann-Whitney U Test for non-parametric variables and Student's t-test for parametric variables. Comparisons of independent variables between groups were evaluated using the Kruskal-Wallis test for non-parametric data. A comparison of dependent variables between the two groups was performed using the Wilcoxon test. For all analyses, significance was set at $P<0.05$.

RESULTS

The study population consisted of 103 CKD patients [n = 103, 46 females, mean ± standard error of the mean (SEM) age 58.63 ± 11.8] and 59 healthy volunteers [n = 59, 29 females, mean (± SEM) age 49.0 ± 10.5]. The demographic and biochemical characteristics of the study population are given in Table 1.

As for the comorbidities, 61 (59.2 %) patients had diabetes mellitus (DM), 90 (87.4%) hypertension, 24 (23.3%) coronary heart disease (CHD), 13 (12.6%) congestive heart failure (CHF), 5 (4.9%) cerebrovascular disease (CVD), 3 (2.9%) pulmonary artery hypertension (PAH), 9 (8.7%) hypothyroidism, and 4 (3.9%) nephrolithiasis.

Out of 103 patients, 63 (61.2%) were using Angiotensin II receptor blockers (ARB), 25 (24.3%) angiotensin-converting-enzyme inhibitors (ACEinh), 26 (25.2%) Ca channel blockers, 7 (6.8%) essential amino-acids, 14 (13.6%) alfa blockers, 29 (28.2%) diuretics, 42 (40.8%) beta-blockers, 34 (33%) statins, 11 (10.7%) phosphate binders, 8 (7.8%) active vitamin D, 30 (29.1%) insulin, 21 (20.4%) oral antidiabetics, 31 (30.1%) acetylsalicylic acid, and 25 (24.3%) proton pomp inhibitors. The association between hepcidin and treatment with bicarbonate (p=0.001), essential aminoacids (p=0.016), phospate binders (p=0.002), allopurinol (p=0.001), active vitamin D (p=0.002), and oral antidiabetics (p=0.013) were significant while there was no association between hepcidin and treatment with statins, ACEinh, ARB, Ca channel blockers, alfa blocker, diuretics, beta blockers, and vitamin D.

No correlation was found between hepcidin and glucose levels. But a negative correlation between glucose and iron (P=0.004, r=-0.227) was observed. There was no significant difference between hepcidin levels based on the presence or absence of diabetes mellitus (Mann-Whitney U, P=0.072, r=-0.178, P=0.072).

TABLE 1. DEMOGRAPHIC AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY GROUP

	Patients (n = 103)	Control (n = 59)	P
Age, years	58.6 ± 11.8	49.0 ± 10.5	0.0001
Male, n (%)	57 (55.3)	30 (50.8)	
Female, n (%)	46 (44.7)	29 (49.2)	
Height, cm	163.3 ± 8.7	163.4 ± 9.5	0.947
Weight, kg	77.3 ± 13.5	72.9 ± 12.1	0.083
BMI, kg/m²	28.3 ± 5.5	27.0 ± 4.4	0.302
Glucose, mg/dL	131.7 ± 65.8	88.5 ± 13.3	0.0001
Creatinine, mg/dL	2.1 ± 2.1	0.7 ± 0.1	0.0001
Urea, mg/dL	74.8 ± 56.5	29.9 ± 6.6	0.0001
TSH, uIU/mL	1.9 ± 2.9	2.3 ± 4.5	0.246
Vitamin B12, pg/mL	328 ± 252	234 ± 134	0.005
Folic acid, ng/mL	9.2 ± 13.4	8 ± 2.8	0.405
HDL, mg/dL	42.7 ± 11.4	46.8 ± 11.6	0.721
LDL, mg/dL	118.1 ± 35.1	127.9 ± 32.4	0.307
Creatinine clearance, ml/min	52.1 ± 38.4		
Albuminuria in 24-h urine, mg/day	900.6 ± 1787.0		
Proteinuria in 24-h urine, mg/day	1454.1 ± 2632.4		
PTH, pg/mL	101.0 ± 114.7	47.5 ± 17.7	0.0001
Vitamin D 25 OH, ng/mL	18.7 ± 11.8	17.0 ± 6.9	0.671
Albumin, mg/dL	4.0 ± 0.4	4.4 ± 0.2	0.001
Calcium, mg/dL	9.1 ± 0.6	8.8 ± 0.3	0.0001
Phosphate, mg/dL	3.7 ± 1.2	3.1 ± 0.5	0.001
Magnesium, mg/dL	2.2 ± 0.2	2.3 ± 0.3	0.262
ALP, U/L	86.3 ± 28.9	84.2 ± 28.9	0.469
Uric acid, mg/dL	6.3 ± 1.9	4.6 ± 1.1	0.0001
Hepcidin, ng/ml	30.3 ± 24.7	17.8 ± 8.4	0.016
eGFR, ml/min	44.2 ± 30.6	100.8 ± 15.8	0.0001
Haemoglobin, g/dL	12.6 ± 1.8	14.2 ± 1.3	0.01
Haematocrit, %	38.3 ± 5.6	43.2 ± 3.7	0.002
MCV,fL	90.3 ± 5.4	91.4 ± 4.6	0.368
MPV, fL	7.9 ± 1	7.7 ± 1.1	0.545
Serum ferritin, ng/mL	90.6 ± 149.7	47.4 ± 44.1	0.041
Serum iron, µg/dL	61.7 ± 25.7	82.5 ± 30.8	0.0001
Serum transferrin saturation, %	21.7%± 9.0	27.6 ± 11.0	0.001

BMI= body mass index; TSH= thyroid-stimulating hormone; HDL= high-density lipoprotein; PTH= parathyroid hormone; ALP = alkaline phosphatase, eGFR = estimated glomerular filtration rate; MCV = mean corpuscle volume; MPV = mean platelet volume.

P-value determined by the Mann-Whitney U test;

However, the mean hepcidin level of non-diabetic patients (22.8 ± 26.4) was higher than those with DM (19.2 ± 23).

The mean hepcidin level (30.3 ± 24.7 ng/ml) of patients was higher than the control group (17.8 ± 8.4 ng/ml), (P<0.05). The hepcidin level was significantly associated with hypertension (P<0.05). There was a positive correlation between hepcidin and urea, uric acid, creatinine, ferritin, CRP, ESR, phosphorus, proteinuria, and albuminuria in 24-hour urine collection. A negative correlation was found between hepcidin and creatinine clearance, eGFR, hemoglobin, hematocrit, calcium, 25 OH vitamin D, Ph, and bicarbonate levels (Table 2 and Figure 1).

DISCUSSION

Hepcidin is a well-known regulator of body iron metabolism. In recent years more evidence supports its effect on glucose and lipid metabolism. In a study by Aigner et al.[17], serum iron concentrations decreased following 75g oral glucose, and hepcidin concentrations increased within the first 120 minutes of oral glucose. These results indicate that glucose negatively regulates iron metabolism and positively stimulates hepcidin release from pancreatic beta cells. However, in our study, we did not find such a correlation between hepcidin and glucose levels. However, a significant negative correlation between glucose and iron was observed, which is consistent with the previous studies. Diabetes mellitus by itself was not related to low hepcidin levels according to our study. Similarly, there was no correlation between BMI and hepcidin levels. There are many conditions like chronic inflammation associated with high hepcidin levels besides high glucose levels and dyslipidemia in CKD.

TABLE 2. CORRELATION BETWEEN HEPCIDIN LEVEL AND LABORATORY-BASED VARIABLES IN THE CHRONIC KIDNEY DISEASE (CKD) GROUP

Correlation of hepcidin	R	P
Glucose	-0.051	0.611
BUN	0.615	0.0001
Urea	0.617	0.0001
eGFR	-0.522	0.0001
Hb	-0.431	0.0001
Sedimentation	0.374	0.0001
sCRP	0.397	0.0001
CRP	0.343	0.0001
Ferritin	0.737	0.0001
Iron	-0.177	0.024
Transferrin Saturation	0.155	0.119
LDL	0.477	0.002
Uric acid	0.152	0.002
Creatinine clearance in 24-h urine	-0.549	0.0001
Albuminuria in 24-h urine	0.305	0.002
Proteinuria in 24-h urine	0.300	0.002
Serum albumin	-0.396	0.0001
Calcium	-0.428	0.0001
Phosphate	0.454	0.0001
TG	0.367	0.002

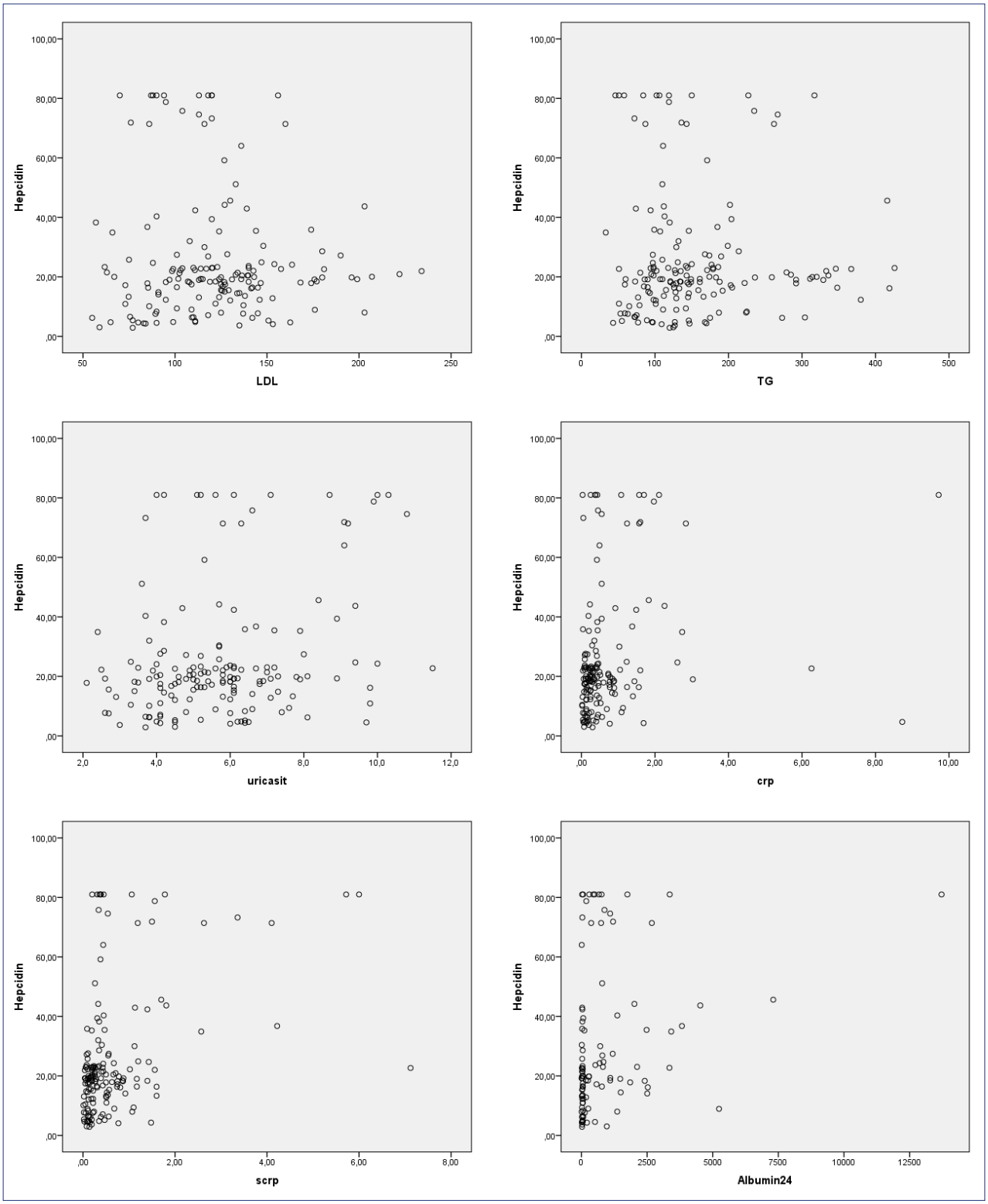
eGFR = estimated glomerular filtration rate; BUN= blood urea nitrogen; Hb = haemoglobin; sCRP = sensitive C-reactive protein; CRP= C-reactive protein; LDL = low-density lipoprotein; TG = triglyceride.

Insulin resistance, but not insulin deficiency or hyperglycemia, is associated with inadequate hepcidin levels. Reduced hepcidin concentrations may cause increased body iron stores in insulin-resistant states.[18,19] Patients with Type 2 diabetes had significantly lower serum hepcidin and hepcidin/ferritin ratio than weight-matched controls. There was a significant negative correlation between the serum hepcidin/ferritin ratio and homeostasis model assessment of insulin resistance.[14,19] Inadequate hepcidin levels for the iron load in patients with Type 2 diabetes were related to increased body iron and insulin resistance.[18] Hepcidin levels that are inadequately low in relation to body iron stores are an independent predictor for incident Type 2 diabetes and may contribute to diabetes-related tissue iron overload. In conclusion, inadequately low hepcidin is a probable independent predictor of incident Type 2 diabetes.[20,21]

An increased iron load is a risk factor for hypertension, cardiovascular diseases, and MetS.[21-23] It also destroys pancreatic beta cells and leads to insulin resistance and the development of Type 2 diabetes.[24] In a study conducted by Wang et al.[10], in streptozocin-induced diabetic rats, hepatic hepcidin levels decreased prominently. As a result, intestinal iron absorption, serum iron, and hepatic iron increased. Similarly, in diabetic patients after pancreatic cell damage, hepcidin levels decrease and iron accumulation is commonly observed. In our patients who were taking oral antidiabetic treatment, we found significantly lower levels of hepcidin compared to those not taking the drug. A low level of hepcidin in diabetic patients with decreased beta-cells is an expected finding.

Hyperuricemia is associated with so-called "cardio-metabolic diseases" including cardiovascular disease and MetS.[25] In hyperuricemic patients; hypertension, hyperinsulinemia, hypertriglyceridemia, and diabetes are reported with a higher prevalence of MetS.

FIGURE 1. CORRELATIONS OF HEPCIDIN WITH LOW-DENSITY LIPOPROTEIN (LDL), TRIGLYCERIDES (TG), C-REACTIVE PROTEIN (CRP), SERUM C-REACTIVE PROTEIN (SCRP), 24-HOUR URINARY ALBUMIN



Hyperuricemia is an indicator for early diagnosis of MetS and its clinical manifestations.[26-28] In a meta-analysis of prospective cohort studies, it is shown that hyperuricemia is a risk factor for developing Type 2 diabetes in middle-aged and older people, independently of other established metabolic risk factors.[29]

In our study, there was a statistically significant positive correlation between hepcidin and serum uric acid levels in CKD patients and healthy volunteers. We also found a statistically significant positive correlation between uric acid and sCRP, CRP, and sedimentation levels in the patient group. Hyperuricemia

is a significant risk factor for atherosclerosis, MetS, hypertension, and worsening of insulin resistance. Lobo et al.[30] investigated the relationship between uric acid and inflammation in hemodialysis patients and reported a positive correlation between the uric acid level and inflammatory markers. In hemodialysis patients, uric acid levels were found to be higher than in the control group. There are no similar studies in the literature comparing hepcidin with uric acid levels. However, it can be considered that uric acid levels might increase indirectly with inflammation. In fact, the relationship between uric acid and hepcidin is more like a vicious cycle affecting both sides, ultimately leading to adverse effects on cardiovascular health and increasing the risk of MetS in CKD.

In our study, there was a positive correlation between triglyceride (TG) and hepcidin and negative correlation between HDL and hepcidin. As an important contributor to MetS, lipid levels were found to be significantly associated with hepcidin. In the literature, there is one study evaluating this relationship in kidney transplant patients reporting a positive correlation between hepcidin and hyperlipidemia.[31] In a study evaluating the effect of hepcidin on systolic blood pressure in healthy individuals, Suárez-Ortegón et al.[32] reported a significant association between hepcidin and TG in healthy women. In our study, we found significantly higher levels of inflammatory markers in CKD patients, thus supporting the presence of ongoing chronic inflammation, commonly seen in these patients. Chronic inflammation leads to increased hepcidin and this, in turn, leads to disorders of iron homeostasis, inducing iron overload with elevated serum ferritin. The presence of hyperlipidemia with chronic inflammation leads to further renal injury and cardiovascular diseases. In a study by Li et al.[33] hepcidin levels were analyzed in hemodialysis patients with diabetes mellitus. Increased serum hepcidin levels in hemodialysis patients with diabetes mellitus, when compared to non-diabetic patients and the general population, were found to be an important contributor to atherosclerosis and cardiovascular risk factors. In a hyperlipidemic mouse model, hepcidin deficiency was found to be associated with low macrophage iron, a decreased aortic macrophage inflammatory phenotype, and protection against atherosclerosis. This shows that low hepcidin activity and related reduced macrophage iron might be a potential therapeutic strategy in the prevention of atherosclerosis[34].

Similar to our findings, the relationship between hepcidin and metabolic syndrome in CKD was shown in previous studies [7,14]. Hypertension was also found to be related to high hepcidin levels in our study. The effect of hepcidin levels on systolic blood pressure, especially in men, was also demonstrated in healthy indivuals[32]. Proteinuria was positively correlated with hepcidin independently from blood pressure readings, blood glucose levels, and eGFR in our patients. It is known that serum hepcidin-25 levels are not dependent on eGFR but hepcidin isoforms can accumulate with renal impairment[35]. Similarly, in a study evaluating the hepcidin effect on the progression of CKD, hepcidin was related to mortality, along with greater proteinuria and elevated CRP[36]. In this study, hepcidin was considered to be a predictor for the progression of CKD, besides baseline eGFR, proteinuria, low albumin, and Hb levels and the presence of CKD. These facts also strengthen the possible role of hepcidin in predicting CKD progression.

It is well documented that hepcidin is a marker in the MetS associated with hyperferritinemia[37]. Our results on CKD patients suggest that hepcidin is involved in the MetS pathophysiology and, thus, it might be used as an early marker for MetS in these patients.

In conclusion, hyperhepcidinemia might be another important prognostic feature of MetS. The exact role of hepcidin in the progression of insulin resistance, atherosclerosis, and CKD needs to be further clarified in future research.

Author's Contribution

Conceptualization: Sibel Bek; Data curation: Berna Ustuner, Necmi Eren, Zeynep Senturk, Sibel Bek; Formal analysis: Berna Ustuner, Sibel Bek; Funding acquisition: Sibel Bek; Investigation: Berna Ustuner, Necmi Eren, Sibel Bek; Methods: Berna Ustuner, Necmi Eren, Zeynep Senturk, Sibel Bek; Project administration: Sibel Bek; Resources: Berna Ustuner, Sibel Bek; Software: Berna Ustuner, Sibel Bek; Supervision: Necmi Eren, Betul Kalender, Sibel Bek; Validation: Sibel Bek; Visualization: Sibel Bek; Writing of the original draft: Berna Ustuner, Sibel Bek; Review and editting: Berna Ustuner, Necmi Eren, Betul Kalender, Sibel Bek

RESUMO

FUNDAMENTO: *A hepcidina é um importante regulador da homeostase do ferro.*

OBJETIVOS: *Este estudo transversal foi realizado para avaliar a associação entre hepcidina e componentes da síndrome metabólica em pacientes com doença renal crônica (DRC).*

PROJETO E LOCAL: *Cento e três pacientes com DRC e 59 voluntários saudáveis foram incluídos no estudo no Hospital Universitário.*

MÉTODOS: *Os níveis séricos de hepcidina foram medidos pelo teste imunoenzimático (Elisa). Quanto aos parâmetros do estudo, idade, sexo, índice de massa corporal, doenças renais, bioquímica sérica, hemograma completo, capacidade de ligação total de ferro e ferro, ferritina, proteína C reativa altamente sensível (hsCRP), proteína C reativa (PCR) e taxa de sedimentação de eritrócitos (VHS) foram avaliados.*

RESULTADOS: *A idade média dos pacientes foi de 58,63±11,8 anos. Número de pacientes em cada estágio da DRC, do estágio I ao estágio V (não em terapia renal substitutiva). O nível de hepcidina foi significativamente associado à hipertensão e níveis mais altos de ácido úrico (P <0,05). Houve correlação positiva entre hepcidina e ureia, ácido úrico, creatinina, ferritina, PCR, VHS, fósforo, triglicerídeo, lipoproteína de baixa densidade (LDL), proteinúria e albuminúria na coleta de urina de 24 horas. Foi encontrada correlação negativa entre hepcidina e taxa de filtração glomerular estimada (TFGe), hemoglobina, hematócrito, cálcio, 25 OH de vitamina D, pH e níveis de bicarbonato.*

CONCLUSÃO: *A hepcidina é um hormônio bem conhecido que regula o metabolismo do ferro, mas também pode ser um importante contribuinte para os componentes da síndrome metabólica em pacientes com DRC.*

PALAVRAS-CHAVE: *Hepcidinas. Insuficiência renal crônica. Síndrome metabólica.*

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ORIGINAL ARTICLE

Hematological detraining-related changes among elderly individuals with high blood pressure

José M. Cancela[1,2]
Miguel A. Sanchez-Lastra[1,2]
Miguel Camôes[3]
Pedro Bezerra[3]

1. HealthyFit Research Group, Department of Special Didactics, Faculty of Education and Sports Sciences, University of Vigo, Pontevedra, Spain.
2. Galicia Sur Health Research Institute (IIS Galicia Sur) Sergas-UVIGO, Spain.
3. Escola Superior de Desporto e Lazer Complexo Desportivo e de Lazer.

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SUMMARY

AIM: *The aim of the present study was to compare the effects of detraining on physical performance, blood pressure, biologic and anthropometric variables of hypertensive elderly individuals, grouped by two levels of previous physical activity.*

METHODS: *A total of 87 elderly individuals (70 to 93 years old) with systolic/diastolic blood pressure levels above 120/80 mmHg who participated during 18 non-consecutive months in 2 years in physical exercise programs offered in northern Portugal communities were included in the study. Tests were performed before and after three months of no exercise. Attendance to the exercise sessions, hematological markers, cardiorespiratory function, and anthropometric variables were assessed. The results were analyzed according to the fulfillment of the WHO recommendations on moderate physical activity (at least 150 minutes/week).*

RESULTS: *Weight, total cholesterol, and glucose were influenced by the amount of physical activity performed previously to the detraining period. After the detraining period, the total cholesterol, glucose, insulin, and weight had significant differences influenced by the amount of physical activity previously performed ($p<0.05$).*

CONCLUSIONS: *The number of minutes per week of aerobic and resistance exercise training over 18 non-consecutive months was not a significant determinant factor in the development of hypertension during the three months of detraining.*

KEYWORDS: *Aged. Quality of life. Exercise. Hypertension.*

INTRODUCTION

Physical inactivity is regarded as the greatest public health problem of the 21st century[1] because of its high prevalence in the world population and associated problems. The negative effect of a sedentary lifestyle is associated with an increased risk of morbidity and worsening of many chronic diseases such as cardiovascular disease (CVD), congestive heart failure (CHF), stroke, osteoporosis, obesity, type 2 diabetes, some types of cancer and hypertension[2]. The many consequences of this problem led the World Health Organization (WHO)[3] in 2014 to set, within the 9 global non-communicable diseases (NCDs), targets for the year 2025, of which two were related to physical activity (PA). First, a relative reduction of 25% in premature mortality from cardiovascular diseases, cancers, and diabetes by 2025, in which PA plays an important role[4]. Second, in Target 3, a 10% relative reduction in the prevalence of insufficient physical activity.

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CORRESPONDING AUTHOR: Miguel A. Sánchez-Lastra
Department of Special Didactics, Faculty of Education and Sport Sciences
University of Vigo, Campus A Xunqueira, s/n, Pontevedra, Spain – E-36005
Tel: +34 986 802054
E-mail: misanchez@uvigo.es

One of the most common health complications in which physical inactivity plays an important role is high blood pressure (HBP). A person is considered to have HBP when the systolic blood pressure (SBP) and diastolic blood pressure (DBP) are greater than 120 and 80 mmHg, respectively. A chronic or persistent increase of blood pressure (BP) can be classified as prehypertension (120-139/80-89 mm Hg) or arterial hypertension (AH) (≥140/90 mm Hg)[5]. Prehypertension is already associated with an increased risk of cardiovascular diseases (CVD)[6]. Although age, male sex, and family history are non-modifiable risk factors for CVD, there are also other relevant factors, such as unhealthy eating habits (e.g. excessive salt consumption), smoking, and sedentarism, which can be prevented. Indeed, WHO's aims for 2025 focused also on a 30% relative reduction in the mean intake of salt/sodium (Target 4) and a 25% relative reduction in the prevalence of increased blood pressure (Target 5).

Scientific literature has reported the benefits of adapted physical exercise in patients with this health condition, demonstrating that a single session of aerobic exercise at an intensity of 50-100% of VO_2max produces a lowering of 18-20 mm Hg in systolic and 7-9 mm Hg in diastolic blood pressure[7]. These changes remain for 12–16 h after exercise, and the maximum changes in blood pressure have been observed in individuals with mild (Stage I) hypertension. Therefore, the promotion of healthy habits is especially important at this stage due to the frequently observed benefits in both prehypertensive and hypertensive patients[7,8].

However, the authors understand that there is little knowledge demonstrated on how these and other AH related variables change when regular physical exercise is stopped over a period of time. Hence, the importance of knowing the extent to which the effects of physical exercise are sustained over time after suspension of exercise, and whether such detraining poses a risk to individuals.

Therefore, the aim of the present study was to compare the effects of detraining on physical performance, blood pressure, biologic and anthropometric variables of hypertensive elderly individuals, grouped by two levels of previous physical activity.

METHODS

Participants

The sample was taken from an already published study with a high number of participants[9]. A total of 87 elderly individuals aged between 70 and 93 years were recruited from several cities in the region of Minho in Portugal, where the city council offered physical exercise programs for the elderly in municipal sports centers.

The inclusion criteria were: (a) Levels of SBP/DBP >120/80 mm Hg at the end of the exercise programs; (b) voluntary participation and signing of written informed consent. All individuals with BP lower than the aforementioned and those who had declined voluntary participation were excluded from the study.

Procedure

All potential participants who met the inclusion criteria were first informed about the study characteristics and protocol. Then, they were asked to sign a written informed consent prior to participation. The study was approved by the Scientific Council's Ethics Committee of the Polytechnic Institute of Viana do Castelo and was carried out in accordance with the Declaration of Helsinki Standards (World Medical Association, 2013), following the European Community guidelines for Good Clinical Practice (111/3976/88, July 1990).

A general clinical interview and a fitness test were conducted, besides obtaining data on attendance to the exercise programs, as well as anthropometric and biochemical variables.

In order to analyze detraining effects, variables were analyzed in the last month of training: June (T0) prior to finishing the exercise program, and after physical inactivity for three months, in October (T1), during the first month of activity. During this period, participants led a normal life and did not participate in said exercise programs. The researchers who analyzed the data were blinded to the amount of physical activity carried out by the subjects.

Assessment

The Six-Minute Walking Test (6MWT) was used to assess the physical condition in terms of cardiovascular function. This test is an amendment to the original Cooper's 12-Minute Walk-Run Test[10] and evaluates the maximum distance a person is able to walk in 6 minutes. It is widely used in people with cardiovascular or pulmonary diseases and it has been proven to be a good predictor of peak oxygen consumption (VO_2max) and survival in patients with heart failure ($r=0.64$; $p<0.001$)[11].

Blood samples were obtained after a minimum of eight hours of fasting, with the subject in a

sitting position, using standard clinical laboratory methods. Serum variables were measured with COBAS 711 and 6000 analyzers (Roche Diagnostics, Mannheim, Germany) and included glycated hemoglobin (HbA1c), cholesterol (HDL, LDL and total), triacylglycerol, blood glucose, insulin, and D-Vitamin levels.

The anthropometric variables analyzed were weight (Tanita BC-418 Body Composition Analyser, Tanita Corporation, Tokyo, Japan), height (Seca 202 Measuring Rod, Seca Gmbh & Co, Hamburg, Germany), and Body Mass Index (BMI). Evaluation of SBP and DBP was done with an electronic sphygmomanometer (Omron M6, Omron Healthcare Group, Kyoto, Japan) following the recommendations from international guidelines.

Exercise program

The exercise program carried out comprised fitness exercises from Monday to Saturday, with 50 minutes/week sessions held in the mornings over 9 months of the year. The sessions were supervised by Physical Education graduates, wherein exercises had a (65%) aerobic component and included lower limb strengthening exercises (35%), performed at moderate intensity. The program was carried out over a two-year period, in 18 non-consecutive months.

Statistical analysis

For statistical analysis of the results, subjects were divided into two groups depending on whether the amount of workout in minutes/week was higher (G>150) or lower (G<150) than 150 minutes/week, which is a cut-off point based on the WHO recommendations[3]. Data analysis was divided into two parts. The first part consisted of a descriptive analysis of the study sample based on the frequency of physical exercise per week (<150 min/week; ≥150 min/week). The Kolmogorov-Smirnov test was used to verify the normality ($p>0.005$) of the variables studied. The Student's t-test was used for independent data, both at T0 and T1, in order to establish the presence or absence of differences between the two groups. Subsequently, an inferential covariance analysis (ANCOVA 2X2) was performed with the following covariates: age, sex, and education level. A p-value <0.05 was considered significant. The SPSS Statistical package 20.0 for Windows (IBM Corp., Armonk, N.Y., USA) software was used for statistical analysis.

RESULTS

A total of 87 participants (69.4% female and 30.6% male) completed the follow-up phase carried out at the end of the exercise program, during which the average age of the sample was 78.5 ± 2.6 years.

Start of the follow-up

Table 1 shows the values of the physical variables, blood pressure, and complete blood count (CBC) at the start of the follow-up. Data are categorized by minutes of physical activity per week. No significant differences in SBP and DBP were observed between the two groups. However, the G>150 presented SBP values considered as high-normal (130-139 mm Hg) while the average values in G<150 exceeded 140 mm Hg, which is considered as Stage 1 hypertension. DBP values were optimal (<80 mm Hg) in both groups. In the remaining variables, significant differences were seen only in weight, BMI, and in the distance covered in the 6MWT test, in which BMI presented lower values while more distance was covered in the 6MWT, in the G>150.

As far as cardiovascular risk factors (CRF) are concerned, despite total cholesterol in both groups being higher than 190mg/dL, the LDL, HDL, or triglyceride levels did not reach values that indicate the presence of dyslipidemia. On the other hand, the fasting plasma glucose in both groups was higher than 102 mg/dL, values considered as CRF (102-125 mg/dL)[5]. Moreover, in the G<150 group, the BMI was higher than 30 kg/m^2. A second evaluation was performed after the 3-month inactivity period (detraining). Significant differences were found between the groups: the total distance covered in 6MWT and vitamin D levels were higher, while the total cholesterol, triglycerides, and insulin were lower in the G>150 as compared to the G<150 (Figure 1).

Table 2 shows a comparative analysis of the two evaluation times (T0 and T1) for the two groups. Significant differences were found in the development of some variables for T0 and T1 in both groups. These differences were lower weight gain ($p = 0.010$) and BMI ($p = 0.002$) in the G<150 versus the G>150, as well as a decrease in total cholesterol ($p = 0.003$) in the G>150 compared to an increase in the G<150, a further reduction in HDL-cholesterol levels ($p = 0.023$) in the G>150 versus the G<150, a slight increase in glucose ($p = 0.046$) in the G>150 versus the G<150 and a greater increase in insulin ($p = 0.004$) in the G>150 versus the G<150.

TABLE 1. ANTHROPOMETRIC, PHYSICAL PERFORMANCE, BLOOD PRESSURE, AND BIOCHEMICAL VARIABLES AFTER 18 NON-CONSECUTIVE MONTHS OF EXERCISE TRAINING IN SUBJECTS EXERCISING <150 OR ≥150 MINUTES PER WEEK (N = 87).

			Amount of exercise			
	Total n=87		<150 min/week n=41		≥150 min/week n=46	
	Mean	Sd	Mean	Sd	Mean	Sd
Age (years)	78.5	2.6	78.3	2.4	78.6	2.7
Sex (n Female)	60		28		32	
Frequency (n/week)	3.3	0.9	1.6	0.5	5.1	1.3
Physical variables						
Weight (kg)	69.9	11.7	73.4	11.7	66.3	10.4*
Height (cm)	155.7	8.2	156.0	8.6	155.3	7.3
BMI (kg/m^2)	28.8	4.1	30.3	5.0	27.4	3.4*
Total distance walked in 6 minutes (m)	408.8	122.7	360.2	144.2	457.5	101.1**
Blood Pressure						
Systolic Blood Pressure (mmHg)	139.2	21.3	141.9	14.9	136.5	15.5
Diastolic Blood Pressure (mmHg)	73.9	10.0	74.8	9.1	73.0	8.3
Blood-count						
Glycosylated Hemoglobin (%)	6.1	1.0	5.8	0.7	6.3	1.3
Cholesterol total (mg/dL)	198.1	44.3	200.9	33.6	193.3	43.3
Cholesterol HDL (mg/dL)	62.8	19.2	62.9	14.2	62.6	25.6
Cholesterol LDL (mg/dL)	109.3	41.1	109.0	39.3	109.7	36.4
Triacylglycerol (mg/dL)	125.3	47.8	145.3	141.0	105.3	55.0
Glucose (mg/dL)	108.9	29.5	102.1	16.7	115.7	37.7
Insulin (µUI/mL)	7.1	3.3	6.9	3.0	7.2	3.3
D-Vitamin (OH25) (ng/mL)	15.9	7.4	17.6	7.8	14.2	7.2

*p<0.05;**p<0.001

TABLE 2. TIME X GROUP INTERACTION ANALYSIS.

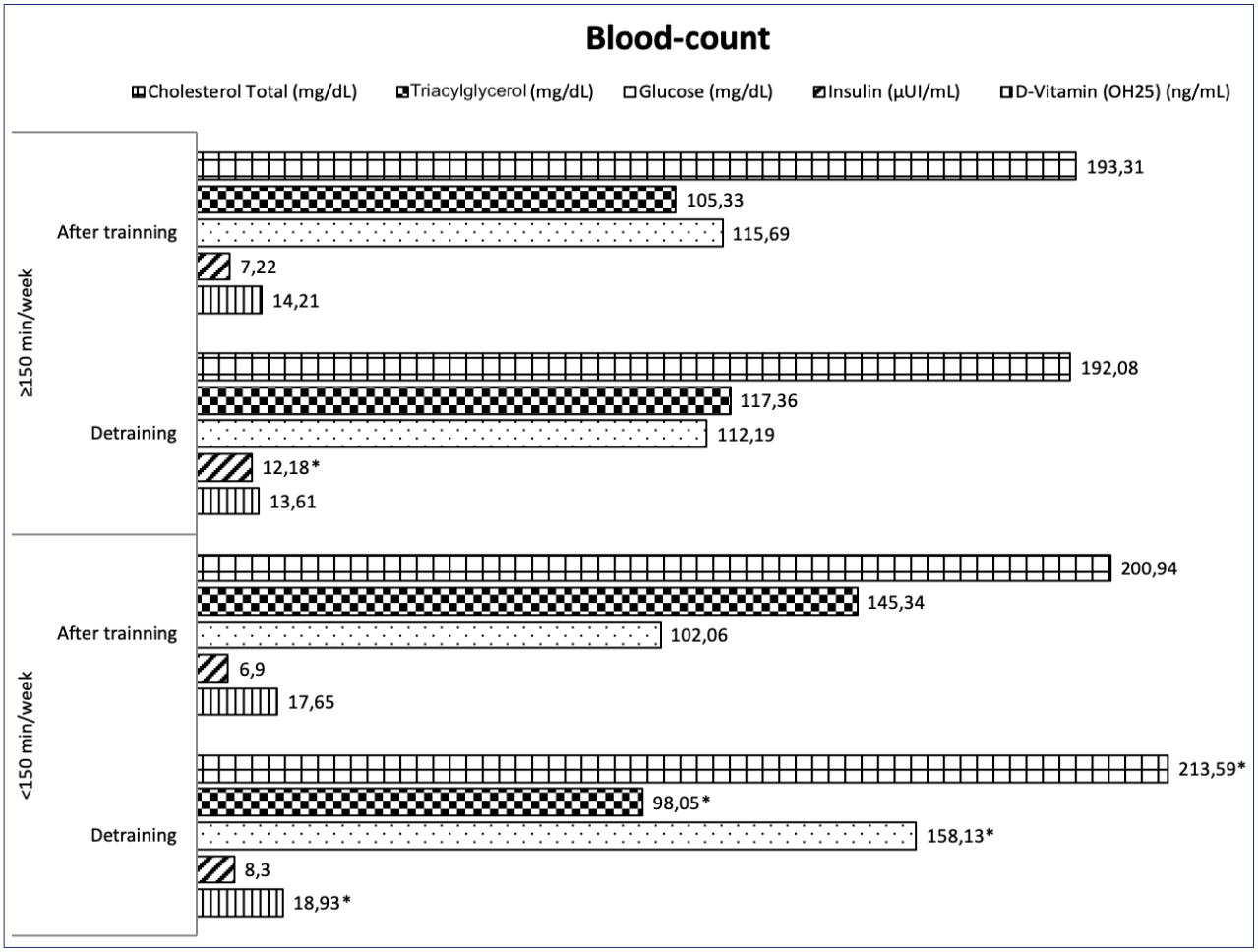
	Mean difference from Baseline (95% CI)		Factor Moment x Group p-Value ANCOVA
	<150 min/week n=41	≥150 min/week n=46	
Weight (kg)	0.6 (-1.8 to 1.6)	5.3 (3.0 to 7.6)	$F_{2,185}$=6.125; p =0.010
Height (cm)	-0.4 (-0.8 to 0.1)	-0.1 (-0.8 to 0.6)	$F_{2,185}$=3.198 p =0.161
BMI (kg(m^2)	0.1 (0.9 to -0.7)	2.2 (-0.0 to 4.5)	$F_{2,185}$=8.695; p =0.002
Total distance walked in 6 minutes (m)	-36.1 (-16.4 to 55.7)	-31.0 (-11.0 to -50.9)	$F_{2,185}$=1.222 p =0.675
Sistolic Blood Pressure (mmHg)	3.7 (-7.2 to 14.7)	6.0 (-0.5 to 12.5)	$F_{2,185}$=1.879 p =0.456
Diastolic Blood Pressure (mmHg)	2.0 (-2.2 to 6.4)	1.6 (-0.6 to 3.8)	$F_{2,185}$=2.058 p =0.321
Glycosylated Hemoglobin (%)	0.1 (-0.1 to 0.3)	-0.3 (-0.0 to -0.6)	$F_{2,185}$=3.214 p =0.111
Cholesterol total (mg/dL)	12.6 (-2.6 to 27.9)	-1.2 (-7.1 to 9.6)	$F_{2,185}$=7.621 p =0.003
Cholesterol HDL (mg/dL)	-0.3 (-0.2 to -0.8)	-4.5 (-2.4 to -6.5)	$F_{2,185}$=4.698 p =0.023
Cholesterol LDL (mg/dL)	10.4 (2.0 to 18.8)	1.1 (-1.5 to 3.7)	$F_{2,185}$=1.789 p =0.210
Triacylglycerol (mg/dL)	-11.1 (-1.6 to 23.8)	-14.1 (14.7 to -42.9)	$F_{2,185}$=2.221 p =0.581
Glucose (Jujum) (mg/dL)	12.8 (10.6 to 14.9)	6.9 (-9.3 to 23.1)	$F_{2,185}$=1.879 p =0.459
Insulin (µUI/mL)	1.4 (-0.3 to 3.1)	5.0 (-1.6 to 8.3)	$F_{2,185}$=6.657; p =0.004
D-Vitamin (OH25) (ng/mL)	1.3 (-1.7 to 4.2)	-0.6 (-1.8 to 3.0)	$F_{2,185}$=3.874; p =0.326

Repeated measures for the ANCOVA analysis and analysis of variance F tests were used. Covariates included age, sex, education level.

DISCUSSION

The aim of the present study was to compare the effects of detraining on physical performance, blood pressure, biologic and anthropometric variables of hypertensive elderly individuals, grouped by two levels of previous physical activity. They participated in a 2-year physical exercise program promoted by different city councils in the North of Portugal. The analysis

FIGURE 1. ANALYSIS OF BIOCHEMICAL VARIABLES IN THE GROUPS <150MIN/WEEK AND ≥150 MIN/WEEK (AFTER TRAINING - DETRAINING).



*p<0.05

was carried out three months after completion of the program and focused on the number of exercise minutes per week.

Numerous studies have documented the usefulness of physical exercise for reducing cardiovascular risk factors, among which is HBP[12,13]. The mechanism involved seems to be the reduction of arterial stiffness due to physical exercise by increasing serum levels of nitric oxide (determinant factor in endothelial relaxation), and also by producing adropine, whose levels increase with exercise[14].

The findings from our study are of special relevance for several reasons. Firstly, studies that have analyzed the effects of physical exercise on SBP/DBP in different population groups did not regularly report on any follow-up periods after the intervention, which may be a limitation for studying the residual effects of detraining on these variables[7]. This study intends to provide relevant information on this issue. Secondly, the follow-up periods subsequent to physical exercise programs with a duration greater than 6 or 12 months are even less analyzed. Cornelissen and Smart[7], in their systematic review and meta-analysis, reported that prolonged exercise programs of more than 6 months were associated with a lower reduction in BP values than those of shorter duration. They suggest that this may be due to longer programs having fewer supervised sessions, and hence lower attendance at sessions. Therefore, in order to analyze the residual effects of exercise on subjects that participated routinely in the programs, this study focused on the number of minutes per week, representative of the adherence to the exercise sessions. Thirdly, as indicated by Cornelissen and Smart[7], there are very few studies that have actually carried out combined physical exercise programs, and our study is one such example. Moreover, recent research indicates that the effects of combined strength and cardiovascular endurance programs may produce better results than programs that focus only on a single physical capacity[12,13,15].

Participants were initially assessed after completing the exercise programs and significant differences were only found between groups in the 6MWT and in their weight. The G<150 presented a higher weight, BMI>30 kg/m^2, considered as obesity level I, and its relationship with physical activity is unmistakable[4]. Literature also indicates that weight reduction is associated with a higher reduction in SBP and SDP values[7]. There was no significant difference between groups for SBP. In the G>150 group, average values did not reach the cut-off point established for Stage 1 hypertension (SBP/DBP ≥140 mm Hg); however, this did happen in the G<150 group. The effect that the more physical exercise the better the BP has been amply demonstrated in the literature, hence this situation is probably influenced by the previous physical activity levels[7]. Another important aspect worth mentioning is that exercise intensity has been identified as a determinant factor for reducing BP[7]. Therefore, the present study groups were engaged in exercise programs of similar intensity, duration, and type, the only difference being in the program carried out, the frequency per week, and consequently the accumulated physical exercise in minutes/week. This is why the present results are exempt from this influence. After a 3-month detraining period, no significant SBP/DBP differences were found in any of the groups or in the analysis of interaction time/group. There is nevertheless an increasing trend in it, which is more pronounced in the G>150. The authors feel that this rebound effect of BP recovery conditioned by the amount of physical exercise performed has not been reported previously[7]. One of the explanations could be related to the increase in weight and BMI after the detraining period, which was more pronounced in the G>150 compared to the G<150. Considering that BMI has been positively associated with BP[16], it is reasonable to think that the increase in BMI could have influenced the increase of BP in the G>150. Nevertheless, more research is needed in order to identify how this or other parameters, such as the previous levels of physical activity, could influence this rebound effect.

In terms of the 6MWT, at baseline, the G>150 group covered more distance in the 6MWT, something expected due to the widely documented relationship between the test and the amount of physical activity undertaken[17,18]. The distance covered by both groups in this test was lower than that reported by Araújo et al.[19] in the Portuguese high BP population with an average age of 71 years. The differences in the results of the 6MWT may be because the average age in our sample was higher than that in Araújo et al.[19] (78 years vs. 71 years), and the average BMI value in our sample was higher. Both these factors have been established as influential in the results of the 6MWT[20]. After the three months of detraining, significant differences were again observed in terms of higher total distance covered in the test, but no significant differences were identified in the analysis of interaction time/group. This indicates a trend towards greater maintenance of physical activity-related functional capacity after a detraining period. These results are in line with those reported by other studies[21], considering that health condition is a determining factor in the results of the 6MWT[22] and that the G<150 group had higher BP values.

The relationship between the cumulative level of physical activity and its influence on other biochemical variables is well documented in the literature and is present in the remaining variables, where significant differences were observed in the case of low total cholesterol (significantly higher in the G<150), as well as in triglycerides[23]. A lower increase in glucose level was observed in the G>150 group compared with the G<150, and results are in line with other studies[24]. Given that there was no nutritional intervention and assuming that eating habits did not change in the participants, the explanation for the high D-vitamin concentration is perhaps because the baseline evaluation (start of the follow-up) was carried out before the summer, while the second evaluation was performed later, hence the higher exposure to UVB rays. The differences between the groups could be due to the fact that participants who did more activity were outdoors in sunlight during the three months of follow-up, and the vitamin D concentration can also be related to the level of physical activity[25].

Even though the findings from this study may be of relevance for the reasons outlined above, there are still a number of limitations that need to be considered. In the first place, we were unable to obtain data prior to the exercise program, which would have facilitated analysis of their development and better contextualization of the differences between both groups during the follow-up period. Secondly, no information was collected on whether participants smoked or not, and the amount of tobacco consumed during the study period.

In conclusion, the number of minutes per week of aerobic and resistance exercise training over 18

non-consecutive months was not a significant determinant factor in the HBP development during the three months of detraining after a 2-year exercise program in the sample of 87 elderly HBP individuals aged 70 to 93 years. However, other variables such as weight, total cholesterol, and glucose may be influenced by the amount of physical activity undertaken and thus have a protective effect at a cardiovascular level in this population.

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Author's Contribution

José M. Cancela contributed to the design, conceptualization, data analysis, and supervised the writing. Miguel A. Sanchez-Lastra contributed to the design, conceptualization, and data collection. Miguel Camões contributed to the design, conceptualization, and data collection. Pedro Bezerra contributed to the design, conceptualization, and data collection.

This work was conducted in the Escola Superior de Desporto e Lazer Complexo Desportivo e de Lazer Comendador Rui Solheiro, Melgaço (Portugal).

RESUMO

OBJETIVO: *O objetivo do presente estudo foi comparar os efeitos da desvalorização do desempenho físico, da pressão arterial e das variáveis bioquímicas e antropométricas dos idosos hipertensivos, dependendo de duas categorias de atividade física prévia.*

MÉTODOS: *Foram incluídos no estudo 87 idosos (70 a 93 anos) com níveis de pressão arterial sistólica/diastólica superiores a 120/80 mmHg que participaram durante 18 meses não consecutivos em dois anos em programas de exercício físico. Os testes foram realizados antes e depois de três meses sem programas de exercícios. Foram avaliados a frequência das sessões de exercício, marcadores hematológicos, função cardiorrespiratória e parâmetros antropométricos. Os resultados foram analisados de acordo com o cumprimento das recomendações da OMS sobre atividade física moderada (pelo menos 150 minutos/semana).*

RESULTADOS: *O peso, o colesterol total e a glicose foram influenciados pela quantidade de atividade física realizada previamente ao período de destreinamento. Posteriormente, o colesterol total, a glicose, a insulina e o peso apresentaram diferenças significativas influenciadas pela quantidade de atividade física previamente realizada (p<0,05).*

CONCLUSÕES: *O número de minutos por semana de treinamento aeróbico e de exercícios resistidos durante 18 meses não consecutivos não foi um fator determinante significativo na evolução da hipertensão durante os três meses de destreinamento.*

PALAVRAS-CHAVE: *Idoso. Qualidade de vida. Exercício. Hipertensão.*

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ORIGINAL ARTICLE

Comparison of saliva and oro-nasopharyngeal swab sample in the molecular diagnosis of COVID-19

Ertuğrul Güçlü[1]
Mehmet Koroglu[2]
Yusuf Yürümez[3]
Hande Toptan[2]
Elif Kose[4]
Fatih Güneysu[5]
Oğuz Karabay[1]

1. Sakarya University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Sakarya, Turkey.
2. Sakarya University, Faculty of Medicine, Department of Clinic Microbiology, Sakarya, Turkey.
3. Sakarya University, Faculty of Medicine, Department of Emergency Medicine, Sakarya, Turkey.
4. Sakarya University, Faculty of Medicine, Department of Public Health, Sakarya, Turkey.
5. Sakarya University, Training and Research Hospital, Clinic of Emergency Medicine, Sakarya, Turkey.

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SUMMARY

BACKGROUND: *Healthcare personnel are at risk of becoming infected while taking upper and/or lower respiratory tract specimens. Therefore, there is a need for sampling methods that do not risk infecting them. In this study, we aimed to compare the saliva and Oro-Nasopharyngeal Swab (ONS) sampling methods.*

METHODS: *Patients were divided into three groups. Group 1 included patients whose diagnosis of COVID-19 was confirmed by polymerase chain reaction (PCR). Group 2 included patients with COVID-19 compatible findings in lung computed tomography (CT), but with a negative PCR. Group 3 included patients who presented to the emergency department with COVID-19 compatible complaints but had normal CT. Saliva and ONS samples were taken on the third day of hospitalization in groups 1 and 2, whereas in group 3, they were taken at the time of admission to the hospital.*

RESULTS: *A total of 64 patients were included in the study. The average age was 51.04 ± 17.9 years, and 37 (57.8%) were male. SARS-CoV-2 was detected in 27 (42.2%) patients' saliva samples. While the sensitivity and positive predictive value of saliva samples were 85.2%, specificity and negative predictive value were 89.2%. The value of kappa was in substantial agreement (0.744), and it was found statistically significant (<0.001).*

CONCLUSIONS: *Saliva samples can be used instead of ONS samples in detecting SARS-CoV-2. Investigating SARS-CoV-2 with saliva is cheaper, easier for the patient and overall, and, most importantly, it poses much less risk of SARS-CoV-2 contamination to healthcare personnel.*

KEYWORDS: *Coronavirus Infections/diagnosis. Saliva. Health Personnel. Betacoronavirus.*

INTRODUCTION

After the novel 2019 coronavirus disease (COVID-19) was first detected in China in December 2019, the World Health Organization (WHO) declared a pandemic on March 11th, 2020, after the identification of >118,000 cases in 114 countries[1]. As of 7 April 2020, a total of 1,331,032 cases were identified in 184 countries, and unfortunately, 73,917 patients died[2].

The novel coronavirus (SARS-CoV-2) spread mainly

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CORRESPONDING AUTHOR: Fatih Güneysu
Sakarya University Training and Research Hospital, Clinic of Emergency Medicine, Sakarya
Sakarya, Turkey, 56000 - Tel.: +905454711555
E-mail: fatihguneysu55@hotmail.com

through respiratory droplets and close contact. It leads to pneumonia and Acute Respiratory Distress Syndrome (ARDS) in patients who have risk factors such as advanced age and underlying comorbidities such as hypertension, diabetes mellitus, cardiovascular disease, and cerebrovascular disease[3]. Molecular-based approaches are the first-line methods to detect this novel coronavirus in suspected cases. Nucleic acid testing (Polymerase Chain Reaction – PCR) is the main technique for laboratory diagnosis. Other methods with a short test time, such as virus antigen or serological antibody testing, are also valuable assays for the detection of the novel coronavirus infection[4].

The World Health Organization (WHO) currently recommends that all patient samples with suspected COVID-19 should be isolated from upper and/or lower respiratory tract specimens such as nasal and pharyngeal swabs, sputum, or bronchoalveolar lavage fluid for nucleic acid amplification diagnostic testing[5]. Since COVID-19 is mainly transmitted through droplets, healthcare personnel are at risk of becoming infected while taking these samples. Therefore, there is a need for sampling methods that do not risk infecting healthcare professionals. In this study, we aimed to compare the saliva samples provided by patients, and the Oro-Nasopharyngeal Swab (ONS) samples taken by the medical staff.

METHODS

Study Place

This study was conducted in Sakarya University Training and Research Hospital, where only the treatment and follow-up of COVID-2019 patients have been carried out since March 2020. Samples were taken after obtaining the consent of the patient. The study protocol was approved by the institutional review board of the Sakarya University (IRB No:16214662/050.01.04/78).

Patients and Study Design

Lung Computed Tomography (CT) is performed to the patients who attend the emergency department with complaints compatible with COVID-19 clinical symptoms, such as fever, cough, and shortness of breath, after examination by the head doctor. At the same time, ONS samples are taken from the patients for molecular analysis to obtain a definitive diagnosis. While patients with moderate and advanced pneumonia in lung CT are followed-up in the hospital, patients with mild clinical symptoms are followed-up in the outpatient clinic. The second swab sample was taken from hospitalized patients who had a negative first sample. When one of the two samples taken was positive, the patient was diagnosed with COVID-19, and if both were negative, COVID-19 was excluded.

In this study, patients were divided into three groups.

Group 1 (30 patients): Hospitalized patients with a finding consistent with COVID-19 in the CT scan of the lung and detected SARS-CoV-2 by PCR in at least one ONS sample.

Group 2 (15 patients): Hospitalized patients with a finding compatible with COVID-19 in lung CT examination, but in whom SARS-CoV-2 were not detected in at least two ONS samples by PCR.

Group 3 (19 patients): Patients who present to the emergency department with complaints compatible with COVID-19 (fever, cough, shortness of breath) but have normal CT.

Collecting study samples

The study samples were taken on the third day of hospitalization in Groups 1 and 2, whereas in Group 3, they were taken at the time of admission to the hospital. ONS and saliva samples for the study were taken simultaneously by the same doctor. As aerosolization may occur while taking a swab sample, the staff who took the sample wear complete personnel protective equipment (N95/FFP2 respirator, glasses or face shield, apron, and gloves). Dacron-flocked swabs were used for the collection of ONS. A single swab was used to take oropharyngeal and nasopharyngeal samples. Firstly, the swab was inserted into the oropharynx and then into the nasopharynx. Oropharyngeal swabs were collected by inserting the swab into the posterior oropharynx and swabbing the posterior pharynx for 2–3 seconds. Then, the swab was inserted through the nostril with a rotation movement until the nasopharynx was reached, and the sample was obtained by rotating the swab gently for 2-3 seconds. Then, the swab was placed into a 5 ml tube containing 2 ml viral transport medium (VTM).

The patients were asked to collect the saliva sample themselves. They were given a sterile dry container and told to close the lid of the container after placing the saliva in it. The staff cleaned the outside of the container with 1/10 diluted bleach-impregnated cloth, after taking the container while wearing gloves. After taking both samples, they were delivered to the

laboratory inside the triple transport system within one hour.

Nucleic Acid Isolation and RT-PCR Study

After the samples were brought to the microbiology laboratory, they were registered in the laboratory operating system, and the ONS and the saliva samples from the same patient were sequentially arranged to coincide with the same PCR set-up. The isolation of all samples was carried out in a negative pressure room, in a class 2-a biosafety cabinet.

RNA isolation from ONS samples was performed with the EZ1 (Qiagen, Germany) device. Elution of 60 µl of 400 µl sample was taken and used as a template in RT-PCR reaction.

RNA isolation from saliva samples was also performed using the EZ1 device. 10 samples were selected to optimize RNA isolation and RT-PCR process from saliva samples, and isolation was achieved both directly and by diluting it with 300 microliter Type-1 water in a 1:1 ratio. Because the positivity rates and Cycle Threshold (CT) values of the diluted samples were closer with the ONS, the study was optimized using the latter method. Elution of 60 µl of 400 µl sample (from a mix of 300 µl saliva sample and 300 µl Type 1 water) was taken and used as a template in RT-PCR reaction.

For the Real-Time PCR (RT-PCR) study, a 10 µl master mix, 2 µl primer, and 8 µl RNA mixture were prepared per sample with genesis RT-PCR SARS-CoV-2 (Primer Design, UK) kit. The reaction was carried out at the following time and temperature with a total reaction volume of 20 µl.

At the end of the reaction, CT values were used as an approximate indicator of the number of copies of the SARS-CoV-2 RNA. A CT value of less than 45 was interpreted as positive for the SARS-CoV-2 RNA.

Statistical analysis

The SPSS software version 21.0 was used for statistical analyses. The variables were investigated by using visual (histogram) and analytic methods (Kolmogorov-Smirnov/ Shapiro Wilk's test) to determine the distribution. Variables that exhibited normal distribution were presented as mean and standard deviation (mean ± SD). The cycle numbers of the PCR assay according to sampling methods were compared by using the paired sample t-test since they showed normal distribution.

The agreement between the two sampling methods was evaluated with the kappa test. Kappa is a measure of this difference, where 1 is a perfect agreement. Kappa values denote the following levels of agreement: 0–0.2, poor; 0.21–0.4, fair; 0.41–0.6, moderate; 0.61–0.8, substantial; 0.81–1, almost perfect[6]. A p-value of less than 0.05 was considered a statistically significant result.

RESULTS

A total of 64 patients were included in the study. Thirty patients were in Group 1, 19 were in Group 2, and 15 were in Group 3. The mean age of the patients was 51.04 ±17.9 years, and 37 (57.8%) were men. In 23

TABLE 1. TIME AND TEMPERATURE VALUES OF COVID-19 REAL-TIME PCR ASSAY.

Cycles		Temperature	Time
Reverse transcription		55 °C	10 minute
Enzyme activation		95 °C	2 minute
X50 cycles	Denaturation	95 °C	10 second
	Annealing and extension	60 °C	60 second

TABLE 2.STATISTICAL ANALYSIS RESULTS OF THE SALIVA SAMPLING METHOD. (N=64)

Properties	Saliva Sample (%)
Sensitivity	85.19
Specificity	89.19
PPV*	85.19
NPV**	89.19

*PPV: Positive predictive value, ** NPV: Negative predictive value

TABLE 3. CYCLE THRESHOLD VALUES OF POSITIVE RESULTED SAMPLES BY TWO SAMPLING METHODS.

Method (n=23)	Mean	Standard Deviation	Min-max	P*
CT of Oral/Nasal swab samples	28.26	4.70	21-35	<0.001
CT of saliva samples	32.91	4.99	25-43	

CT: Cycle threshold, *Paired sample t test applied

TABLE 4. STATISTICAL ANALYSIS RESULTS OF THE SALIVA SAMPLING METHOD IN GROUP 1.

Properties	Saliva Sample (%)
Sensitivity	87.50
Specificity	100.00
PPV*	100.00
NPV**	66.66

*PPV: Positive predictive value, ** NPV: Negative predictive value

(35.9%) of the patients, both saliva and ONS samples were positive at the same time, in 4 (6.25%) patients, only the saliva, and in another 4 (6.25%) patients, only the ONS was positive. SARS-CoV-2 was detected in the saliva samples of 27 (42.2%) patients. The value of kappa was substantial in agreement as 0.744 and it was found to be statistically significant (<0.001). The sensitivity, specificity, positive predictive value, and negative predictive values of saliva samples are presented in Table 2, and the mean PCR cycles of saliva and ONS samples are presented in Table 3.

Both saliva and ONS samples were positive in 21 (70%) of the 30 patients in Group 1, and the specificity and positive predictive value was 100% (Table 4). The value of kappa was 0.737, and it was found to be statistically significant (<0.001).

In group 2 patients, test positivity was detected in two of the saliva samples, but not in any of the ONS samples.

In Group 3, both saliva and ONS samples were positive in two patients. In this group, SARS-CoV-2 was detected only in saliva in two patients, and only in ONS in one patient. The sensitivity, specificity, PPV, and NPV were 66.7%, 83.3%, 50%, and 90.9%, respectively. The value of kappa indicated moderate agreement as 0.444 and was not statistically significant (p=0.080).

DISCUSSION

In this study, we have demonstrated that saliva samples could be used instead of ONS samples in the diagnosis of COVID-19. The sensitivity, specificity, positive predictive value, and negative predictive value of saliva samples were found to be more than 85%. The value of kappa was in substantial agreement as 0.744, and it was found statistically significant (<0.001). Accordingly, the results obtained with the saliva sample are quite parallel to the results obtained with the ONS sample.

In this study, the ONS samples were taken as a reference method when comparing them with the saliva samples. Unfortunately, the sensitivity of the upper respiratory tract samples was low (32-66%)[7]. Although the lower respiratory tract samples were more sensitive, the study design was conceived in this way due to the difficulties associated with sampling and the risks it poses. This causes a decrease in the compliance rate and kappa value in statistical analysis. If this study were to be be done with lower respiratory tract samples, the saliva samples' sensitivity and specificity found would be higher, since saliva samples were positive in only four patients.

Our findings contribute significantly to the diagnosis and follow-up of COVID-19. There are some advantages of using saliva samples in the diagnosis of COVID-19. First of all, its contribution to the safety of healthcare personnel is the main advantage. On 14 February 2020, the National Health Commission of China reported that a total of 1,716 health workers had been infected with this virus[8]. In Spain, COVID-19 has been identified in at least 12,298 healthcare professionals (14.4% of total reported cases). Until March 23, 4824 healthcare personnel were infected with the new coronavirus (SARS-CoV-2) in Italy[9,10]. Healthcare personnel is exposed to upper respiratory tract secretions very intensely during the sampling process. Since the oropharynx and nasopharynx are stimulated during the correct swabbing process, the patient can cough or sneeze. So, considering the necessity of close contact between healthcare workers and infected patients to collect nasopharyngeal or oropharyngeal samples, self-collection of saliva by the patient can strongly reduce the risk of COVID-19 contamination. Also, experienced staff is essential while collecting ONS with a swab. On the other hand, saliva collection is a very simple process, and there is no need for healthcare personnel since it is done by patients themselves.

Secondly, we think our results are important for dentists. A study with SARS-CoV showed that salivary gland epithelial cells can be infected with SARS-CoV shortly after infection in rhesus macaques[11]. This suggests that salivary gland cells could be a significant source of virus in saliva, particularly early in infection[11]. To et al.[12] recently identified SARS-CoV-2 in the saliva of infected patients.SARS-CoV-2 can be transmitted from asymptomatic patients and infected patients just before symptoms begin. In a mathematical modeling study in China, authors declared that 86.2% of all infections from 10–23 January 2020 were from undocumented cases, many of whom were likely not severely symptomatic. They conclude that asymptomatic patients appear to have facilitated the rapid spread of the virus throughout China[13]. Our findings prove that SARS-CoV-2 is in saliva. Consequently, dentists who have a high risk of exposure to their patients' saliva face the risk of becoming infected even if their patients do not have symptoms such as fever and cough. Dentists should use maximum personnel protective equipment consisting of N95/FFP2 respirator, face shield, cap, apron, and gloves when

performing intraoral intervention even if their patients have no symptoms.

Thirdly, cotton and calcium alginate swabs, or swabs with wooden shafts may contain substances that inactivate some viruses and inhibit PCR testing. Therefore, these swabs are not recommended for use in the diagnosis of COVID-19. Dacron or polyester flocked swabs with plastic shafts should be used for collecting standard ONS samples. For the transport of samples, the use of VTM containing antifungal and antibiotic supplements is strongly recommended[5]. In the case of a pandemic, since these materials are used in large amounts all over the world, there may be difficulties in the provision of these materials from time to time, and sometimes when they can't found, there may be disruptions of the correct diagnosis. Conversely, since only a sterile dry container is needed to take a saliva sample, there will be no problem in the supply of material and there will be no delays in patient diagnosis. Also, this method is cost-effective compared to the standard ONS method. Considering that swab and VTM cost at least 1 $ per sample, taking saliva samples will save millions of dollars.

In our study, the PCR cycle of saliva samples was more than ONS samples. This may be due to two reasons. First, the viral load in saliva may be less than the oropharynx and nasopharynx. Secondly, the enzymes in saliva could be suppressing the reproduction of the virus in the mouth. Although further studies are needed regarding these hypotheses, these causes could not greatly affect the sensitivity and PPV in identifying SARS-CoV-2 in saliva.

CONCLUSION

In conclusion, saliva samples can be used instead of ONS samples in detecting SARS-CoV-2. Investigating SARS-CoV-2 with saliva is cheaper, more effortless for the patient, easier, and most importantly, it poses much less risk of SARS-CoV-2 contamination to healthcare professionals. We believe that saliva is a good alternative to the ONS in the diagnosis of COVID-19, especially in less developed countries with limited resources.

RESUMO

OBJETIVO: *Funcionários da saúde correm risco de infecção ao coletar amostras do trato superior e/ou inferior. Portanto, existe a necessidade de métodos de coleta de amostras que não representem um risco de infecção. Neste estudo, nosso objetivo foi comparar as métodos de coleta de saliva e swab de naso e orofaringe (ONS).*

MÉTODOS: *Os pacientes foram divididos em três grupos. O Grupo 1 incluiu pacientes cujo diagnóstico de COVID-19 foi confirmado por reação em cadeia da polimerase (PCR). O Grupo 2 incluiu pacientes com achados compatíveis com COVID-19 em exames de tomografia computadorizada (TC), mas com PCR negativo. O Grupo 3 incluiu pacientes que compareceram ao departamento de emergência com queixas compatíveis com COVID-19, mas TC normal. Amostras de saliva e ONS foram coletadas no terceiro dia de internação, nos Grupos 1 e 2, já no Grupo 3, foram coletadas no momento da internação.*

RESULTADOS: *Um total de 64 pacientes foram incluídos no estudo. A média de idade foi de 51,04 ± 17,9 anos, e 37 (57,8%) eram do sexo masculino. SARS-CoV-2 foi detectado em 27 (42,2%) amostras de saliva dos pacientes. A sensibilidade e valor preditivo positivo foi de 85,2% nas amostras de saliva, já a especificidade e o valor preditivo negativo foi 89,2%. O valor de Kappa estava substancialmente de acordo (0,744) e era estatisticamente significante (<0,001).*

CONCLUSÃO: *Amostras de saliva podem ser usada em vez de ONS na detecção de SARS-CoV-2. O uso de amostras de saliva para detecção de SARS-CoV-2 é mais barato, mais fácil para o paciente e em geral e, mais importante, representa um risco muito menor de contaminação de SARS-CoV-2 para os profissionais da saúde.*

PALAVRAS-CHAVE: *Infecções por Coronavirus/diagnóstico. Saliva. Pessoal de saúde. Betacoronavirus.*

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ORIGINAL ARTICLE

Effect of COVID-19 on platelet count and its indices

Ertuğrul Güçlü[1]
Havva Kocayiğit[2]
Hüseyin Doğuş Okan[1]
Unal Erkorkmaz[3]
Yusuf Yürümez[4]
Selcuk Yaylaci[5]
Mehmet Koroglu[6]
Cem Uzun[1]
Oğuz Karabay[1]

1. Sakarya University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Sakarya, Turkey.
2. Sakarya University Training and Research Hospital, Division of Anesthesiology, Sakarya, Turkey.
3. Sakarya University Faculty of Medicine, Department of Biostatistics, Sakarya, Turkey.
4. Sakarya University Faculty of Medicine, Department of Emergency Medicine, Sakarya, Turkey.
5. Sakarya University Faculty of Medicine, Department of Internal Medicine, Sakarya, Turkey.
6. Sakarya University Faculty of Medicine, Department of Medical Microbiology, Sakarya, Turkey.

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SUMMARY

BACKGROUND: *Easily accessible, inexpensive, and widely used laboratory tests that demonstrate the severity of COVID-19 are important. Therefore, in this study, we aimed to investigate the relationship between mortality in COVID-19 and platelet count, Mean Platelet Volume (MPV), and platelet distribution width.*

METHODS: *In total, 215 COVID-19 patients were included in this study. The patients were divided into two groups. Patients with room air oxygen saturation < 90% were considered as severe COVID-19, and patients with ≥90% were considered moderate COVID-19. Patient medical records and the electronic patient data monitoring system were examined retrospectively. Analyses were performed using the SPSS statistical software. A p-value <0.05 was considered significant.*

RESULTS: *The patients' mean age was 64,32 ± 16,07 years. According to oxygen saturation, 81 patients had moderate and 134 had severe COVID-19. Our findings revealed that oxygen saturation at admission and the MPV difference between the first and third days of hospitalization were significant parameters in COVID-19 patients for predicting mortality. While mortality was 8.4 times higher in patients who had oxygen saturation under 90 % at hospital admission, 1 unit increase in MPV increased mortality 1.76 times.*

CONCLUSION: *In addition to the lung capacity of patients, the mean platelet volume may be used as an auxiliary test in predicting the mortality in COVID-19 patients.*

KEYWORDS: *Coronavirus Infections. Blood Platelets. Mean platelet volume. Mortality.*

INTRODUCTION

The World Health Organization (WHO)[1] declared a pandemic on March 11th, 2020, after the identification of > 118,000 novel 2019 coronavirus disease (COVID-19) cases in 114 countries. As of 7 May 2020, a total of 3,825,028 cases had been identified in 187 countries, and unfortunately, 267,996 patients had died[2].

The clinical spectrum of COVID-19 appears to be wide, encompassing asymptomatic infection, mild

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CORRESPONDING AUTHOR:Hüseyin Doğuş Okan
Adnan Menderes Caddesi Sağlık Sokak No: 195 Adapazarı - Sakarya –Turkey - 54000
E-mail: okanhd@hotmail.com

upper respiratory tract illness, severe viral pneumonia with respiratory failure, and even death. In particular, older age, d-dimer levels greater than 1 μg/mL, higher SOFA score on admission, and comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease, and oncological diseases were associated with worse prognosis and in-hospital death[3,4]. Treatment strategies including drugs, vaccines, or targeted therapy approaches have been limited until now[5]. Easily accessible, inexpensive, and widely used laboratory tests that show the severity of COVID-19 are important. Mean platelet volume (MPV) and platelet distribution width (PDW) are widely and routinely used in clinical practice worldwide. Higher MPV and increased PDW have been found in sepsis, and PDW was found to be a poor prognostic factor in severe sepsis[6]. However, the role of these parameters in COVID-19 has not been investigated. In this study, we aimed to investigate the relationship between mortality in COVID-19 and platelet count, MPV, and PDW.

METHODS

Study setting

This is a retrospective cohort study that was conducted between April 01, 2020, and April 15, 2020, in a tertiary training and research hospital. The hospital where the study was conducted was designated as the coronavirus pandemic hospital in the province by the Ministry of Health. The hospital has a total of 400 patient beds, 85 of which are intensive-care beds. Patient medical records and the electronic patient data monitoring system were examined retrospectively. The study protocol was approved by the institutional review board of Sakarya University (IRB No:71522473/050.01.04/105).

Study Group

Patients diagnosed with COVID-19 were included. Complete blood count, C-reactive protein (CRP), and biochemistry tests are routinely performed on patients who attend the emergency department with complaints compatible with COVID-19 such as cough, fever, and shortness of breath. Also, Lung Computed Tomography (CT) is performed on patients who have shortness of breath, after their examination by the responsible doctor. At the same time, oro-nasopharyngeal swab (ONS) samples are taken from the patients for molecular analysis to reach a definitive diagnosis. Patients with advanced bilateral pneumonia, and/or tachypnea (respiratory rate > 26/minute), and/or arterial oxygen saturation < 90% in room air are followed up in the intensive-care unit, while patients with moderate clinical symptoms are followed up in the hospital wards. A second swab sample was taken from hospitalized patients with a negative first sample. When one of the two samples taken was positive, the patient was diagnosed with COVID-19, and if both were negative, COVID-19 was excluded.

Study design

The patients were divided into two groups according to the lowest oxygen saturation during their first two days after hospital admission. Patients with oxygen saturation < 90% in room air were considered severe COVID-19, and patients with ≥90% were considered moderate COVID-19. Complete blood count and CRP values were obtained from patients on the day of hospital admission and on the third day of hospital follow-up. Patients who were discharged within 28 days after diagnosis of COVID-19 and who continued to undergo follow-up in the hospital on the 28th day of patient monitoring were accepted as survivors. Patients who died within the 28 days of patient monitoring were recorded as non-survivors. Thrombocytopenia was defined as grade 1: absolute platelet count (APC) 150,000 – 100,000/mm^3; grade 2: 99,000 – 50,000/mm^3; grade 3: < 49,000/mm^3. Lymphopenia was defined as grade 1: absolute lymphocytes count (ALC) 1500- 1000/ul; grade 2: ALC 999–750/ul; grade 3: ALC < 750/ul.

Statistical Analysis

Descriptive analyses were performed to provide information on the general characteristics of the study population. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of numerical variables was normal. Accordingly, two independent sample t-tests and one way ANOVA were used to compare the age between/among groups. The Mann Whitney U test and Kruskal Wallis H test were used to compare the non-normally distributed numeric variables between/among groups. The numeric variables were presented as the mean and standard deviations. Categorical variables were compared by the Chi-Square test. Categorical variables were presented as a count and percentage. A multiple logistic regression model was implemented to determine the risk factors independently associated with exit status and hospitalization time. A p-value < 0.05 was considered significant.

Analyses were performed using SPSS statistical software (IBM SPSS Statistics, Version 23.0. Armonk, NY: IBM Corp.)

RESULTS

In total, 215 COVID-19 patients were included in this study. The study population consisted of 95 females and 120 males, and their mean age was 64,32 ± 16,07 years. According to oxygen saturation, 81 patients had moderate and 134 had severe COVID-19. Since nine of the patients were discharged ≤ 3 days, they did not have a third-day analysis. Thrombocytopenia was observed in 54 (25.1 %) patients on the hospital admission day and in 52 (24.1 %) patients on the third follow-up day. On admission day, 43 patients had grade 1, 9 patients had grade 2, and two patients had grade 3 APC. On the third follow-up day, 40 patients had grade 1, 8 patients had grade 2, and four patients had grade 3 APC. On admission day, severe COVID-19 patients had significantly higher white blood count (WBC), neutrophil, and CRP values than moderate COVID-19 patients ($p < 0.05$). On the third follow-up day, WBC, neutrophil, platelet, MPV, and CMV values were significantly higher in severe patients than moderate COVID-19 patients ($p < 0.05$). On admission day, 62 patients had grade 1, 34 patients had grade 2, and 44 patients had grade 3 ALC. On the third follow-up day, 52, 44, and 57 patients had grade 1, 2, and 3 ALC, respectively. The mean lymphocyte value was lower in severe COVID-19 cases compared to moderate COVID-19 cases both on the day of hospital admission and on the third follow-up day ($p < 0.05$). The difference among WBC, neutrophil, platelet, and CRP between two days in severe and moderate COVID-19 patients was significant ($p < 0.05$) (Table 1).

Among the 215 COVID-19 patients, 56 (26.04 %) of them died within the 28-day follow-up. The age of the deceased patients was greater that that of the survivors. Thrombocytopenia was observed in 22 (39.3 %) of the non-survivors and in 31 (19.5 %) of the survivors ($p=0.003$). WBC, neutrophil, CRP, and PDW in non-survivors were significantly higher than in survivors in both admission day and the third day of follow-up

TABLE 1. COMPARISON RESULTS OF THE HEMATOLOGICAL CHARACTERISTICS AND OTHER FEATURES BETWEEN SEVERE AND MODERATE COVID-19 PATIENTS.

	Moderate COVID-19 (≥ 90 SaO2)		Severe COVID-19 (< 90 SaO2)		p
	n	Mean±SD	n	Mean±SD	
Gender (Male)	81	44 (54.3)	134	76 (56.7)	0.732
Age (years)	81	56.52 ± 15.95	134	69.04 ± 14.26	< 0.001
White blood count (K/uL)	81	6485.11 ± 2016.83	134	8960.72 ± 5016.91	< 0.001
Neutrophil (K/uL)	81	4283.67 ± 1846.83	130	7792.15 ± 7948.32	< 0.001
Lymphocyte (K/uL)	81	1573.53 ± 523.33	134	1389.35 ± 1607.33	< 0.001
Platelet (K/uL)	81	187.4 ± 59.82	134	208.63 ± 135.72	0.573
Mean Platelet Volume (f/l)	80	9.18 ± 1.24	132	9.61 ± 1.76	0.129
Platelet Distribution Width (%)	80	17.37 ± 2.32	132	17.72 ± 2.52	0.142
C-reactive protein (mg/L)	81	34.69 ± 43.05	133	107.53 ± 84.33	< 0.001
White Blood Count 2 (K/uL)	70	5714.4 ± 2191.37	129	9855.51 ± 8228.18	< 0.001
Neutrophil 2 (K/uL)	69	3795.51 ± 1959.37	127	8254.4 ± 7233.19	< 0.001
Lymphocyte 2 (K/uL)	70	1501.57 ± 892.05	128	922.66 ± 486	< 0.001
Platelet 2 (K/uL)	70	172.93 ± 67.58	128	217.82 ± 92.18	< 0.001
Mean Platelet Volume 2 (f/l)	69	9.38 ± 1.42	123	9.85 ± 1.79	0.043
Platelet Distribution Width 2 (%)	70	17.96 ± 1.43	124	18.13 ± 1.66	0.144
C-reactive protein 2 (mg/L)	64	36.92 ± 41.73	124	119.57 ± 82.52	< 0.001
White Blood count difference (K/uL)	70	-757.19 ± 2359.37	129	1004.3 ± 8033.14	0.003
Neutrophil difference (K/uL)	69	-511.11 ± 2048.64	123	585.45 ± 9756.85	0.006
Lymphocyte difference (K/uL)	70	-29.19 ± 851.87	128	-473.22 ± 1578.33	0.341
Platelet difference (K/uL)	70	-10.57 ± 52.39	128	10.24 ± 126.49	0.001
Mean Platelet Volume difference (f/l)	68	0.08 ± 0.89	122	0.37 ± 1.59	0.301
Platelet Distribution Width difference (%)	69	0.61 ± 2.34	123	0.55 ± 2.45	0.913
C-Reactive Protein difference (mg/L)	64	0.85 ± 24.14	124	14.4 ± 94.77	0.040
Hospitalization day	81	6.01 ± 3.49	134	14.75 ± 8.6	< 0.001

TABLE 2. DIFFERENCE IN AGE, GENDER AND HEMATOLOGICAL CHARACTERISTICS BETWEEN SURVIVORS AND NON-SURVIVORS OF PATIENTS WITH COVID-19

	Survivors		Non-survivors		P
	n	Mean ± SD	n	Mean ± SD	
Gender (Male)	159	87 (54.7)	56	33 (58.9)	0.697
Age (years)	159	61.15 ± 16	56	73.34 ± 12.58	< 0.001
White blood count (K/uL)	159	7569.78 ± 3940.36	56	9329.21 ± 5046.97	0.011
Neutrophil (K/uL)	157	6037.69 ± 7077.53	54	7630.35 ± 4598.34	< 0.001
Lymphocyte (K/uL)	159	1563.2 ± 1432.63	56	1162.14 ± 812.23	0.004
Platelet (K/uL)	159	207.69 ± 123.06	56	180.59 ± 78.14	0.094
Mean Platelet Volume (f/l)	158	9.34 ± 1.37	54	9.77 ± 2.11	0.189
Platelet Distribution Width (%)	158	17.44 ± 2.35	54	18.02 ± 2.69	0.040
C-reactive protein (mg/L)	159	67.87 ± 70.21	55	114.92 ± 94.73	< 0.001
White Blood Count 2 (K/uL)	148	7499.39 ± 4804.14	51	11009.02 ± 10877.1	0.001
Neutrophil 2 (K/uL)	146	5777.12 ± 4695.94	50	9334.8 ± 9117.4	< 0.001
Lymphocyte 2 (K/uL)	147	1259.44 ± 734.31	51	746.55 ± 477.17	< 0.001
Platelet 2 (K/uL)	147	206.52 ± 84.72	51	188.78 ± 92.26	0.348
Mean Platelet Volume 2 (f/l)	146	9.45 ± 1.47	46	10.41 ± 2.07	0.005
Platelet Distribution Width 2 (%)	147	17.89 ± 1.55	47	18.63 ± 1.56	0.006
C-reactive protein 2 (mg/L)	137	78.3 ± 79.75	51	126.71 ± 75.31	< 0.001
White Blood count difference (K/uL)	148	-144.61 ± 3963.6	51	1920.67 ± 11237.2	0.182
Neutrophil difference (K/uL)	144	-435.75 ± 7132.57	48	2072.73 ± 9731.54	0.175
Lymphocyte difference (K/uL)	147	-285.1 ± 1545.15	51	-406 ± 724.5	0.048
Platelet difference (K/uL)	147	-0.05 ± 117.13	51	11.33 ± 68.25	0.561
Mean Platelet Volume difference (f/l)	145	0.05 ± 1.08	45	0.96 ± 1.94	0.005
Platelet Distribution Width difference (%)	146	0.46 ± 2.35	46	0.93 ± 2.57	0.389
C-Reactive Protein difference (mg/L)	137	8.15 ± 68.63	51	14.19 ± 100.72	0.091
Hospitalization day	159	11.92 ± 8.63	56	10.13 ± 7.08	0.456

*: Shown as count and percentage

(p=0.001). On the other hand, MPV in non-survivors was significantly lower than in survivors only in the third follow-up day (P < 0.005). The demographic and laboratory findings of survivors and non-survivors are seen in table 2.

According to the multiple logistic regression model for mortality, in case of an increase of 1 unit MPV difference (MPV differences between 1st and 3rd day), the probability of death increases 1.762 times. In addition, the probability of death of patients with oxygen saturation < 90 % is 8.405 times higher than that of patients with oxygen saturation ≥ 90 % (table 3).

TABLE 3. MULTIPLE LOGISTIC REGRESSION MODEL FOR MORTALITY.

	β	SE of β	p	OR	95% CI for OR
Age	-0.042	0.007	< 0.001	0.959	0.945-0.973
Oxygen saturation	2.129	0.528	< 0.001	8.405	2.987-23.646
MPV diff	0.566	0.166	0.001	1.762	1.272-2.440

β: regression coefficient, SE: standard error, OR: odds ratio, CI: confidence interval, MPV: Mean platelet volume

DISCUSSION

Our findings revealed that oxygen saturation at admission and MPV difference between the first and third days of hospitalization were significant parameters in COVID-19 patients for predicting mortality. While, mortality was 8.4 times higher in patients who had oxygen saturation under 90 % at hospital admission, 1 unit increase in MPV between the first and third days of hospitalization increases mortality 1.76 times. In addition to the lung capacity of the patient, MPV may be used as an auxiliary test in predicting the mortality in COVID-19 patients.

Primary inflammation triggered by rapid viral replication and release of potent proinflammatory cytokines occurs in the early stages of COVID-19 infection[7]. In addition to pulmonary infiltrate and diffuse alveolar damage, widespread endothelial inflammation due to viral infection of the endothelial cell can strengthen the further secretion of various inflammatory cytokines[8]. Neutrophils and leukocytes might reinforce the cytokine storm other

than lymphocytes in COVID-19 because prominent lymphopenia has been developed in most COVID-19 patients, especially in severe ones[9]. In a meta-analysis, researchers found that severe illness was associated with lower lymphocyte and higher leukocyte counts[10]. In our study, while the leukocyte and neutrophil values of severe cases on the day of admission to hospital were higher than in mild cases, the lymphocyte values were low, too ($p < 0.05$). Moreover, on the third day of hospitalization, leukocyte and neutrophil levels were increased even more in severe cases and decreased in mild cases ($p < 0.05$). However, on the third day, although the lymphocyte values in severe cases decreased much more than in mild cases, the difference was not significant.

Zhao et al.[11] reported that a lymphocyte count of less than 1.5×10^9/L may be useful in predicting the severity of clinical outcomes. They found that there was a three-fold increased risk of severe COVID-19 with the presence of lymphopenia. Our study revealed that leukocyte and neutrophil values in non-surviving patients were higher than in survivors both on the day of admission and on the third day of the follow-up, but the difference in the increase between the first and third days was not significant. On the other hand, the decrease in lymphocyte values of the patients who died was significant. Therefore, the power of the decrease in lymphocyte value in showing mortality was higher than that of the elevation in leukocyte and neutrophils. So, clinicians should closely monitor patients with lymphopenia.

Some studies have found a relationship between thrombocytopenia and the severity of the COVID-19 and related mortality. It has been reported that mortality increases as platelet count decreases[12,13]. Interestingly in our study, although thrombocytopenia was more likely to occur in non-survivors than in survivors, we did not find any correlation between platelet level and disease severity or mortality.

TABLE 4. MULTIPLE LINEAR REGRESSION MODEL FOR HOSPITALIZATION DAY.

	β	SE of β	p	95% CI for β
Age	0.053	0.035	0.130	-0.016-0.121
Oxygen saturation	8.548	1.134	< 0.001	6.310-10.786
MPV difference	-0.905	0.382	0.019	-1.658--0.152
Constant	3.508	2.173	0.108	-0.780-7.796

β: regression coefficient, SE: standard error, CI: confidence interval, MPV: Mean platelet volume

Non-survivors had lower platelet counts than survivors on both admission day and third follow-up day, but this difference was not statistically significant. Similar to our study, other studies reported that platelet values were found to be normal in many patients at the time of hospital admission[14]. These differences between studies may be related to the time of the tests. Also, hydroxychloroquine, azithromycin, and enoxaparin treatment have been started in most countries when COVID-19 is suspected. These drugs can cause thrombocytopenia[15,16]. Another reason for the difference between studies may be that thrombocytopenia caused by drugs and thrombocytopenia caused by the disease present an intricate structure.

On the other hand, platelet indices, MPV, and PDW, were found to be higher in non-survivors on both admission day and third follow-up days. To our knowledge, this study is the first one specialized in the association between platelet indices and in-hospital mortality in patients with COVID-19. According to our results, every 1 unit increase in MPV increased mortality by 1.76 times. The mechanism of change in platelet indices in COVID-19 patients is probably multifactorial. Three hypotheses related to platelet count and structure are proposed in COVID-19. Firstly, as with other coronavıruses, thrombocytopenia is possibly due to infection of the bone marrow. Secondly, platelet destruction by the immune system. Thirdly, platelet consumption due to aggregation in the lungs[17]. Generally, platelet production increases as platelet count decreases. An increased number of young platelets is also functionally more active than older platelets. These changes may explain the increase in platelet indices, MPV, and PDW.

LIMITATIONS

In this rapidly emerging new non-characteristic infection of the modern medical age, it is necessary to identify biomarkers that can predict the severity and prognosis of the disease. MPV and PDW can be a simple, economical, fast, and widely available laboratory parameter that can distinguish directly between COVID patients with and without a severe presentation of the disease. Moreover, MPV can also be used to predict the mortality in COVID-19. In order to show the strength of these parameters more clearly, studies with a large number of patients are needed.

RESUMO

OBJETIVO: *Testes laboratoriais de fácil acesso, baixo custo e amplamente utilizados capazes de demonstrar a gravidade da COVID-19 são importantes. Portanto, neste estudo, o nosso objetivo foi investigar a relação entre a mortalidade na COVID-19 e a contagem de plaquetas, volume plaquetário médio (VMP) e largura de distribuição de plaquetas.*

MÉTODOS: *No total, 215 pacientes com COVID-19 foram incluídos no estudo. Os pacientes foram divididos em dois grupos. Pacientes com saturação de oxigênio < 90% em ar ambiente foram considerados casos graves de COVID-19 e pacientes com valores ≥90% foram considerados casos moderados. Os registros médicos dos pacientes e o sistema eletrônico de monitoramento de dados de pacientes foram analisados retrospectivamente. As análises foram realizadas utilizando o software estatístico SPSS. Um valor de p <0,05 foi considerado significativo.*

RESULTADOS: *A média de idade dos pacientes foi de 64,32 ± 16,07 anos. Com base na saturação de oxigênio, 81 pacientes eram casos moderados e 134 tinham COVID-19 grave. Nosso estudo revelou que a saturação de oxigênio no momento da internação e a diferença nos valores de VPM entre o primeiro e terceiro dia de internação foram parâmetros significativos para predizer mortalidade de pacientes com COVID-19. A mortalidade foi 8,4 vezes maior nos pacientes com saturação abaixo de 90% no momento da internação, mas um aumento de apenas 1 unidade no valor de VPM aumentou a mortalidade 1,76 vezes.*

CONCLUSÃO: *Além da capacidade pulmonar dos pacientes, o volume plaquetário médio pode ser utilizado como um teste auxiliar para prever a mortalidade de pacientes com COVID-19.*

PALAVRAS-CHAVE: *Infecções por Coronavirus. Plaquetas. Volume Plaquetário Médio. Mortalidade.*

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ORIGINAL ARTICLE

The effect of nitric oxide, endothelial nitric oxide synthetase, and asymmetric dimethylarginine in hemorrhoidal disease

Aylin Hande Gokce[1]
Feridun Suat Gokce[2]
Sinem Durmus[3]
Ramila Hajiyeva[4]
Feyzullah Ersoz[5]
Remise Gelisgen[6]
Hafize Uzun[6]

1. Assistant Professor. Department of General Surgery, Istanbul Atlas University Faculty of Medicine, Istanbul, Turkey.
2. Department of General Surgery, Balıklı Rum Hospital, Istanbul, Turkey.
3. Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey
4. Assistant Doctor. Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey.
5. Associate Professor. Department of General Surgery. Istanbul Training Research Hospital, Istanbul, Turkey..
6. Professor Doctor. Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey

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SUMMARY

AIM: *The aim of this study was to examine the roles of nitric oxide (NOx), endothelial nitric oxide synthetase (eNOS), and asymmetric dimethylarginine (ADMA), which is the major endogenous inhibitor of nitric oxide synthases (NOS), in the pathophysiology of hemorrhoidal disease.*

METHODS: *This study included 54 patients with grades 3 and 4 internal hemorrhoidal disease and 54 patients without the disease who attended the General Surgery Clinic. NOx, eNOS, and ADMA levels were measured with the Enzyme-Linked ImmunoSorbent Assay (ELISA) method.*

RESULTS: *The patients had higher NO and eNOS levels and lower ADMA levels than the control subjects ($p<0.001$). A significant highly positive correlation was found between NO and eNOS ($p<0.001$). Nevertheless, there was a highly negative correlation between ADMA and NO-eNOS($p<0.001$, $p<0.001$).*

CONCLUSION: *This preliminary study reveals that higher NOx and eNOS activities and lower ADMA levels in the rectal mucosa are observed in patients with hemorrhoidal disease than in those with normal rectal tissue. The imbalance between endothelium-derived relaxing factors, such as NO and endogenous competitive inhibitor of NOS, ADMA, may cause hemorrhoidal disease. Our study proposes that hemorrhoids display apparent vascular dilatation and present with bleeding or swelling. ADMA is an effective NOS inhibitor and may be a promising therapeutic option for hemorrhoidal disease.*

KEYWORDS: *Arginine/analogs & derivatives. Nitric oxide synthase type III. Hemorrhoids. Nitric oxide. Nitric oxide synthase.*

INTRODUCTION

Hemorrhoidal tissues are normal anatomical and structural elements of the anal canal consisting of veins and muscle fibers. Hemorrhoidal disease is caused by the extension of these tissues due to several factors, including constipation, diarrhea, straining, and pregnancy. In recent studies, increasing microvascular density in hemorrhoidal tissue has been observed, suggesting that neovascularization might

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CORRESPONDING AUTHOR: Aylin Gokce
Amatya /Istanbul – Istanbul – 34098
E-mail: ahgokce79@hotmail.com

be another important factor of hemorrhoidal disease1.

Nitric oxide (NO) is a potent vasodilator that is synthesized from L-arginine by one of the following three nitric oxide synthases (NOS): inducible NOS (iNOS), neuronal NOS (nNOS), or endothelial NOS (eNOS). NOS was reported to increase significantly in hemorrhoids2. Although the vasodilator functions of NO are amply studied in the gastrointestinal tract, the relative contributions of NOS isoforms to hemorrhoids are unclear.

Asymmetric dimethylarginine (ADMA) is the major endogenous inhibitor of all three NOS isoforms. ADMA is produced from the proteolysis of the proteins that contain methylated arginine[3]. A competitive inhibitor of endogenous NOS, ADMA results in a reduction of NO production[4]. Increasing plasma ADMA levels have been reported in disease pathology in a variety of conditions that were characterized by endothelial dysfunction, including hypertension, hypercholesterolemia, renal failure, tobacco exposure, and hyperglycemia[5,6]. ADMA has also been indicated as an independent risk factor for coronary heart disease and endothelial dysfunction[7].

There is no adequate information in the literature regarding the relationship between human hemorrhoids and ADMA. Therefore, this study aimed to investigate the roles of NO, eNOS, and ADMA in the pathophysiology of hemorrhoidal disease.

METHODS

The protocol was approved by the local Ethics Committee of the Istanbul Education and Research Hospital (verdict number: 2019/2037) and was conducted in accordance with the Declaration of Helsinki. This study included 54 patients with grades 3 and 4 internal hemorrhoidal disease who attended the General Surgery Clinic. All subjects were of Turkish descent. They all provided informed consent for inclusion before study participation was initiated. In our clinic, the Milligan Morgan procedure was performed on patients with grades 3 and 4 internal hemorrhoids under general anesthesia. All patients with hemorrhoidal disease in the rectal mucosa underwent rectal biopsy. The area was washed with saline solution and evaluated. The control group was comprised of patients who underwent colonoscopy; those with hemorrhoidal disease, malign disease, and inflammatory bowel disease were excluded from the study. Patients who underwent rectal biopsy and whose histopathological results were normal were included in the study as the control group. The biopsies of the control group were endoscopically performed with biopsy forceps on the normal rectal mucosa close to the anus. Resected pieces were washed with saline solution. The study and control groups were referred to as Group 1 and Group 2, respectively. The samples were stored at −80°C for biochemical evaluation.

Preparation of tissue samples

The Group 1 specimens were homogenized in a four-fold volume of phosphate-buffered solution (PBS, pH: 7.4) using a homogenizer (Next Advance Bullet Blender Storm 24). To remove debris, the homogenate was centrifuged at 3000 *g* for 10 minutes. The clear upper supernatant was taken, and tissue analyses (NOx, eNOS, and ADMA) were performed. All the experimental procedures were performed at +4°C.

Measurement of NOx, eNOS, and ADMA levels in tissue

Tissue NOx, eNOS, and ADMA levels are measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (SinoGeneClon Biotech Co., Ltd., HangZhou, China) as per the manufacturer's instructions. The coefficients of intra- and inter-assay variation (% CVs) of NO, eNOS, and ADMA were less than 10%.

Statistical analysis

The number of patients in the groups was calculated for NO, eNOS 3, and ADMA parameters using a testing power of 95%. The minimum number of patients in the groups with and without the disease (hemorrhoidal disease) was calculated as 16 for NO and eNOS and 18 for ADMA according to Power analysis. In our study, a total of 108 patients were measured, 54 patients in each group.

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 21.0). All data were first checked for normality. Then, the categorical variables were analyzed using the chi-square test ($\chi^{2)\ test.}$ Normally distributed continuous variables were presented as mean ± standard deviation. Differences in the two groups were analyzed with the Student's *t*-test. To test the relationship between the variables, the Pearson Correlation was used. Indicative accuracy of distant markers was contrasted by analyzing the area below the receiver operating characteristic (ROC) curve, which was used to compare the diagnostic accuracy of the various markers. From the

results of the ROC curve, odds ratios were calculated for cut-off points by multivariate analysis. Differences were considered significant when $p< 0.05$.

RESULTS

The subject characteristics and circulating concentrations of biochemical parameters are found in Table 1. The patients (Group 1) had statistically significantly higher NOx and eNOS levels (for both $p<0.001$) and lower ADMA levels ($p<0.001$) than the control subjects (Group 2).

We concluded that there was a significant highly positive correlation between NOx and eNOS (r=0.830, $p<0.001$) (Table 2). Furthermore, we assessed that ADMA is highly negatively correlated with NOx ($r=-0.676$, $p<0.001$) and eNOS ($r=-0.684$, $p<0.001$) (Table 2). We noted that the eNOS levels had an excellent diagnostic performance in the differentiation between the groups [(AUC) = 1.000, 95% confidence interval (CI): 1.00–1.00)] $p<0.001$, with both 100% sensitivity and specificity for a cut-off point at 140.65 pg/mL.

In addition, the AUC of NOx was 0.959 (95% CI: 0.909–1.00) with 100% specificity and 94.4% sensitivity for a cut-off point at 125.60 mmol/L (Table 3). Multivariate analysis showed that if eNOS is greater than 140.65 pg/mL, the disease risk increases 2010-fold ($p=0.0002$), and if NOx is greater than 125.60 mmol/L, the disease risk increases 185-fold ($p=0.0006$).

TABLE 1. DEMOGRAPHIC CHARACTERISTICS, NOX, ENOS, AND ADMA LEVELS OF GROUPS.

	Group 1 (n=54)	Group 2 (n=54)	p
Age	37.5±7.1	37.3±6.0	0.849
Gender (Female/Male)	29/25	26/28	0.568
NOx (mmol/L)	176.2±18.2	109.7±19.8	<0.001
eNOS (pg/mL)	182.0±14.0	116.4±10.3	<0.001
ADMA (ng/mL)	66.4±13.8	100.8±10.3	<0.001

NOx: Total nitric oxide; eNOS: Endothelial nitric oxide synthetase; ADMA: Asymmetric dimethylarginine.

TABLE 2. CORRELATION OF PARAMETER DATA IN GROUP 1

		eNOS (pg/mL)	ADMA (ng/mL)	Age
NOx (mmol/L)	r p	0. 830** 0.000	-0.676 0.000	-0.069 0.621
eNOS (pg/mL)	r p	-	-0.684 0.000	-0.127 0.360
ADMA (ng/mL)	r p	-0.684 0.000	- -	-0.119 0.390

NOx: Total nitric oxide; eNOS: Endothelial nitric oxide synthetase; ADMA: Asymmetric dimethylarginine. Spearman correlation analysis was used. *p<0.05; **p<0.01; ***p<0.001

DISCUSSION

This preliminary study revealed that higher NOx and eNOS activities and lower ADMA levels in the rectal mucosa were observed in patients with hemorrhoidal disease than in those with normal rectal tissue. An imbalance between a endothelium-derived relaxing factor, such as NO, and the endogenous competitive inhibitor of NOS, ADMA, may cause hemorrhoidal disease. This study found that hemorrhoids display apparent vascular dilatation and manifest with bleeding or swelling. ADMA is an effective NOS inhibitor and it may be a promising therapeutic option for hemorrhoidal disease.

Hemorrhoidal disease is more common in people over 30 years old8. In this study, the mean age of the subjects was 37.5 years in Group 1 and 37.3 years in Group 2, consistent with what is found in the literature. There was no remarkable difference in age between Groups 1 and 2. There was also no remarkable difference in the incidence rates of hemorrhoidal disease in men and women8. the sex distribution of the patients included in this study was approximately

TABLE 3. CUT-OFF POINTS ACCORDING TO ROC CURVE, SENSITIVITY, SPECIFICITY, AND AUC FOR NITRIC OXIDE (NOX) AND ENDOTHELIAL NITRIC OXIDE SYNTHETASE (ENOS).

			Asymptotic 95% Confidence Interval				
Variable(s)	AUC	p	Lower Threshold	Upper Threshold	Cut-off points	Sensivity	Specificity
NOx (mmol/L)	0.959	0.000	0.909	1.000	125.60	% 94.4	% 100
eNOS (pg/mL)	1.000	0.000	1.000	1.000	140.65	%100	%100

NOx: Total nitric oxide; eNOS: Endothelial nitric oxide synthetase; AUC: Area under the curve.

the same, consistent also with the literature. There was no remarkable difference in sex between Groups 1 and 2.

NO exerts physiological functions in the nervous and immune systems, contributing to behavior regulation, defense mechanisms against infectious disease, tumors, and gastrointestinal motility9. In this study, the amount of NO in Group 1 was remarkably higher than that in Group 2. Hemorrhoidal disease occurs due to the dilatation of hemorrhoid lumps that normally exist in the rectal area10. We consider that NO has an important role in the etiology of hemorrhoids, since it is released to lower pressure against causes that increase the pressure, like coughing, straining, and pregnancy. It supports our opinion that the NO level in rectal tissue excised from the patients with hemorrhoidal disease was higher than that of the control group.

Indeed, eNOS has received even more attention than NO due to its instability and the regulatory mechanisms of eNOS on NO production11. In hemorrhoids, NOS, an enzyme that synthesizes nitric oxide from L-arginine, was reported to increase remarkably12. García-Martín et al.13 reported that the eNOS level was higher in people with migraines and had a history of migraines in their families and that eNOS inhibitors could be used in the treatment. There are publications revealing that a low level of eNOS is related to coronary artery disease14,15, essential hypertension16, and multiple sclerosis17. In our study, the eNOS level of Group 1 was remarkably higher than that of Group 2. It supports the opinion that eNOS and NOx have an effect on the occurrence of hemorrhoidal disease.

Lohsiriwat et al.2 observed NOS protein expression in tissue extracts of hemorrhoid and rectal tissue by Western blot analysis. Furthermore, they compared the expression levels to those of human microvascular endothelial cells. They also studied the distribution of all NOS isoforms in the tissue sections using immunohistochemistry. They provided further evidence that hemorrhoids have a higher protein expression of all NOS isoforms than the rectal tissue. There are considerably higher levels of nNOS and eNOS in the rectal tissue of patients with hemorrhoidal disease than in those with normal rectal tissue, suggesting that blood vessels in hemorrhoids are exposed to higher NO concentrations than those of normal rectal tissue. It appears that the bleeding or swelling caused by vascular dilatation might play an important role in hemorrhoidal symptoms and could be a potential target for medical treatment. NOS reduction, by applying NOS inhibitors, could likely improve these symptoms. There are publications revealing that ADMA, released as an endogenic and natural inhibitor of eNOS, increases hypercholesterolemia, coronary artery disease, and diabetes mellitus18. Even in a healthy population, high levels of circulating ADMA may be associated with higher rates of all-cause death19. Nevertheless, there is no adequate information in the literature regarding the relationship between human hemorrhoids and ADMA. Ragina et al.20 reported a clear and remarkable rise in systemic ADMA levels after laparoscopic colorectal surgery, even in the absence of surgical complications. The ADMA level of Group 1 was remarkably lower than that of Group 2. ADMA was very strongly negatively correlated with NO and eNOS. It is considered that vasodilatation of the hemorrhoidal masses is caused by the increase in ADMA release against increased NO and eNOS. Thus, NO release inhibition or insufficient ADMA levels can be one of the underlying causes.

Vasodilation in hemorrhoidal veins develops due to causes such as constipation, pregnancy, and coughing, which increase intraabdominal pressure. Therefore, the usability of NOS inhibitors in hemorrhoidal disease against increasing vasodilator NO and eNOS should be further studied.

We firmly believe that if our study had been conducted with a larger patient series, the results would have provided more guidance. The fact that ADMA, an endogenous NOS inhibitor, is associated with hemorrhoidal disease and other diseases has limited our ability to compare our study with others.

This study shows more detailed evidence that the rectal tissue of patients with hemorrhoidal disease has intense NOx and eNOS activities and lower ADMA levels than the normal rectal tissue, indicating that hemorrhoids are associated with noticeable vascular dilatation, high blood perfusion, tissue swelling, and bleeding tendency. If known changes in the blood flow in hemorrhoids can be explained by changes in NOx, eNOS, and ADMA levels described herein, a decrease in eNOS could potentially improve the hemorrhoid symptoms.

ADMA is an effective NOS inhibitor that may be a promising therapeutic option for hemorrhoidal disease. Further investigations are necessary to elucidate this hypothesis.

Conflict of interest

The authors declare that there is no conflict of interest related to the publication of this manuscript. No competing financial interests exist. The authors received no specific funding for this article.

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Author's Contribution

Planning: Aylin Hande Gokce, Feridun Suat Gokce, Hafize Uzun, Sinem Durmus, Feyzullah Ersoz.

Data collection: Aylin Hande Gokce, Feridun Suat Gokce, Hafize Uzun,Ramila Hajiyeva, Remise Gelişgen.

Data entry: Aylin Hande Gokce, Feridun Suat Gokce, Ramila Hajiyeva, Feyzullah Ersoz.

Data analysis: Aylin Hande Gokce, Feridun Suat Gokce, Hafize Uzun, Ramila Hajiyeva, Sinem Durmus, Remise Gelisgen.

Article writing: Aylin Hande Gokce, Feridun Suat Gokce, Hafize Uzun, Sinem Durmus.

Manuscript analysis and interpretation: Aylin Hande Gokce, Feridun Suat Gokce, Ramila Hajiyeva, Feyzullah Ersoz, Remise Gelisgen.

RESUMO

OBJETIVO: *O objetivo deste estudo foi examinar os papéis do óxido nítrico (NOx), do óxido nítrico sintetase endotelial (eNOS) e da dimetilarginina assimétrica (ADMA), que é o principal inibidor endógeno das óxido nítrico sintase (NOS) na fisiopatologia da doença hemorróida.*

MÉTODOS: *Este estudo incluiu 54 pacientes com doença hemorróida interna de grau 3 e 4 e 54 pacientes sem a doença que se inscreveram na Clínica Geral de Cirurgia. Os níveis de NOx, eNOS e ADMA foram medidos com o método de Ensaio Imuno absorvente ligado a enzima (ELISA).*

RESULTADOS: *Os pacientes têm níveis mais altos de NO e eNOS e níveis mais baixos de ADMA do que os indivíduos controle (p <0,001). Uma correlação altamente positiva significativa foi encontrada entre o NO-eNOS (p <0,001). No entanto, houve uma correlação negativa muito séria entre ADMA e NO-eNOS (p <0,001, p <0,001).*

CONCLUSÃO: *Este estudo preliminar revela que os pacientes com doença hemorróida têm atividades mais altas de NOx e eNOS e níveis mais baixos de ADMA na mucosa retal do que os pacientes com tecido retal normal. Desequilíbrio entre o fator relaxante derivado do endotélio, como; O NO e o inibidor competitivo endógeno da NOS, ADMA, podem causar doenças hemorróidas. Nosso estudo propõe que as hemorróidas exibam aparente dilatação vascular e apresentam sangramento ou inchaço, a ADMA é um inibidor eficaz da NOS e pode ser uma opção terapêutica promissora para a doença hemorróida.*

PALAVRAS-CHAVE: *Arginina/análogos & derivados. Óxido nítrico sintase tipo III. Hemorroidas. Óxido nítrico. Óxido nítrico sintase.*

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REVIEW ARTICLE

Hormone therapy after risk-reducing surgery in patients with BRCA1/BRCA2 mutation: evaluation of potential benefits and safety

Agnaldo Lopes da Silva Filho[1]
Gabriella Martins Carvalho[2]
Larissa Cristelli de Sena[2]
Lívia Passini Guimarães Gomes[2]
Marcos Flávio Habib Valério[2]
Raíssa Isabelle Leão Martins[3]
Eduardo Batista Cândido[4]

1. Professor Titular do Departamento de Ginecologia e Obstetrícia da UFMG, Belo Horizonte, MG, Brasil.
2. Acadêmico da Faculdade de Medicina da UFMG, Belo Horizonte, MG, Brasil.
3. Médica Residente em Ginecologia e Obstetrícia do Hospital das Clínicas da UFMG, Belo Horizonte, MG, Brasil.
4. Professor Adjunto do Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina da UFMG, Belo Horizonte, MG, Brasil.

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SUMMARY

Women with mutations in the BRCA 1 and 2 genes are at increased risk for ovarian and breast cancer and therefore candidates for risk-reducing surgery, including salpingo-oophorectomy and mastectomy. Risk-reducing salpingo-oophorectomy (RRSO) is considered the most effective prophylactic measure for ovarian cancer prevention in this group of patients. This procedure involves loss of ovarian function and induced menopause. Estrogen therapy is the most effective treatment for controlling vasomotor symptoms and improving the quality of life of climacteric women. However, the potential hormonal stimulation of these tumors and the risk of breast cancer are a concern regarding the safety of hormone replacement therapy (HRT) in this population. This article aims to review the current evidence regarding the potential benefits and safety of HRT after RRSO.

KEYWORDS: *Mutation. Estrogen replacement therapy. Salpingostomy. Ovariectomy. Salpingo-oophorectomy. Breast neoplasms. Ovarian neoplasms.*

INTRODUCTION

Epithelial ovarian cancer is responsible for approximately 125,000 deaths annually worldwide,[1] with a higher incidence in more developed countries. In Brasil, it is estimated there are 6,150 new cases and 3,879 deaths due to ovarian cancer every year.[2] Approximately 70% of women with ovarian cancer are diagnosed with metastatic or locally advanced disease (stages III and IV), with approximately 30% of five-year survival. Despite the high rates of response to chemotherapy initially, approximately 80% of the women with advanced disease present recurrence within two years after the initial treatment.[3,4]

Among the most important risk factors for the development of ovarian cancer is a family history

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CORRESPONDING AUTHOR: Agnaldo Lopes da Silva Filho
Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina da Universidade Federal de Minas Gerais
Avenida Professor Alfredo Balena, 190, Santa Efigênia, Belo Horizonte, MG, Brasil – 30130-100
E-mail: agnaldo.ufmg@gmail.com

of breast or ovarian cancer, particularly in women with two or more first-degree relatives affected by it.[5] Mutations in the DNA repair pathways, as well as in the genes of susceptibility to breast cancer 1 and 2 (BRCA 1 and 2), predispose to an increased risk of breast and ovarian cancer. Women with mutations in these genes have an increased risk of developing ovarian cancer (15-56%) and breast cancer (45-80%) throughout life when compared to the general population (ovarian cancer, 1.4%; breast cancer, 12%).[6] However, some evidence suggests that the risk of developing ovarian cancer is different in those with BRCA1 mutations (45-60%) and BRCA2 mutations (11-35%).[6]

Risk-reducing salpingo-oophorectomy (RRSO)

RRSO provides patients with BRCA mutations a significant reduction in the risk of ovarian cancer, i.e., approximately 80%, and of breast cancer, i.e., approximately 50%,[6,7] in addition to a reduction in mortality.[8] Considering the substantial difference in the phenotypes of breast and ovarian cancers associated with BRCA mutations, further studies are necessary to determine separately the risk reduction in patients with BRCA1 and BRCA2 mutations. The indication for this procedure should take into account various factors, such as patient age, current, and desired parity, and the current risk of malignancy development.[6] It is recommended to perform RRSO in women with mutations in BRCA1 between the ages of 35-40 years and, for those with BRCA2 mutations, between 40-45 years.[6]

The risk for breast and ovarian cancer in patients with BRCA1 mutations begins to increase around 30-40 years, while in patients with BRCA2 mutations, it increases approximately ten years later, allowing for a postponement of surgery in the latter.[6,8] However, delaying RRSO should be avoided to prevent reducing its protective effect against breast cancer, so it should be indicated for women aged 35-40 years with defined offspring or earlier when there is a family history of early-onset cancer.[6]

Induced menopause

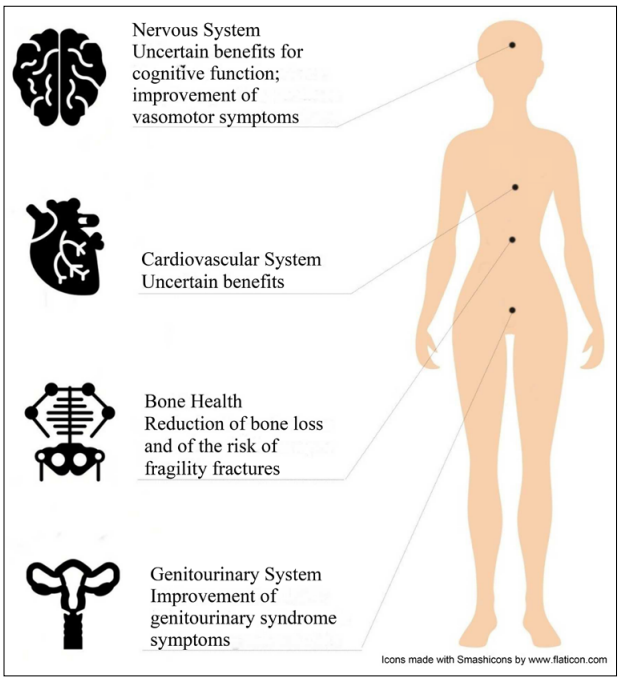
Induced menopause is the term recommended by North American Menopause Society (NAMS) to define the cessation of menstruation after bilateral oophorectomy or iatrogenic ovarian ablation after chemotherapy or radiotherapy.[9] The symptoms of induced menopause are usually more intense than those associated with natural menopause due to the sudden onset of symptoms, younger age, and their effects on physical and psychological problems characteristic of cancer therapy, such as concerns with body image and sexual dysfunction.[10] In these patients, induced menopause can lead to adverse health outcomes, including cardiovascular diseases, osteoporosis, and cognitive impairment. Estrogen deficiency can also result in vasomotor symptoms such as vaginal dryness, fatigue, and mood and sleep changes, adversely affecting the quality of life.[10,11]

Thus, the management of the climacteric symptoms is essential to optimize the quality of life of patients. Estrogen replacement therapy is considered the most effective treatment for vasomotor symptoms, in addition to providing benefits regarding osteoporosis and cardiovascular disease.[12] However, the potential hormonal stimulation of these tumors and the risk of breast cancer pose a concern regarding the safety of hormone therapy (HRT) in this population. The decision to indicate or contraindicate HRT for patients with mutations in the genes BRCA 1 and 2 after RRSO should be based on the best available evidence.[10]

Impacts of hormone replacement therapy

If there is no contraindication due to cardiovascular risk, the first choice of treatment for vasomotor

FIGURE 1. BENEFITS OF HORMONE REPLACEMENT THERAPY (HRT) AFTER RISK-REDUCING SURGERY IN PATIENTS WITH BRCA1/BRCA2 MUTATIONS.



symptoms is systemic estrogen replacement therapy. In patients with hormone receptor-negative tumors submitted to hysterectomy, it is recommended to use the lowest dose of estrogen for the minimum possible time. If the uterus was preserved, it is necessary to associate progestogen.[12]

Genitourinary and vasomotor symptoms and quality of life

Vasomotor symptoms are usually characterized by a sudden onset of heat sensation in the upper chest and face, which can become widespread. It usually lasts between 2 and 4 minutes and may be associated with profuse sweating, palpitation, and anxiety.[13] The combination of vulvovaginal atrophy and urinary tract dysfunction constitutes the "genitourinary syndrome of menopause".[13] This is clinically manifested as vaginal dryness, dyspareunia, dysuria, bladder hyperactivity, and recurrent urinary infections.

Estrogen replacement is the most effective treatment for controlling vasomotor symptoms and improving the quality of life of symptomatic women.[13] It is also associated with an improvement of symptoms of vulvovaginal atrophy, bladder hyperactivity, and recurrent urinary tract infections.[13] Studies show there are benefits from HRT to the quality of life of women with BRCA mutations who undergo risk-reducing salpingo-oophorectomy, with a reduction of vasomotor symptoms and improvement of sexual function.[8]

Bone health

The decline in the levels of estradiol after menopause increases bone resorption, contributing to fractures.[13] In this perspective, estrogen replacement reduces the bone loss associated with menopause and, consequently, the risk of fractures due to fragility.[13] In the case of women who underwent RRSO, the use of HRT also decreases the likelihood of bone disease.[8]

Cardiovascular health and cognitive function

During the reproductive period, women have a lower incidence of cardiovascular diseases in comparison with men, but this difference no longer exists after menopause due to the loss of the protective effect of estrogen on the lipid profile. However, there is not enough evidence to recommend the use of HRT for primary or secondary prevention of cardiovascular diseases. Even so, it is important to highlight that HRT can be linked to the reduction of cardiovascular risk in women who underwent RRSO in pre-menopause.[8]

The existence of benefits from HRT on the cognitive function of women after menopause is still uncertain[13]. Specific studies on the impact of HRT on cardiovascular health and cognitive function in women with BRCA mutations are scarce.[8]

Risk of breast cancer

Several studies have shown an increased risk of breast cancer with HRT after menopause, particularly with the combined replacement of estrogen and progesterone.[8,13] These findings led to a great concern regarding hormone replacement in women who already have an increased risk of developing the disease, such as carriers of BRCA mutations.

However, based on studies carried out in this specific population, the reduction of breast cancer risk with pre-menopause RRSO in women with BRCA mutations and no personal history of breast neoplasias is not affected by the use of HRT.[8]

Among these studies, there is a meta-analysis conducted by Marchetti et al.[14] based on three cohorts (Kotsopoulos et al.[17]; Gabriel et al.[18]; Rebbeck et al.[19]), which together included 1,100 women with BRCA1 and BRCA2 mutations who underwent RRSO. It was concluded that HRT did not significantly affect the risk of breast cancer in this population, both when considering all cohorts (RR = 1.01; 95% CI = 0.16-1.54) or only the prospective cohorts (RR = 0.98; 95% CI = 0.63-1.52).

In addition, the analysis of a subgroup of women based on the formulation of the HRT used demonstrated that estrogen alone presented a lower risk for breast cancer in comparison to combined HRT. In total, among users of HRT, 326 used estrogen replacement alone and 114 used a combination of estrogen and progesterone and found no significant difference regarding the risk of breast cancer when comparing both groups. However, the risk of breast cancer risk was lower for women who used estrogen alone, both in the study population as a whole (OR = 0.62; 95% CI = 0.29-1.31) and in that included only in prospective studies (OR = 0.53; 95% CI = 0.25-1.15).[14]

In this perspective, the systematic review carried out by Gordhandas et al.[8] based on 100 papers with the keywords "hormone replacement therapy", "BRCA" and "risk reduction" came to similar conclusions both on the absence of significant differences in the risk of breast cancer with the use of hormone replacement

therapy in women carriers of BRCA mutations and regarding the possible superiority of the use of estrogen alone in this population.

It is also important to highlight that the breast cancer in women with BRCA1 mutations usually has a triple-negative phenotype, i.e., hormone receptor-negative, while women with BRCA2 mutations generally present neoplasms with the expression of estrogen and progesterone receptors. So, is it possible that hormone replacement therapy impacts the risk of breast cancer differently in these two groups of patients.[8] In this context, it is necessary to observe that most patients in the studies mentioned above include BRCA1 mutations and that usually there are scarce data on the use of hormone replacement therapy in patients with BRCA2 mutations. Therefore, although HRT seems a plausible option for managing the symptoms of menopause, the data available on its safety must be analyzed with caution in the context of women with BRCA2 mutations.[14]

Other prophylactic surgeries

Hysterectomy

Prophylactic hysterectomy for patients with BRCA mutations is not recommended as routine.[15] However, estrogen replacement alone is associated with increased risk of endometrial cancer and RRSO without concomitant hysterectomy increases the risk of this cancer being of the serous type and histologically more aggressive.[14]

Some evidence suggests that the hysterectomy concomitant to RRSO could be considered for the following reasons: theoretical risk of cancer of the remnant uterine tube; reducing the risk of endometrial pathology in women with breast cancer prior to RRSO who are on tamoxifen, and simplification of a possible HRT, making it possible to use estrogen alone, which does not alter the risk of breast cancer[6].

Salpingectomy

Recent data indicate the distal portion of the uterine tube as the place of origin of most serous ovarian cancers of high degree. This etiopathogenic hypothesis increases the possibility of two-step surgery for patients with BRCA1/2 mutations: a pre-menopausal risk-reducing salpingectomy and a late oophorectomy[11,16]. However, there are still important questions on the effectiveness, potential impact on ovarian function, and most appropriate allocation of this strategy in medium- and high-risk women[16].

Non-hormonal alternatives for the relief of symptoms

As illustrated in Table 3, among the non-hormonal medications that have demonstrated effectiveness in controlling vasomotor symptoms are paroxetine (7.5 mg/day), venlafaxine (75 mg/day), and gabapentin (900-1.200 mg/day). For the management of genitourinary syndrome symptoms, topical estrogen therapy may be the most appropriate, particularly in cases of moderate or severe symptoms that did not improve with the use of vaginal moisturizers.[12]

TABLE 3. NON-HORMONAL OPTIONS FOR THE GENITOURINARY SYNDROME OF MENOPAUSE.

Preparation	Composition	Effects
Vaginal moisturizers	Preparations based on polycarbophil, hyaluronic acid, polyacrylic acid, or pectin.[13]	When used regularly (at least twice a week) can provide an effective non-hormonal approach for the relief of symptoms of vaginal atrophy. Although useful, these are probably inferior to therapy with estrogen. In addition, they did not provide any reduction in the symptoms of the lower urinary tract or asymptomatic bacteriuria.[13]
Vaginal lubricants	Various presentations, usually water- or silicone-based.[21]	They are used to temporarily alleviate (during sexual activity) dryness and dyspareunia. They do not revert changes caused by vaginal atrophy.[13]

FINAL CONSIDERATIONS

Women with mutations in the genes BRCA1 and BRCA2 have an increased risk of developing ovarian and breast cancer throughout life compared to the general population. The most effective strategy for reducing this risk is RRSO. However, induced menopause is one of its consequences, which generates negative impacts, affecting the quality of life and longevity. HRT is the only measure capable of significantly compensating hormonal deprivation and neutralizing the symptoms of menopause.

Although evidence suggests that HRT does not decrease the protective effect of RRSO in patients with mutations, the security concerns regarding estrogen and progesterone intake reduce their use in this scenario. There are also data demonstrating that the use of estrogen alone after RRSO does not increase the risk of breast cancer among women with BRCA1 mutations. The effects of progestogen hormone replacement therapy in patients with mutations, however, require further studies.

RESUMO

Mulheres portadoras de mutações nos genes BRCA 1 e 2 possuem risco aumentado para cânceres de ovário e mama e, portanto, são candidatas às cirurgias redutoras de risco, incluindo a salpingo-ooforectomia e a mastectomia. A salpingo-ooforectomia redutora de risco (SORR) é considerada a medida profilática mais efetiva para prevenção do câncer de ovário nesse grupo de pacientes. Esse procedimento implica a perda da função ovariana e menopausa induzida. A estrogenioterapia é o tratamento mais efetivo para o controle de sintomas vasomotores e melhora da qualidade de vida de mulheres no climatério. No entanto, a potencial estimulação hormonal desses tumores e o risco de câncer de mama constituem uma preocupação com a segurança da terapia hormonal (TH) nesta população. Este artigo tem como objetivo uma revisão das evidências atuais quanto aos benefícios potenciais e segurança da TH após SORR.

PALAVRAS-CHAVE: *Mutação. Terapia de reposição de estrogênios. Salpingostomia. Ovariectomia. Salpingo-ooforectomia. Neoplasias da mama. Neoplasias ovarianas.*

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REVIEW ARTICLE

The south american context of diagnostic disclosure of adolescents infected by HIV/AIDS: a systematic literature review

Patrícia Neyva da Costa Pinheiro[1]
Bernard Carl Kendall[2]
Ligia Regina Franco Sansigolo Kerr[3]
Keith Michael Pickett[4]
Izaildo Tavares Luna[1]
Maria Isabelly Fernandes da Costa[1]
Luisa Fânia da Costa Luz[5]

1. Universidade Federal do Ceará – UFC, Fortaleza, CE, Brasil.
2. Tulane University, Research Support & Education Librarian – Tulane, New Orleans, EUA.
3. Doutora em Medicina pela Universidade de Ribeirão Preto. Professora titular da Universidade Federal do Ceará – UFC, Fortaleza, CE, Brasil.
4. Master of Library and Information Science. Research Support & Education Librarian, Tulane University, New Orleans, LA, EUA.
5. Licenciada em Letras. Especialista em Língua Portuguesa pela Universidade Estadual do Ceará – UECE, Fortaleza, CE, Brasil.

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SUMMARY

OBJECTIVE: *To analyze the scientific evidence on the disclosure of the diagnostic of adolescents infected by HIV/AIDS in the South American context.*

DATABASE: *Systematic literature review using the PubMed, Cinahal, Embase, Cochrane, BVS, and Global Health databases and the descriptors: adolescent and HIV and family and Argentina or Bolivia or Brasil or Chile or Colombia or Ecuador or French Guiana or Paraguay or Peru or Uruguay or Venezuela.*

DATA SYNTHESIS: *Brasil was the country highlighted. It was verified that parents have a direct and indirect influence over the adolescents' life, especially regarding behaviors and health care. Dialog among family members can reduce adolescents' vulnerability to HIV and encourage diagnostic disclosure.*

CONCLUSION: *It is necessary to amplify research involving adolescents with HIV/AIDS and their parents/caregivers and family members to improve care and reduce the cases of the disease. It is suggested that policies of prevention and treatment should involve families, caregivers, partners, and the community.*

KEYWORDS: *Family. Adolescent. HIV. South America.*

INTRODUCTION

Adolescence is characterized by various transformations that permeate the biological, psychological, social, and emotional aspects that influence decision making regarding adulthood and the construction of individual, sexual, and social identity.[1,2] The changes that occur in the life of adolescents expose them to the

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CORRESPONDING AUTHOR: Patrícia Neyva da Costa Pinheiro
Rua Alexandre Baraúna, 1115, Rodolfo Teófilo, CE , Brasil – 60430-160
Tel: +55 85 3366-8455 / Fax: +55 85 3366-8456
E-mail: neyva.pinheiro@yahoo.com.br

most diverse types of vulnerabilities, among these, the infection by HIV/aids.[3]

HIV/AIDS is considered a serious public health issue, and among adolescents it becomes even more worrying, considering the incidence of over 2,400 adolescents/young people infected daily and the over 10 million people aged between 15 and 24 years living with HIV/aids worldwide. The index of aids among young people and adolescents aged from 15 to 19 years increased from 2.8 cases per 100 thousand inhabitants to 5.8 cases.[4] In addition, aids is considered the eighth leading cause of death among adolescents throughout the world, and its prevention and treatment are some of the most important challenges for public health.[5]

The strategies targeted at the prevention and treatment of seropositive adolescents are directly linked to their families, considering that the participation of this support network influences the treatment, with adherence to the antiretroviral therapy (ART) and changes in routine and behaviors, often based on beliefs, values, and habits learned from an early age.[6]

However, crises arising from difficulties in the relationship between parents and adolescents are closely related to the absence of dialog and difficulty of understanding between these two worlds. In this sense, parents and children create barriers for dialog, as a result, adolescents seek other sources of information, mainly friends and the media.[7,8]

Dialog can be an important tool when it comes to safe sex practices since the family can positively contribute to prevention and treatment, minimizing prejudices and taboos related to the coexistence with a seropositive adolescent. Thus, it is necessary to have an open and welcoming dialog, especially during the disclosure of the diagnosis. In addition, it is necessary to highlight the importance of research on the disclosure of diagnosis that results in evidence that provides refuge to adolescents infected by HIV.[9,10]

Thus, this study aims to analyze the scientific evidence on the disclosure of the diagnosis of adolescents infected by HIV/aids in a South-American scenario.

METHODS

A systematic review of the literature was carried out outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Prism), organized and completed independently by the researchers.[11] The search was performed in the following databases: PubMed, Cinahal (Cumulative Index to Nursing & Allied Health Literature), Embase (Excerpta Medica para banco de dados), Cochrane (Central Register of Controlled Trials), BVS (Biblioteca Virtual em Saúde), and Global Health, using the controlled descriptors indexed in the DeCS (*Descritores em Ciências da Saúde*)[12] along with the name of each country, in April and May 2014.

The descriptors used in the search were: *adolescent and HIV and family and Argentina or Bolivia or Brasil or Chile or Colombia or Equador or French Guiana or Paraguay or Peru or Uruguay or Venezuela*. Only the boolean operator (delimiter) *and* was used, assuming that, during the search, each country acted uniquely in the combination with the other descriptors.

We used End Note®, an online tool for managing references integrated with the Web of Knowledge, and various databases as aiding tools for data organization. To achieve the objective proposed, a question was formulated based on the PICO (Patient Intervention Comparison Outcome) strategy: What is the scientific evidence published in the following databases on the disclosure of the diagnosis of South American adolescents infected by HIV/AIDS?

We compared a database of 430 papers generated by the keywords *adolescent and HIV and family and names of South American countries,* with a database of 4,553 papers generated by the descriptors *adolescent and HIV and names of South American countries* and there were no differences, i.e., the papers found were the same. It is necessary to highlight that these studies were all carried out in Brasil. The analysis of the studies was carried out independently, based on their critical reading and the interpretation of the information, comparing the findings with the literature.

RESULTS

In total, 430 papers were found; however, from the reading of their titles, 415 were excluded, remaining only 15 papers. Of these, five were duplicated, so there were ten papers left. After reading the abstracts, four papers were excluded (extended abstract in annals; population of the study related to children up to 6 months of age; research conducted in Canada and without any relation with South American countries, and a book chapter). Of the six papers selected, all were carefully read and none were excluded (Figure 1).

Among the six studies, there was a prevalence of studies published in 2008, followed by 2006, 2012, and 2013. The summary of the six studies identified

regarding their authors, years, periodicals, titles, designs, locations, and samples are presented in Table 1. The scope, intervention, comparison, results, and evidence can be found in Table 2.

Regarding the design of the studies, qualitative studies were prevalent, and there was only a single quantitative study. The sample comprised 338 adolescents; of these, 296 were part of the cross-sectional quantitative study, and 42 were distributed in the qualitative studies, ranging from 4 to 22 adolescents per study. Among the 12 South American countries, only Brasil, most prominently the state of São Paulo, had studies that met our criteria.

The disclosure of the diagnosis was considered a milestone in the life of adolescents and is permeated by the fear of prejudice and social isolation, in addition to influencing the quality of life of adolescents and their families. Vertically infected adolescents receive a different treatment, and the adherence to the treatment and questions made were considered facilitators.

However, it was observed that adolescents who live with their parents are less exposed to risk situations; it is considered that the influence of parents contributes to the building and adoption of healthy behaviors among adolescents. However, the difficulty in dialogs on sex and sexuality exposes adolescents to situations of vulnerability. This difficulty in dialog may be associated with low parental schooling.

FIGURE 1. FLOWCHART OF PAPER IDENTIFICATION AND SELECTION FOR A SYSTEMATIC REVIEW ON THE ROLE OF FAMILIES WITH ADOLESCENTS IN THE CONTEXT OF SOUTH AMERICAN COUNTRIES, 2014.

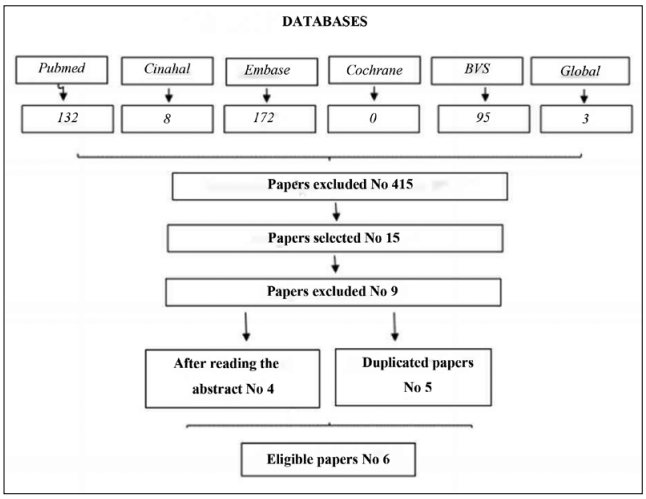

TABLE 1. CHARACTERISTICS OF STUDIES ON THE FAMILIES OF ADOLESCENTS INFECTED BY HIV/AIDS IN BRASIL, 1996 TO 2014.

Code	Author, Year, Place	Title/ Journal	Design/Sample/Problem
1	Marques *et al.*, 2006; São Paulo and Santos	Disclosure of HIV infection from the perspective of adolescents living with HIV/aids and their parents and caregivers- *Cad. Saúde Pública*	- Qualitative; Study; 22 adolescents and 13 cares - HIV Seropositivity
2	Peres *et al.*, 2008; São Paulo	Family Structure and Adolescent Sexual Behavior in a Poor Area of São Paulo, Brazil- *Journal of Adolescet Health*	- Cross-sectional study; 296 young people/adolescents- Vulnerability to HIV
3	Lima & Pedro, 2008; Porto Alegre, RS	Growing up with HIV/aids: a study on adolescents with HIV/aids and their family caregivers- *Rev Latino-am Enfermagem*	Exploratory study with a qualitative approach - Growth and development with HIV
4	Barbosa *et al.*, 2008; Fortaleza	Stages of change in parents discussions with their children about HIV/aids prevention- Revista Latino America de Enfermagem	- HIV Prevention
5	Galano *et al.*, 2012; São Paulo	Interviews with family members: the essential tool for planning the disclosure of the diagnosis of HIV/aids for children and adolescents- *Ciência & Saúde Coletiva*	Qualitative study; 23 parents/relatives- HIV Seropositivity
6	Cammarano Ribeiro *et al.*, 2013; Rio Grande do Sul	Therapeutic everyday of adolescent who has HIV/aids: self-caring occupation and family solitude- *Ciência, Cuidado & Saúde*	Qualitative study with phenomenological approach and philosophical theoretical methodological; 16 adolescents- Growth and development with HIV

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TABLE 2. CHARACTERISTICS OF STUDIES ON FAMILY AND TEENAGERS IN THE SCENARIO OF THE AIDS PANDEMIC, ACCORDING TO PROBLEM, INTERVENTION(S), COMPARISON, RESULTS, AND EVIDENCE, BRASIL, 1996 TO 2014.

Intervention	Code/Comparison	Result/Evidence
1	Disclosure of the diagnosis/ *Enhancing Care Initiative* (ECI)	The disclosure of the diagnosis is a milestone in the life of the adolescents and families. The fear of prejudice, discrimination, and social isolation are complicating factors in the disclosure of the diagnosis. On the other hand, the need for adherence to treatment and questions are facilitators. Adolescents who became infected by vertical transmission have different care in comparison to others. The demand is created by the family and health team.
2	Influence of parents on the behavior of children/ Studies from Latin America, Caribbean and United States	Adolescents living with parents are less exposed to risks. The influence of one or both parents in the life of adolescents contributes to reducing the risks to which they are exposed. Efforts focused on the reduction of risks in adolescence should involve the parents.
3	Treatment, disclosure of the diagnosis, influence of parents and teachers;	Changes in the quality of life of adolescents and their relatives. Changes in lifestyle due to the treatment and care. Confidentiality regarding the disclosure of the diagnosis to society for fear of prejudice, discrimination, and social isolation. No approach from the family, school and health services regarding bodily changes , sexual and reproductive health of these adolescents. Strong influence of the media.
4	Dialog	Adolescents are vulnerable to risks because parents find it difficult to talk with their children about sex/sexuality. Mothers are more present than fathers in the education of their children. Low parental schooling hinders dialog. The inability of parents to establish dialog increases adolescents' vulnerability to HIV/aids. Efforts focused on the prevention of HIV should involve adolescents, families, schools, and health units.
5	Disclosure of the diagnosis; Handbook prepared by members of the Institute of Health of New York	The decision to disclose the diagnosis is a slow and painful process Fear of prejudice, discrimination, and social isolation, guilt, changes in mental health, and desire to live are complicating factors in the disclosure of diagnosis. On the other hand, the need for adherence to treatment, the ability to keep a secret, and questions are facilitators.
6	Treatment	Changes in the quality of life of adolescents. Changes in lifestyle due to the treatment and care. Need for strengthening of self-care strategies. Family was the main support source.

DISCUSSION

The disclosure of the diagnosis as a milestone in the life of the adolescents and their families was one of the main findings of the study. Three of the six studies addressed the disclosure of the diagnosis, with emphasis on studies carried out at Harvard University and the Merck Foundation. Some countries became participants of this project, among them Brasil (São Paulo and Santos), Senegal (Dakar), South Africa (KwaZulu-Natal), Thailand (Northern Region), and Puerto Rico. Ten priority areas of care were established, among them, the service diagnosis support to adolescents and nine recommendations to improve health care services for adolescents and their caregivers, highlighting relevant aspects such as the importance of raising awareness about the rights of young people, improving dialogs about stigmatization and discrimination, providing clear and updated information, and involving family members and close friends in the process of disclosure of the diagnosis.[13]

It is possible to have subsidies to disclose the diagnosis, with proper support, thus minimizing the negative aspects and strengthening the positive ones. The main negative factors that hinder the disclosure of the diagnosis were stigma, prejudice, and discrimination; the positive ones were adherence to the treatment and care.[2,4]

The stigma, prejudice, and discrimination experienced by many people living with HIV/aids and their families have been discussed internationally, mainly by the United States, since it has a discriminatory and exclusive nature that can compromise the life conditions and health of individuals and communities.[14]

Therefore, this stage of the lives of theses adolescents requires care, efficiency, and awareness of the professionals and families involved. It is important to take into account the influence of parents in their children's risky behaviors because this has a direct and indirect impact on the lifestyle and daily routines of seropositive adolescents.

One of the studies that stood out was developed by the Aids Institute of the New York State Department of Health and included the preparation of a handbook with recommendations on the disclosure of the diagnosis, the type of approach, best time, need for a multi-professional team and family support. The handbook also covers intra-related topics, such as that of caregivers to establish a care plan, specific ages and considerations for the disclosure; ways to prepare the family, considerations for disclosure with adolescents, and monitoring and support visits to adolescents.[15]

This condition, caused by disadvantaged situations, such as seropositivity for HIV, can lead to social rejection and endanger the physical and mental health of individuals. Therefore, social psychology seeks to highlight ways of tackling stigma, prejudice, and discrimination.[16] However, oftentimes, this aspect is not valued, considering the great importance given to studies on the perception of risk and behaviors in specific groups and, in particular, on the adherence to the treatment.[16]

The possibility of better adherence to treatment, through awareness and positive health behavior, is seen as a positive factor that encourages the disclosure of the diagnosis. It is known that adequate adherence to highly active antiretroviral therapy (ARVT), introduced in 1996, has had a major positive impact in the reduction of morbidity and mortality of people with HIV/aids. In Brasil, it has reduced mortality by approximately 70% and the incidence of opportunistic infections by 80%.[17] However, adherence to the treatment has many complicating factors, such as the conciliation between the daily activities, treatment demands, and the negative connotation that HIV and ARVT have to many individuals.

ARVT influences the prevalence of hyperlipidemia and the redistribution of fat during puberty, being significantly associated with sexual maturity. Changes in the body and self-perception have both hampered the adherence to the treatment as well as increased the incidence of depression. Thus it is necessary to have, in this process, the guidance and support of parents and caregivers, creating support groups and networks, as well as training the professionals involved.[12,18]

The role of friends can also be considered a positive factor, given their importance in the life of adolescents. Young people feel more comfortable talking about various subjects, including sex and sexuality, and feel supported to return to consultations and seek therapeutic assistance in the company of friends. Thus, in addition to increasing support for families and caregivers, it is important to improve strategies targeted at peers. Two of them are counseling and voluntary testing since these favor the approximation between the adolescents and their families, communities, peers, and services.[19]

However, the support of friends is seen as more relevant to teenagers who become infected through sex or blood, because many parents, caregivers, and professionals demonstrate greater care with those infected by vertical transmission, providing, therefore, greater support for them. This leads us to reflect on the symbolic representation of being infected by one's mother or by other means, such as the emotional involvement of professionals and family members, the need to live with the condition from birth, and the high incidence and mortality of transplacental cases.[20]

Limited life conditions as a result of HIV seropositivity may influence psychosocial development, cognitive maturity, and even decrease of pubertal maturation. However, these adolescents need to recognize their condition and the importance of erotic and seductive play using condoms, thus reducing the risk of transmission. It is necessary to support subsidies so that these adolescents can have autonomy and be responsible for their decisions and sexual behaviors. To achieve that it is necessary to increase the discussion around the topic and expand the scope of research on the risks and problems to which this population is exposed.[21]

Parents and caregivers have difficulty in establishing a dialog about sex and sexuality with these adolescents, who still turn to friends for support and answers.[22] It is necessary to strengthen and adjust initiatives involving caregivers and family members, focusing on their physical, psychological, and social well-being and, consequently, providing positive support for adolescents with HIV, thus improving the quality of life of parents, carers, children and adolescents.[23]

Communication between parents and adolescents can, directly and indirectly, influence their attitudes, beliefs, intentions, and behaviors towards sex and condoms. Therefore, it is necessary to identify the communication barriers so that it becomes increasingly effective, thus minimizing the risks and enabling a healthy sex life.[24] Studies developed in Ghana and Tanzania have shown that dialogs between parents and adolescents have grown considerably, particularly concerning the use of condoms and sex. However, it is necessary to expand research on the dialog between children and parents.[22]

Critical and reflexive dialog enables the critical thinking and empowerment of individuals and communities, thus impacting the promotion of health and determinants of health, disease, and care, particularly in South America, which requires changes in the living conditions and health of the population. Self-determination towards change is related to empowerment, which is understood as a social, cultural, psychological

and/or political process that can take place at an individual or community level.[24]

CONCLUSION

Care for HIV seropositive adolescents, as well as to their family members and caregivers, must involve psychological and emotional support, awareness about the treatment, changes in lifestyle and habits, promotion of quality of life, healthy sexuality, use of condoms, combat against prejudice, discrimination, and social isolation.

It is necessary to carry out new studies that allow knowing and comparing results that can assist in the decision making of health policies to the prevention and assistance of adolescents in the midst of the HIV/AIDS pandemic in Brasil and in Latin America.

The scarcity of research on the topic leads us to reflect on some aspects, such as: limited experience in the treatment of children and adolescents, the irrelevance of the problem in South American countries, lack of scientific production in many countries, as well as the possibility of the descriptors used not being indexed in the DeCS or not portraying the content of the research.

Regarding the implications and contributions to practice, the research highlights the need for care initiatives with goals and objectives that enhance interdisciplinary actions guided by scientific knowledge, focusing on the particularities of different cultures, values, and beliefs of adolescents and their families in the context of stigma, prejudices, social isolation and recovery of resilience, social support, and empowerment.

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Author's Contribution

All authors contributed equally to this study.

Statement of conflict of interests

Nothing to declare.

RESUMO

OBJETIVO: *Analisar as evidências científicas acerca da revelação diagnóstica de adolescentes infectados pelo HIV/aids no contexto sul-americano.*

FONTES DE DADOS: *Revisão sistemática da literatura nas bases de dados PubMed, Cinahal, Embase, Cochrane, BVS e Global Health, utilizando os descritores adolescent and HIV and family and Argentina or Bolivia or Brasil or Chile or Colombia or Equador or French Guiana or Paraguay or Peru or Uruguay or Venezuela.*

SÍNTESE DOS DADOS: *O Brasil foi o país de destaque. Verificou-se que os pais exercem influência direta e indireta sobre a vida dos adolescentes, especialmente com relação aos comportamentos e cuidados de saúde. O diálogo entre os membros da família pode reduzir a vulnerabilidade dos adolescentes ao HIV e encorajar a revelação do diagnóstico.*

CONCLUSÃO: *É necessário ampliar a pesquisa envolvendo adolescentes, pais/cuidadores, famílias com HIV/aids para melhorar os cuidados e reduzir os casos da doença. Sugere-se que as políticas de prevenção e tratamento envolvam famílias, cuidadores, parceiros e comunidades.*

PALAVRAS-CHAVE: *Família. Adolescente. HIV. América do Sul.*

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REVIEW ARTICLE

The effectiveness of percutaneous injections of ozonotherapy in low back pain

Luciano Timbó Barbosa [1]
Célio Fernando de Sousa Rodrigues [1]
Raul Ribeiro de Andrade [2]
Fabiano Timbó Barbosa [1]

1. Universidade Federal de Alagoas (Ufal), Maceió, AL, Brasil
2. Centro Universitário Cesmac (Cesmac), Maceió, AL, Brasil

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SUMMARY

INTRODUCTION: *Lumbar pain is one of the main reasons for medical consultation, causing the disruption of daily routines due to its disabling nature, thus resulting in social and personal damage. Among the complementary treatments, ozonotherapy offers analgesia to most patients, with reports of complications. However, great questions about its clinical effectiveness have not been answered yet, and there have been reports of serious complications.*

OBJECTIVE: *To describe the use of ozonotherapy in the treatment of lumbar pain, focusing on its favorable and unfavorable effects, and its analog profile.*

METHODS: *A cross-sectional bibliographic research was performed with scientific articles obtained from the Pubmed, LILACS and Scopus database, using the following descriptors: "Ozone", "Therapy", "Lumbar pain", "complication", "Disk herniation", "Guideline", "Protocol", "Standards", "Criteria".*

RESULTS: *The researched literature corroborates that, in clinical practice, there is safety in the use of oxygen-ozone therapy through percutaneous injections for the treatment of lumbar pain, especially when compared to surgeries and use of medicines, provided that strict criteria are followed.*

CONCLUSION: *The procedure is effective and has a favorable analgesic profile. However, it is necessary to produce a medical guideline that will help in its strict and systematic control.*

KEYWORDS: *Ozone/therapeutic use. Low back pain. Spinal cord compression. Intervertebral disc displacement.*

INTRODUCTION

Low back pain is one of the main reasons for medical consultations, hindering the execution of daily activities, due to its incapacitating nature, thus resulting in social and personal expenditures and requiring preventive treatment[1-3]. Among complementary treatments, ozonotherapy has gained greater prominence after its use was authorized in the entire national territory of Brasil by the Senate Bill no. 9,001/2017[4]. In addition to being little invasive, it is a method that provides analgesia for most patients,

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CORRESPONDING AUTHOR: Luciano Barbosa
Universidade Federal de Alagoas (Ufal) – Campus A. C. Simões. Av. Lourival Melo Mota, S/N,
Tabuleiro do Martins, Maceió - AL, Cep: 57072-970
E-mail: nluciano.timbo@gmail.com

with minimal reports of complications[2,3,5]. However, important questions regarding its clinical effectiveness remain answered, in addition to some reports of serious complications[6], which have given cause for a letter by the Federal Council of Medicine (CFM), on 14 December 2017, condemning the bill, alleging lack of technical and scientific evidence of the method's efficacy and safety[7]. However, advanced studies on the treatment of different musculoskeletal disorders, such as rheumatoid arthritis, lumbar facet syndrome, joint syndrome, subacromial bursitis, carpal tunnel syndrome, osteoarthritis, hip bursitis, adhesive capsulitis of the shoulder, temporomandibular disorder, and mainly on the treatment of diseases of the spinal column, make ozonotherapy an option of effective treatment[8-10].

It is essential to know that around 70-80% of the world population has low back pain. Among these, the most frequent of radicular compressions is the disc disease, whose spontaneous resolution of pain in the acute phase (from 6 to 12 weeks after the onset of pain) has been documented in 60-80% of those affected by it, with the ability to resume work activities three months after the onset of symptoms[1,5,11].

Cases that are not spontaneously resolved are managed by conservative treatments, with good outcomes in 90% of lumbar pain cases[9]. The procedures are minimally invasive, such as corticosteroid, anti-inflammatory and anesthetic injections, chymopapain chemonucleolysis, nucleo-discectomy, intradiscal laser discectomy, intradiscal electrothermal therapy, percutaneous nucleoplasty, and chemodiscolysis using a mixture of oxygen and ozone (O_2O_3), the first choice, after the failure of others. The technique is supported by its favorable effects[4,12,13].

Surgery is performed in cases of intolerable symptoms, progressive neurological deficits, or risk of cauda equina syndrome[14,15].

The whole of the conservative propaedeutics along with minimally invasive procedures conceptualizes complementary treatments for pain relief and accelerated healing[3,5]. The goal of this paper was to describe the use of ozonotherapy in the treatment of lumbar pain, focusing on its favorable e unfavorable effects and analgesic profile.

METHODS

The bibliographic and transversal search for scientific papers was conducted in the PubMed (maintained by the National Center for Biotechnology Information), Lilacs, and Scopus databases. We used the following keywords: "Ozone", "Therapy", "Lumbar Pain," "Complication", "Herniated Disc","Guideline", "Protocol", "Patterns", "Criteria". The inclusion criteria were: scientific studies on humans, male or female, aged 18 years or more, diagnosed with lumbar pain. There were no restrictions regarding dates or languages to the publications included. Scientific studies related to the subject were selected after analysis of their titles and abstracts. Papers such as letters to the editor, communications, editorials, commentaries, and studies with partially published data were excluded.

HISTORICAL PERSPECTIVE

Ozone (O_3) has been studied for a very long time, its first observer was the philosopher and scientist Martin van Marum, who in 1783 felt a peculiar odor, which he subsequently named the "smell of electricity", near his electrostatic machine whenever it was working[16.17]. Another researcher, Cruickshank, reported the same characteristic smell in 1801[16.17]. However, the two only described the smell and did not do ahead with their studies[16.17]. However, a chemistry professor at the University of Basel, Christian Friedrich Schönbein, identified, in 1839, the same smell in water electrolysis and, in 1840, declared it was a gas, which he called ozone[16-19]. The molecular formula of ozone was determined in 1865 by Jacques-Louis Soret and confirmed in 1867 by Schönbein, who is considered its discoverer[16.17].

During the First World War, ozone was used for the first time, by Dr. Albert Wolf, as a groundbreaking therapy even in gangrenous injuries, paving the way for its use in medicine[3,13,14,19,20].

Subsequently, an attempt to cease lumbar pain using ozonotherapy was made in 1989, by Verga, an orthopedic surgeon who applied gas injections into sensitive areas of the paravertebral muscle, treating approximately 8,000 patients aged over 15 years; the recurrence of pain was less than 2%[18]. Eight years later, Fabris et al.[21] published a study reporting a new type of percutaneous treatment for lumbar herniated disc based on intradiscal injections of oxygen-ozone [20]. The first suggestion of using tomographies to guide needle introduction was made in 1998, by Muto et al.[22].

Several studies were published between 1989 and 2018 on the use of percutaneous injections of ozonotherapy for lumbar pain, and good efficacy and safety were observed, with an efficacy rate of 70-90%[20.21].

TYPES OF ADMINISTRATION, DOSES, AND CONCENTRATIONS

Ozone can be administered as a gas mixture of oxygen-ozone (medical-grade ozone), with a concentration between 10 and 40 µg/mL[23-26]. However, a study on the therapy with low (10 µg/mL), medium (40 µg/mL), and high (60 µg/mL) concentrations in patients with disc herniation showed a higher rate of decline in the group who received a volume of 40 µg/ml[25.26]. Table 1 summarizes the studies considered for long-term results.

The richest concentrations, which do not exceed a total of 5% of the total in ozone, are used in disc herniations[2,3,24], and the intramuscular, intradiscal, intraforaminal, and periradicular routes are very widespread in Europe[2,24,27,30]. Table 2 summarizes the application in various musculoskeletal diseases.

In disc diseases, the procedure is guided by computed tomography (CT) to avoid errors in the application and serious complications, and it must be accompanied by an anesthesiologist. However, intramuscular administration in the paravertebral lumbar muscles, bilaterally, with 10 to 20 ml on each side, using the lateral extraspinal approach, with asepsis prior to the procedure using 2% lidocaine and not exceeding 15 seconds in the time for injection, does not require the use of radiology since it is a simple procedure[23.29].

In all other routes, it is essential to be guided by CT, and they differ from the previously mentioned procedure since route used is towards the center of the disc by a posterior-lateral approach using a 10 ml oxygen-ozone mixture injection. Subsequently, the needle is redirected to the epidural space, where the tip of the needle should be in the middle point of the intervertebral foramen under lateral view and in the inner margin of the pedicle under anteroposterior view. Still using radiographic imaging, then another 10 ml are injected in the foramen space[31].

MECHANISM OF ACTION

Its use, in pain management, is based on the exploitation of the chemical properties of ozone (Figure 1) acquired from the unstable quality of oxygen, which allows the formation of one or more different simple substances and their direct (mechanical) and indirect (anti-inflammatory) effects[1,23,29,31].

The first consists of the destruction of the disc

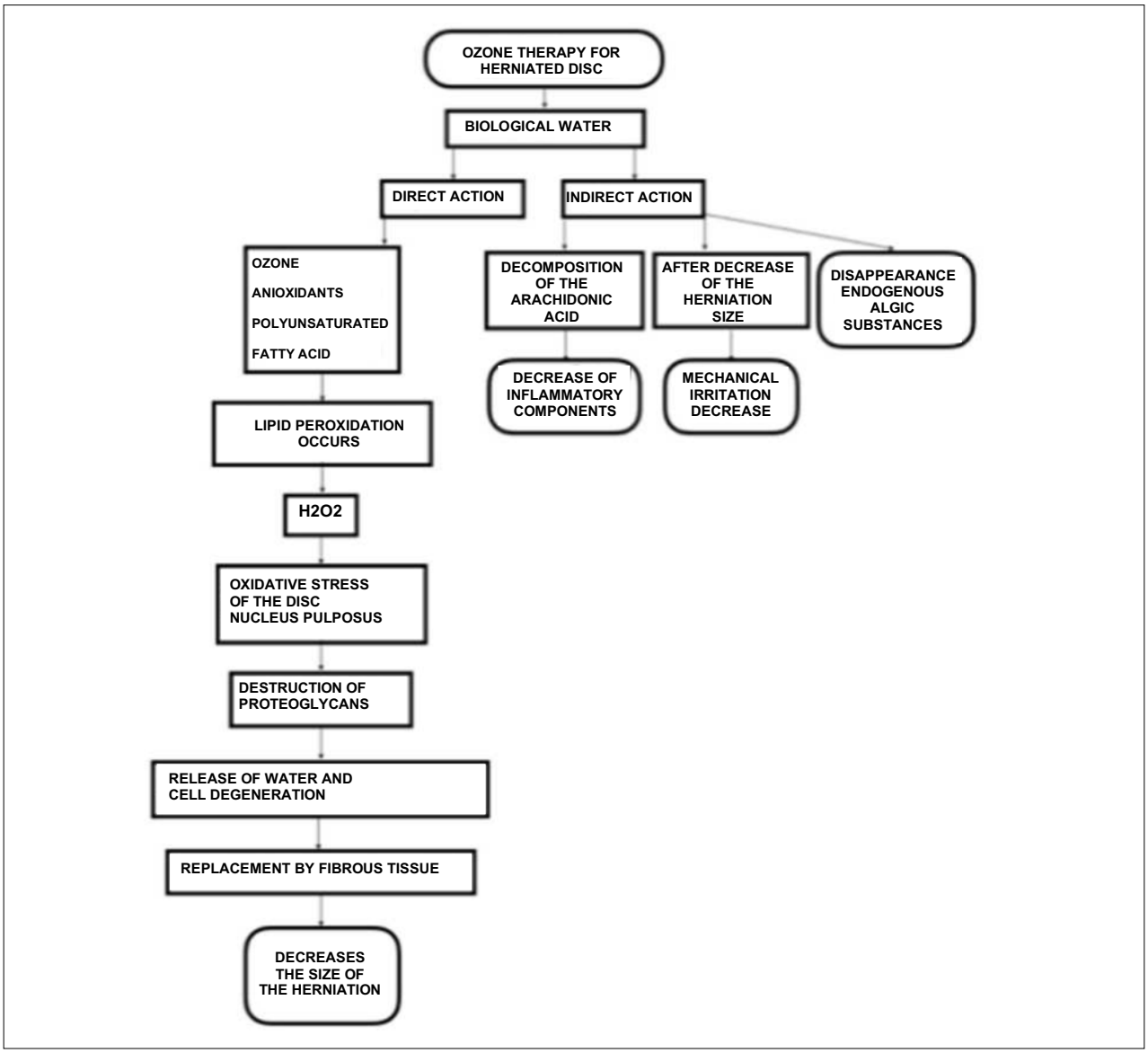
TABLE 1. PAPERS PUBLISHED REPORTING LONG-TERM RESULTS.

STUDY GROUP	YEAR OF PUBLICATION	PATIENTS ENROLLED	PROCEDURE	FOLLOW-UP TIME	CONCENTRATION USED IN THE STUDY
Niu et al [25]	2018	80	Paravertebral intramuscular	1 year	20 pg/ml in Group B, 40 pg/ ml in Group C, and 60 pg/ml in Group D
Hosseini et al [26]	2019	128	Paravertebral intramuscular	1 year	29 to 32 pg/ml
Biazzo et al [23]	2018	109	Paravertebral intramuscular	4 years	27 pg/ml
Bhatia et al [27]	2019	500	Percutaneous injection to the nucleus pulposus	1 years	30 pg/ml
Barbosa et al [3]	2017	19	Epiduroscopy	1 year	30 pg/ml
Bonetti et al [28]	2016	84	Intraforamen	13 years	25 pg/ml
Apuzzo et al [11]	2014	923	Paravertebral intramuscular	12 years	20 pg/ml
Paoloni et al [29]	2009	327	Paravertebral intramuscular	2 years	20 pg/ml

TABLE 2. SUMMARY OF APPLICATIONS IN SEVERAL MUSCULOSKELETAL DISEASES.

MUSCULOSKELETAL DISEASES	ADMINISTRATION	DOSAGE	CONCENTRATION
Lower back pain	Paravertebral Intramuscular	10 to 20 ml on each side	40 pg/ml
Herniated disc	Intradiscal injection	10 ml	40 pg/ml
Shoulder (glenohumeral joint)	Injection with a posterior approach laterally under the lower margin	10 ml	15 pg/ml
Hip bursitis (inflammation)	Injection with a lateral approach with an infiltration method called peritrochanteric.	10a15ml	25 pg/ml
Osteoarthritis (OA) (knee)	Variable approach, predominantly external/ internal lateral parapatellar	5 to 20 ml	15 to 40 pg/ml
Cervical herniated disc	Intradiscal injection	5 ml	20 pg/ml

FIGURE 1. MECHANISMS OF ACTION.



nucleus pulposus components, resulting in a release of water molecules and the subsequent cellular degeneration of the matrix, which is then replaced by fibrous tissue, leading to a reduced disc volume[24,27,28,32].

The ozone reacts with the antioxidants and polyunsaturated fatty acids (PUFAs) present. Lipidic peroxidation leads to the simultaneous formation of reactive oxygen species (ROS) and lipid oxidative products (LOPs)[25].

One of the ROS is hydrogen peroxide (H_2O_2), a non-radical oxidant capable of acting as an ozone messenger, responsible for causing several biological and therapeutic effects[25].

The production of LOP follows the peroxidation of polyunsaturated fatty acids (PUFAs). They are inherently toxic and should be generated at very low concentrations[28].

The ozone reacts as soon as it is dissolved in biological water (the nucleus pulposus is located in the central disc has a gelatinous water content of approximately 75% to 80%), causing an oxidant action and decomposing some of the glycosaminoglycans chains in the nucleus, reducing its ability to retain water, decreasing the size of the herniation and, subsequently, contributing to reducing the impact of the herniation on the venous and arterial flow, causing hyperoxygenation[11,25,33].

The indirect effect is perceived by modifying the decomposition of arachidonic acid in inflammatory prostaglandins. As a result, there is a decrease in inflammatory components and pain regression[8,27,29]. In addition, the disappearance of the herniated material decreases mechanical irritation, reducing axon sensitivity. On the other hand, endogenous analgesic

substances released during perineural ischemia or neural inflammation present in the spinal ganglion and nerve roots that can stimulate the nociceptors cease to exist[3].

A recent study has raised the hypothesis that after the administration of percutaneous injections humoral immunity is suppressed, leading to low levels of IgG and IgM. Consequently, the pain score in the Visual Analog Scale (VAS), which uses a 10 cm ruler and a 10-point scale, with zero indicating no pain and 10 the most severe pain, in addition to intermediate scores from 1 to 3, which indicate mild pain, and 4 to 6, which indicate moderate pain that affects sleep. However, its validation was limited by the short follow-up period of only 12 months, indicating that in order to subscribe to his theory, the long-term effectiveness requires further studies[25].

COMPLICATIONS

Due to its improper use by charlatans and unprepared doctors, erroneous indications in the recent past have made its use dangerous, thus contributing to a bad reputation of the therapy[5,19], causing some states of the United States[7,19], as well as in Brasil[7], to withdraw permissions of its use. Therefore, it is vital to know possible complications.

However infrequent, and even considering the absence of adverse effects when using the concentrations already mentioned in this article, complications should be considered, such as: thunderclap headaches due to pneumocephalus, with only three cases reported in the literature[14,23]; Anton Syndrome, only three cases up until now[6]; reduction of visual acuity; vitreous hemorrhage; subcutaneous hematoma; syncope; air embolism; vertebrobasilar ischemic cerebral accident; pyogenic discitis; lesion to the dorsal and ventral root, causing sciatic pain; and local infection due to lack of proper hygiene and asepsis[3,15,34-36].

FINAL CONSIDERATIONS

The literature confirms that, in clinical practice, it is safe to use oxygen-ozone therapy through percutaneous injections for the treatment of lumbar pain, particularly when compared to surgery and drug therapy[31]. The complications are minimal, provided the strict criteria in the patient selection are followed, with the presence of disc herniations visualized by computed tomography or guided by fluoroscopy, with an accurate diagnosis of spinal level, and trained and experienced physicians to perform the procedure, except for the intramuscular approach. It is also required to use the correct dosage of ozone, and follow-up after the procedure must be conducted by a neuroradiologist[3,7,28,33,37].

CONCLUSION

We conclude that minimally invasive therapy with oxygen-ozone and its minor complications or side effects make this procedure effective and with a favorable analgesic profile. However, it is necessary to draw up medical guidelines to assist in strict control and systematic procedures, thus avoiding damage to patients, since we could not find any in the publications analyzed.

Conflicts of interest

The authors declare there are no conflicts of interest in the present paper.

Author's Contribution

Luciano Timbó Barbosa: Assessment and selection of papers and preparation of the manuscript.

Célio Fernando de Sousa Rodrigues: Assessment and correction of the manuscript.

Raul Ribeiro de Andrade: Assessment and selection of papers. Fabiano Timbó Barbosa: Assessment and correction of the manuscript.

RESUMO

INTRODUÇÃO: *Dor lombar é um dos principais motivos de consultas médicas, provocando afastamento das rotinas diárias, por ser incapacitante, resultando em danos sociais e pessoais. Dentre os tratamentos complementares, a ozonioterapia oferece analgesia para a maioria dos pacientes e com mínimos relatos de complicações. Entretanto, grandes questionamentos sobre sua efetividade clínica ainda não foram respondidos, além de haver relatos de complicações graves.*

OBJETIVO: *Descrever o uso da ozonioterapia no tratamento da dor lombar, enfocando seus efeitos favoráveis e desfavoráveis, e seu perfil analgésico.*

MÉTODOS: *Foi realizada uma pesquisa bibliográfica transversal com artigos científicos obtidos das bases de dados PubMed, Lilacs e Scopus, utilizando os descritores: "Ozônio", "Terapia", "Dor lombar", "Complicação", "Hérnia de Disco","Diretriz", "Protocolo", "Padrões", "Critérios".*

RESULTADOS: *A literatura pesquisada corrobora que, na prática clínica, há segurança na utilização da terapia com a mistura oxigênio-ozônio por meio de injeções percutâneas para o tratamento de dor lombar, principalmente quando comparada às cirurgias e ao uso de medicamentos, desde que sejam seguidos critérios rígidos.*

CONCLUSÃO: *O procedimento é efetivo e tem perfil analgésico favorável. No entanto, é necessária a confecção de uma diretriz médica que auxiliará no controle rígido e sistemático do mesmo.*

PALAVRAS-CHAVE: *Ozônio/uso terapêutico. Dor lombar. Compressão da medula espinal. Deslocamento do disco intervertebral.*

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REVIEW ARTICLE

Laboratory findings in SARS-CoV-2 infections: State of the art

Miguel Augusto Martins Pereira[1]
Isabella Carolina de Almeida Barros[1]
Ana Luiza Veríssimo Jacob[1]
Mayara Lopes de Assis[1]
Salim Kanaan[2,3]
Hye Chung Kang[4]

1. Graduando em Medicina, Faculdade de Medicina, Universidade Federal Fluminense, Niterói, RJ, Brasil
2. Professor Adjunto de Patologia, Departamento de Patologia, Universidade Federal Fluminense, Niterói, RJ, Brasil.
3. Coordenador do Internato e da Residência médica em Patologia Clínica/Medcina Laboratorial, Hospital Universitário Antônio Pedro, Niterói, RJ, Brasil.
4. Professora Titular de Patologia, Departamento de Patologia, Universidade Federal Fluminense, Niterói, RJ, Brasil.

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SUMMARY

OBJECTIVE: *The scientific community is constantly assessing the clinical and laboratory manifestations of COVID-19 in the organism. In view of the fragmentation of the large amount of information, knowledge gaps in relation to laboratory markers, and scarcity of papers in Portuguese, we propose a Literature review on laboratory changes observed in patients infected with SARS-CoV-2.*

METHODS: *Analysis of articles published between December 2019 and May 2020 on the PubMed and SciELO databases. The articles were identified, filtered, and evaluated based on the approach to the subject, language, and impact. Then, the articles were subjected to a thorough reading, in full, by 4 (four) independent researchers.*

RESULTS: *Leukopenia and lymphopenia were included in most studies, even in case definitions. Platelet count and platelet-lymphocyte ratio, at peak platelet, were associated with advanced age and longer hospital stay. Eosinopenia showed a sensitivity of 74.7% and specificity of 68.7% and, together with increased CRP, these are one of the future prospects for screening for disease. A high level of procalcitonin may indicate bacterial co-infection, leading to a worse prognosis. COVID-19 manifests itself with increased levels of many inflammatory markers such as IL-1, IL-2, IL-6, IL-7, IL-12, IP10, IFN-γ, MIP1A, MCP1, GSCF, TNF-α, and MCP1/CCL2, as well as LDH, ESR, D-dimer, CK, ALT, and AST.*

CONCLUSION: *There is a need for further studies on the new SARS-CoV-2. So far, there is no consensus regarding laboratory findings and their usefulness, whether as a prognostic marker, mortality, or disease severity.*

KEYWORDS: *Coronavirus Infections. Betacoronavirus. Blood Cell Count. Leukocyte Count.*

INTRODUCTION

In December 2019, the first cases of a severe acute respiratory infection of unknown etiology by then appeared in the city of Wuhan, the capital of the Hubei province, in China, the first epicenter of the current pandemic[1,2]. After further investigation, it was found that many of the patients had a common exposure to the wholesale seafood market of Huanan, known for the trade of live animals[1]. Shortly, the agent

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CORRESPONDING AUTHOR: Miguel Augusto Martins Pereira
Avenida Marquês do Paraná, 303 – Hematologia Clínica, Niterói, RJ, Brasil – 24033-900; Tel: +552126299095
E-mail: mappereira@icloud.com.br

responsible for the disease was identified. The now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused by the 2019 novel coronavirus (2019-nCoV), which quickly spread throughout China, as well as the world, having reached by April a total of 823,626 cases and 40,598 deaths[1,3].

The coronaviruses are single-stranded RNA viruses, enveloped, that measure around 60nm to 140nm in diameter and feature projections on its surface, hence the name coronaviruses, in allusion to a crown. Other viruses of this same family also circulate in humans and usually cause respiratory syndromes, some examples are the HKU1, NL63, 229E, OC43, MERS-CoV, and SARS-CoV[1,4]. SARS-CoV-2 is the agent responsible for COVID-19, it is 50% genetically compatible with MERS-VOC and 79% compatible with SARS-CoV[2].

The clinical manifestations of this new disease have not been not fully established yet since they vary from mild symptoms to severe pneumonia with extensive alveolar damage, which may result in death[5,6]. The most commonly reported symptoms are fever, dry cough, myalgia, fatigue, dyspnea, and even headache, diarrhea, hemoptysis, coryza, as well as productive cough[5,7]. The fatal cases were, in general, of middle-aged and elderly patients with pre-existing conditions (oncologic surgery, cirrhosis, hypertension, heart disease, coronary disease, diabetes, and Parkinson)[6].

The laboratory findings of COVID-19 show the results of the virus' mechanism of attack to host cells. In this sense, the phenomenon known as amplification of the immune response was confirmed in multiple viral infections. In it, there is a cellular uptake of virus-antibody complexes after virus interaction with FcR, FcγR, or other receptors, resulting in a more objective infection in the target cells. In addition, the interaction of FcγR receivers with the complex of anti-virus S-protein (anti-S-IgG) neutralizing antibodies can facilitate both inflammatory responses and the persistent viral replication in patients' lungs[8].

Since this is a recent pandemic, the scientific community is still evaluating the clinical and laboratory consequences of the infection in the body. Thus, considering the fragmentation of larges amounts of information, the gaps in knowledge regarding laboratory markers in COVID-19, and the scarcity of studies in the Portuguese language, we propose a comprehensive and dynamic literature review on laboratory alterations observed in patients infected by SARS-CoV-2.

METHODS

The review selected articles published from 31 December 2019 until 1 May 2020, indexed in the following databases: PubMed (US National Library of Medicine National Institutes of Health) and SciELO (Scientific Electronic Library Online). The descriptors (MeSH) used were: *"2019 novel coronavirus" or "2019-nCoV" or "COVID-19"*. We considered eligible articles in the English language whose summary included laboratory findings from patients with confirmed positive results by molecular testing for SARS-CoV-2 infection. The references from the studies identified were also analyzed to detect additional studies. Thus, we selected 19 (nineteen) articles based on their approach to the subject and impact. Then, the articles were subjected to a thorough reading, in full, by 4 (four) independent researchers.

Hemogram alterations

Among the first laboratory findings reported in patients diagnosed with COVID-19 is a reduction in the number of white blood cells (leukopenia), which varied, between the studies, from 9.1% to 33.7%[3,4,7,9,10], a reduction in the number of lymphocytes (lymphopenia)[3,4,7,9-12], and, later, of eosinophils[13,14].

The percentage of patients with lymphopenia in the studies discussed[3,4,7,9,10] ranged from 35.3% to 82.1%, the highest value presented by Guan et al.[9], from a study involving 1099 patients. Therefore, lymphopenia is among the most common laboratory findings, illustrating the apoptosis activation mechanism and the signaling pathway of the P53 pathway, induced by SARS-CoV-2 in lymphocytes, providing a decreased immune response to the virus[2,7,15,16]. Indeed, the (Chinese) guidelines began to include lymphopenia and leukopenia in their case definitions. Still, in regard to lymphopenia, numbers below 1000 would be associated with more severe presentations of the disease[1,17]. It was also reported that non-surviving patients developed lymphopenia and leukocytosis more often, along with abnormal values of D-dimer, blood urea nitrogen, and creatinine[18].

The work by Chen et al.[3] analyzed, through a series of 30 cases, the dynamic alterations in the number of platelets during the treatment of COVID-19 patients. The univariate analysis of the study showed that age, platelet peaks, and the platelet-lymphocytes ratio (PLR), during the platelet peak, were influencing factors in severe patients, while a multivariate analysis showed that the PLR value during the

peak was an independent influencing factor in severe patients. The maximum number (mean) of platelets or peaks during the treatment in severe patients was 392 × 10^9/L, significantly higher than the 301 × 109/L of non-severe patients (P=0.047). The platelets/lymphocytes ratio (PLR) of 626 severe patients was significantly greater than the 262 (P=0.001) of non-severe patients. The platelet peaks were also associated with more advanced age and longer hospitalization (P<0.005). This could be related to the cytokines storm phenomenon. In this way, based on the absolute values, the fact that the levels of lymphocytes and platelets are sensitive indicators that reflect the control of infection and inflammation, and the results presented, the PLR could be used in the monitoring of COVID-19 patients.

With respect to eosinopenia, it is important to mention the retrospective comparative study by Li et al.[13], which divided 989 patients based on the nucleic acid test of the polymerase chain reaction for SARS-CoV-2 infection into two groups, one positive and one negative for SARS-CoV-2. The work confirmed some laboratory findings already well described, such as leukopenia, lymphopenia, and increased PCR. However, among the most interesting results, eosinopenia presented a sensitivity of 74.7% and specificity of 68.7%, with an area under the curve (AUC) of 0.717, and the combination of eosinopenia and increased ultra-sensitive C-reactive protein (us-PCR) presented a sensitivity of 67.9% and specificity of 78.2% (AUC of 0.730). Thus, according to the authors, eosinopenia or the combination of eosinopenia and increased us-PCR in the diagnostic parameters recommended by the COVID-19 guidelines would improve predictive and discriminatory capacity and, therefore, could efficiently screen patients suspected of COVID-19, changing, thus, the strategic parameters for managing the disease since it would also decrease the medical resources necessary for molecular and imaging tests.

The eosinophil count is among the laboratory findings that showed a potential for predicting the progression of the coronavirus since, as concluded by some studies, almost all patients presented eosinopenia in the first week of hospitalization. However, the time for eosinophil recovery, in mild patients, was less than that in severe cases; thus, this suggests that a lower and ascending eosinophil count can be a sign of progression and recovery of COVID-19, respectively[14].

The platelet count is usually normal or slightly low[1], but the presence of thrombocytopenia ranged from 5.0% to 36.2% in the studies analyzed[3,4,7,9,10]. As for the red series, Huang et al.[7]reported anemia as one of the most common complications found in COVID-19 patients.

Procalcitonin and troponin I

Increased values of procalcitonin, creatinine, and troponin I are uncommon in new coronavirus infections[7,9,10]. However, in the study by Huang et al.[7], 12% of the cases were diagnosed with myocarditis; thus, the level of high-sensitivity troponin I was significantly higher in those patients. Procalcitonin is a pro-hormone associated with infectious diseases, however, most COVID-19 patients present normal values of serum procalcitonin[1,4,7,11,12]. Thus, a high level of procalcitonin could indicate a co-bacterial infection and, consequently, worse prognosis[1.19]. Following this same line of reasoning, it was observed that the rate of patients with abnormal values admitted to the ICU was three times higher than those with normal values (75% vs. 22%; p <0.001)[18].

Cytokines and cytokine storms

Patients with SARS and COVID-19 have similar patterns of inflammatory damage. In the serum of patients diagnosed with SARS, there are increased levels of pro-inflammatory cytokines {for example, interleukin (IL)-1, IL-6, IL12, interferon-gamma (IFN-γ), interferon γ–induced protein-10 (IP10), macrophage inflammatory proteins 1A (MIP1A), and monocyte chemoattractant protein-1 (MCP1)}, which are associated with lung inflammation and severe pulmonary injury[20].

In addition, other cytokines, such as IL-7, IL-2, granulocyte-colony stimulating factor (GSCF), tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein 1 (MCP1/CCL2) also had higher plasma levels in COVID-19 patients, when compared to healthy adults[7]. Unexpectedly, some anti-inflammatory cytokines such as IL10 and IL4 were also increased in these patients[7], an unusual phenomenon in the acute phase of a viral infection. The same study highlights that ICU patients have a significantly higher level of GSCF, IP10, MCP1, MIP1A, and TNF-α than those not in the ICU, suggesting that the phenomenon of "cytokine storms" may aggravate the disease, so it could be associated with the progression of pneumonia/respiratory failure[3.7].

Other laboratory findings

It is worth mentioning other laboratory findings considered common by some authors, such as the prolonged duration of prothrombin and increased lactate dehydrogenase enzyme (LDH)[1,4]. There are also mentions to increased the values of serum ferritin, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), D-dimer, creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase(AST), total bilirubin, and reduced values of albumin[3,4,7,9,10,21]. High ferritin, ALT, and AST could be the result of liver injury due to an affinity of the virus with the liver in the advanced stage of disease[3].

The plasma D-dimer is a fibrin degradation product, which has a cross-reaction with it and, when dosed by the quantitative ELISA method, has been shown to be highly sensitive (above 99%) in cases of deep venous thrombosis and nonmassive pulmonary thromboembolism (PTE), with a cutoff value of 500 μg/L; therefore, values lower than 500 μg, virtually exclude PTE.[22]

However, the specificity of the fibrin for PTE is very low since its production is increased in situations such as cancer, inflammation, infection, necrosis, and in post-operative periods in general. Therefore, levels above 500 μg/L have a very low predictive value for PTE and are unable to confirm the disease 22· However, in the current pandemic context, a study conducted by Garcia-Olivé et al.[23], pointed out that patients diagnosed with pneumonia caused by COVID-19 and higher levels of D-dimer were associated to a greater probability of developing TEP 3, 6, 9 and 12 days after the identification of the D-dimer levels with an odds ratio (OR) of 1.7, 2.0, 2.4, and 2.4, respectively.

Tang et al.[24] also found that abnormal coagulation parameters were more frequent in patients who died (n=21) than in those who survived. Specifically, the values of PT, D-dimer, and fibrinogen were 1.14, 3.5, and 1.9 times higher in non-survivors than in survivors, respectively. In general, 71.4% of the patients who died met the criteria for disseminated intravascular coagulation (DIC), in comparison with only 0.6% of those who survived[24]. Thus, some authors suggest that an evaluation for DIC should be regarded as a routine part of COVID-19 patient monitoring[18].

The lactate dehydrogenase (LDH) enzyme is another marker that we highlight, since a study suggested, through multivariate regression, that LDH was an independent risk factor for COVID-19 based on a comparison with influenza A (H1N1)[25]. In this study, it was concluded that the LDH was an independent predictor of death in healthy adults and a risk factor for death in patients with cardiovascular diseases, reflecting the direct damage to myocardial cells[25].

Finally, as expected, patients admitted to intensive care units (ICUs) feature more laboratory abnormalities than those who are not[4,7]. Still regarding patients in need of intensive care, we stress the importance of the Italian study by Zangrillo et al.[17], which included 73 ICU patients under mechanical ventilation, i.e., severe patients, in order to identify predictors of early mortality. The analysis found a curious laboratory profile of lymphopenia (average of 770 per mm3; CI: 580-1000 per mm3), hyper inflammation with PCR (average of 184.5 mg/dL; CI: 108.2-269.1 mg/dL), and D-dimer (average of 10.1 μg/m; CI: 5.0-23.8 μg/m).

CONCLUSION

It is evident, therefore, that further studies are needed on the new SARS-CoV-2 and its interactions with the body. The clinical alterations it causes, as well as the laboratory findings resulting from the infection, must be better described. It is also important to investigate the laboratory profile not only of symptomatic patients but also of the little-studied asymptomatic ones. In addition, there are still gaps in our knowledge, such as the contradictory increase of anti-inflammatory cytokines, the usefulness of the PLR, and the curious normal values of procalcitonin in patients with the disease.

In short, despite the limitations of the current scientific literature (small samples, most of the studies involving the same population [Chinese], mostly Chinese operational definitions and reference values, and many clinical studies in progress), it would be utopian to expect the quick identification of new markers of the new coronavirus infections, predictors of severity, and mortality, as well as algorithms and guidelines based on laboratory findings, thus determining new approaches.

RESUMO

OBJETIVO: *A comunidade científica avalia a todo momento, as manifestações clínicas e laboratoriais da COVID-19 no organismo e, em vista da fragmentação da grande quantidade de informações, lacunas de conhecimento em relação aos marcadores laboratoriais e escassez de trabalhos em português, propomos uma revisão de Literatura sobre alterações laboratoriais observadas em pacientes infectados por SARS-CoV-2.*

MÉTODOS: *Análise de artigos publicados entre dezembro de 2019 a maio de 2020 nas plataformas PubMed e SciELO. Os artigos foram identificados, filtrados e avaliados com base na abordagem ao assunto, idioma e impacto. Depois, os artigos foram submetidos a uma minuciosa leitura, na íntegra, por 4 (quatro) pesquisadores independentes.*

RESULTADOS: *A leucopenia e a linfopenia constaram na maioria dos trabalhos, presente até em definições de caso. A contagem de plaquetas e a razão plaquetas-linfócitos, no pico plaquetário, foram associados à idade avançada e maior tempo de hospitalização. A eosinopenia apresentou sensibilidade de 74,7% e especificidade de 68,7% e, juntamente com aumento da PCR, são uma das perspectivas futuras de triagem para doença. O alto nível de procalcitonina pode indicar uma co-infecção bacteriana, levando a pior prognóstico. A COVID-19 se manifesta com níveis aumentados de muitos marcadores inflamatórios como IL-1, IL-2, IL-6, IL-7, IL-12, IP10, IFN-γ, MIP1A, MCP1, GSCF, TNF-α e MCP1/CCL2, bem como LDH, VHS, dímero-D, CK, ALT e AST.*

CONCLUSÃO: *Há necessidade de estudos adicionais sobre o novo SARS-CoV-2. Até agora, não há unanimidade em relação aos achados laboratoriais e sua utilidade, seja como marcador prognóstico, de mortalidade, ou de severidade de doença.*

PALAVRAS-CHAVE: *Infecções por Coronavirus. Betacoronavirus. Contagem de células sanguíneas. Contagem de Leucócitos.*

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REVIEW ARTICLE

A pragmatic approach and treatment of coronavirus disease 2019 (COVID-19) in intensive care unit

Sergio Henrique Loss[1]
Diego Leite Nunes[1]
Oellen Stuani Franzosi[2]
Cassiano Teixeira[1]

1. Médico Intensivista, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil.
2. Nutricionista, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil.

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SUMMARY

There is a new global pandemic that emerged in China in 2019 that is threatening different populations with severe acute respiratory failure. The disease has enormous potential for transmissibility and requires drastic governmental measures, guided by social distancing and the use of protective devices (gloves, masks, and facial shields). Once the need for admission to the ICU is characterized, a set of essentially supportive therapies are adopted in order to offer multi-organic support and allow time for healing. Typically, patients who require ventilatory support have bilateral infiltrates in the chest X-ray and chest computed tomography showing ground-glass pulmonary opacities and subsegmental consolidations. Invasive ventilatory support should not be postponed in a scenario of intense ventilatory distress. The treatment is, in essence, supportive.

KEYWORDS: *Coronavirus Infections. Betacoronavirus. Pandemics. Cuidados Críticos.*

INTRODUCTION

Infections of the new coronavirus (called COVID-19, i.e., coronavirus disease 2019) are caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and are a flu-like infection similar to the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) that occurred in 2002 and 2012, respectively[1,2]. The SARS-CoV-2's genome is a single-stranded positive-sense RNA[3] and it probably originated from bat-derived coronaviruses that directly infected humans or spread to an unknown intermediate host to humans in Wuhan, Hubei Province, China[4-6]. In addition to a similar flu presentation, COVID-19 can manifest itself as a neurological syndrome, heart failure, or acute myocardial infarction[7,8]. Most infections (80%) are mild. However, 6-10% will require transfer to the ICU[9]. Since much controversy involves the different types of therapy for this population we proceeded with a scoping review about therapies for critically ill patients infected with COVID-19 in order to offer intensivists the most consensual approach in an objective and simplified way.

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CORRESPONDING AUTHOR: Sérgio Henrique Loss
Ramiro Barcelos, 2350, Porto Alegre, RS, Brasil – 90035-007
E-mail: sergio.loss@gmail.com

METHODS

This is a scoping review about critical care approaches to patients with COVID-19. A literature search of MEDLINE was conducted in PubMed throughout May 2020, using the terms coronavirus, COVID-19, SARS-CoV-2, pandemic, critical care, treatment. The retrieved papers were assessed and used in the review according to the quality and methodology used.

DISCUSSION

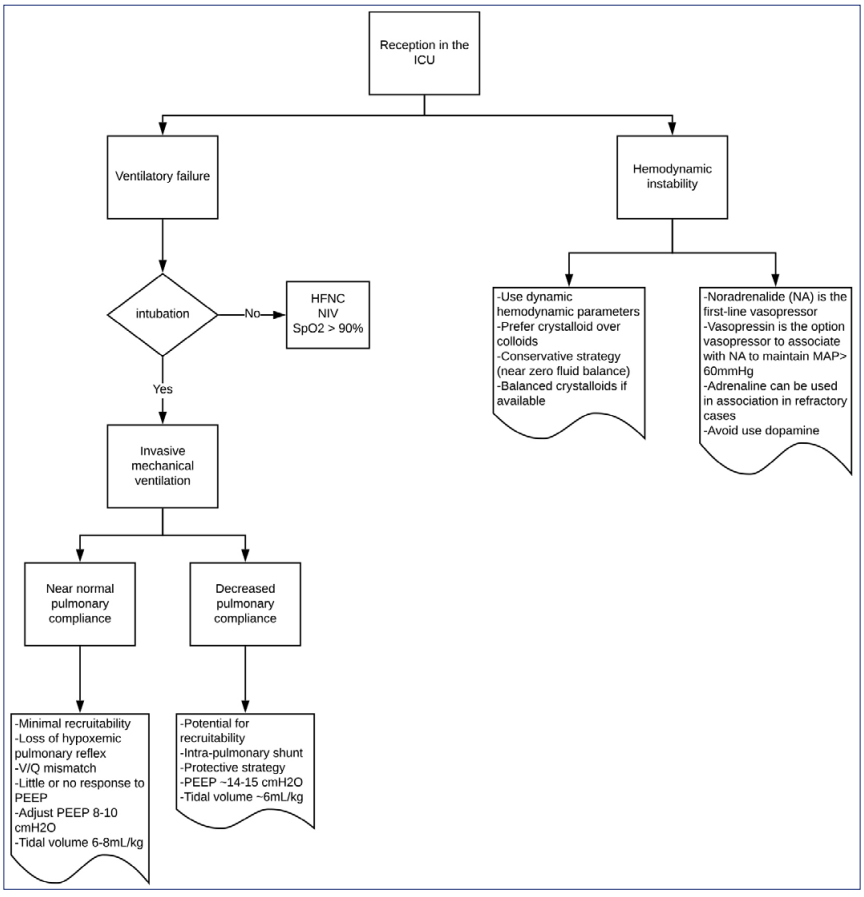
Admission to the unit

Patients with suspected or confirmed COVID-19 with progressive worsening of ventilatory failure or development of multiorgan dysfunction should be referred to the ICU, preferably in beds specifically dedicated to the treatment of this infection[1]. The entire security process for the assistance team must be clear. The institution must provide all necessary safety equipment (PPE – Personal Protective Equipment) [10], including suitable conditions for all staff[11]. Patients should be at least 2 meters apart[12].

Ventilatory support

Hypoxemic respiratory dysfunction is typical of a severe presentation in COVID-19[4]. Supplemental oxygen should be given when SO2 <90%[13]. Indications for ventilatory support, non-invasive or invasive, do not differ from routine indications for ICU. High-flow nasal oxygen supply does not significantly disperse bio-aerosol and is preferable over non-invasive ventilation (NIV). If the patient does not maintain SpO2 above 90%, especially in a context of significant suffering and excessive inspiratory effort, invasive mechanical ventilation is indicated. There are two different phenotypic presentations of ventilatory failure, one with normal or almost normal pulmonary compliance and severe hypoxemia (ventilation/perfusion mismatch), and the other with reduced compliance and intrapulmonary shunt[14-18]. Figure 1 summarizes the approach, types of ventilatory failure, and adjustments to the ventilator parameters.

FIGURE 1. VENTILATORY AND HEMODYNAMIC SUPPORT. HFNC: HIGH FLOW NASAL CANULA; NIV: NON-INVASIVE VENTILATION; V/Q: VENTILATION/PERFUSION; PEEP: POSITIVE END EXPIRATORY PRESSURE; MAP: MEDIUM ARTERIAL PRESSURE



Prone ventilation is indicated in patients with a PO2/FiO2 ratio <150 who were unable to maintain the ventilation strategy with a tidal volume of 4-6mL/Kg[19,20]. In refractory cases, extracorporeal membrane oxygenation (ECMO) with venous cannulation (ECMO V-V) may be attempted. Note that if this therapy is strongly considered, contact with a reference center should be made early in search of guidance and assessment of a window for clinical transfer conditions[21].

Hemodynamic

Hemodynamic instability is managed with crystalloid infusion, preferably using balanced solutions and vasopressors. The goal is to maintain an average blood pressure greater than 60mmHg[16,17]. The strategy is summarized in Figure 1.

Antiviral treatment

Hydroxychloroquine was the first drug proposed as an antiviral treatment due to its proven action in vitro against this virus class[22]. Subsequently, a non-randomized trial with a series of potential biases suggested that the association of hydroxychloroquine with azithromycin would decrease the time and severity of the disease[23]. Geleris et al.[24] included 1,376 patients with COVID-19 in a multivariable Cox model with inverse probability weighting according to the propensity score and they could not find an association with either a greatly lowered or an increased risk of the composite outcome of intubation or death. Rosenberg et al.[25] studied the association of treatment with hydroxychloroquine or azithromycin and hospital mortality in patients with COVID-19 and did not find any association between them. Despite the absence of evidence to support its use, some government protocols have recommended hydroxychloroquine at a dose of 400mg twice daily for 5 days in severe cases. When used, the QT interval must be monitored by electrocardiogram. The association of hydroxychloroquine and azithromycin should be avoided due to the potential cardiovascular effects[26].

The combination of two antiretrovirals (lopinavir-ritonavir) was tested on a randomized clinical trial enrolling 199 placebo-controlled patients. There was no evidence of improvement in mortality outcomes or reduction in the hospital stay. An important criticism of the study was that most participants were allocated 12 days after the onset of symptoms[27]. A recent review on the use of antiviral therapy against COVID-19 highlighted the importance of remdesivir, considering it a promising therapy (which could be confirmed in a randomized, double-blind, placebo-controlled clinical trial in patients with a severe presentation of the disease and expected to be published in May-June 2020)[28]. An excellent review of pharmacological treatments for COVID-19 has recently been published by Sanders et al.[29] and summarizes the current evidence on the main proposed, reused, or experimental treatments, providing a concise review of current clinical experience and treatment guidelines for this new coronavirus epidemic.

Other treatments

Steroids may be beneficial for a broad spectrum of critically ill patients, including those with cardiovascular, respiratory, and neurological conditions[30] and it seems to be associated with better outcomes in septic shock[31]. Since severe forms of COVID-19 have been linked to a cytokine storm, the use of corticosteroids has received special interest[32,33]. However, there is a wide divergence regarding corticosteroid use in patients with COVID-19 and its use should be evaluated on a case-by-case basis[34,35]. Published treatment protocols recommend methylprednisolone 0.5-1mg/kg/day for two weeks. However, until further data are available[36], the routine use of corticosteroid is not recommended[16,29]. However, patients with refractory shock should receive low-dose corticosteroid therapy[37].

Patients with COVID-19 can show a marked increase of D-dimer, meaning a coagulation disruption, which seems to be associated with increased mortality. Heparin use was shown to decrease mortality in this scenarium[38,39]. Thus, its utilization in this population seems to be reasonable. Prophylaxis of deep vein thrombosis/pulmonary thromboembolism is indicated in all patients (enoxaparin 40mg QD)[40,41].

Supportive treatment is often necessary and does not differ from routine practice in intensive care units. Fever is a complex, physiological, and adaptive response to infection that deserves additional assessment as to the need and safety of being medicated. The team must consider that fever can inhibit microbial reproduction, viral replication, and improve leukocyte function. Thus, perhaps fever should be treated only when it reaches values of 38.3-38.5C or higher[42,43].

Nutritional support

Perhaps, this area has the most fanciful proposal regarding immunity or outcomes of patients infected

with SARS-CoV-2 due to the miraculous effects of some micronutrients. In fact, the guidelines for nutritional therapy for critically ill patients published by respected societies, such as ASPEN, ESPEN, or BRASPEN, are perfectly applicable to critically ill patients with COVID-19[44-46]. Nutritional therapy and Intensive Care societies have recently published suggestions based on nutritional therapy guidelines and focused on clinical situations frequently identified in the course of SARS-CoV-2 disease[47-50]. The nutritional recommendations are summarized in Figure 2. Possibly, the COVID-19 pandemic is posing unpreceded challenges regarding nutritional assessment. Nevertheless, patients with SARS-CoV-2 disease should be treated individually, guided by the patient's conditions during intensive care support[47-50].

Regarding nutritional assessment, in the inability to obtain direct objective nutritional data, it may be necessary to evaluate secondary data for nutritional assessment when restrictions of ICU access exist, according to the institution's infection control division instructions. Secondary data can be obtained from the patient's records and by interviewing the family through various platforms. Nutritional risk assessment should be performed with validated tools (e.g. NRS-2002[51] and NUTRIC[52] scores). It is important to consider that ESPEN guidelines suggest that all patients with longer than 48 hours of ICU stay should be considered at nutritional risk[45]. The registered dietitian's findings should be registered on the patient's records and a coordinated nutritional therapy plan should be defined and shared with the medical team in order to provide safe and optimal nutritional therapy.

Objectively, nutritional therapy should be started early, that is, as soon as the patient demonstrates they are resuscitated (or about to be) and perfusion is established, preferably by a high density (> 1.2 kcal/mL) polymeric formula administered by gastric or post-pyloric feeding tubes (avoid endoscopy). Nutrition therapy should not be postponed solely by the use of neuromuscular agents, although deep sedation associated or not with neuromuscular agents may cause nutritional intolerance. Gastric residual monitoring is not recommended as standard care. Nutrition therapy should be given to patients undergoing prone positioning. If gastrointestinal intolerance persists after prokinetic therapy optimization, tropic nutrition may be considered (10-20 mL/h or 500 kcal/day).

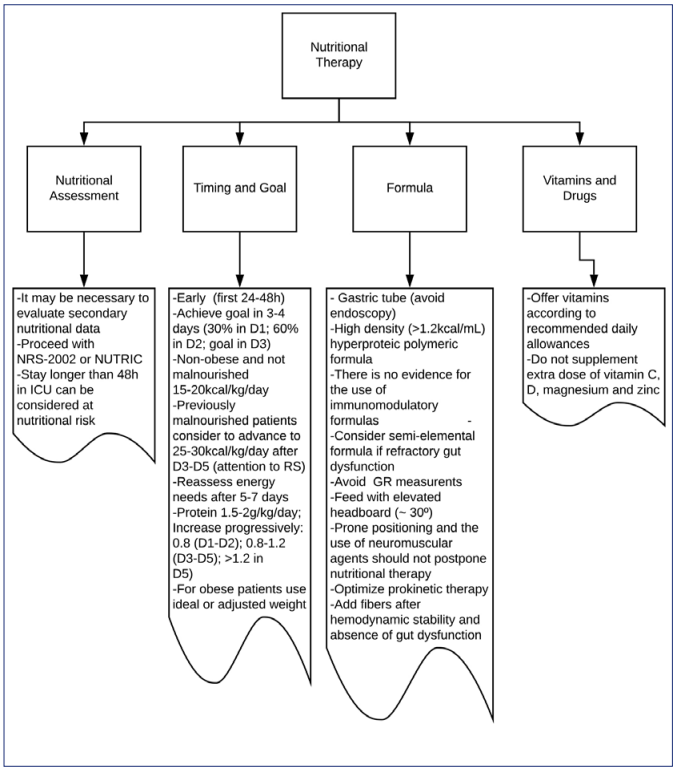
The calorie and protein doses are summarized in Figure 2. Trace elements and vitamins are offered according to the usual repletion practices. Currently, there is no evidence for immunomodulation. Fibers could be given according to the institution's practices as soon as the patient has hemodynamic stability and absence of digestive tract dysfunction (10-20g/day).

Prognosis

Recent cohorts showed rates of ICU admission or severe illness ranging from 4.9 to 26% of cases[53-58]. Most patients with COVID-19 appear to need mechanical ventilation (MV) due to acute respiratory distress syndrome (ARDS). Besides that, data about the duration of ventilation are limited but suggest prolonged MV for two weeks or more[53-55]. Common complications include acute kidney injury, mild transaminitis, cardiomyopathy, pericarditis, pericardial effusions, arrhythmias, sudden cardiac death, and superinfection (e.g., ventilator-associated pneumonia) [53-58].

Early data are emerging describing outcomes from COVID-19 in critically ill patients who develop ARDS[12,53,59,60]. Mortality appears lower than that in patients with severe acute respiratory syndrome (SARS-CoV) or Middle East respiratory syndrome

FIGURE 2. NUTRITIONAL THERAPY. NRS: NUTRITIONAL RISK SCREENING; NUTRIC: NUTRITION RISK IN CRITICALLY ILL; D: DAY; RS: REFEEDING SYNDROME; BMI: BODY MASS INDEX; GR: GASTRIC RESIDUAL VOLUME



(MERS). The mortality from COVID-19 appears driven by the presence of severe ARDS, and it is approximately 50% (range 16 to 78%). In a single-center retrospective cohort of 52 critically ill Chinese patients with COVID-19, 62% had died after 28 days, with a median duration of only seven days from intensive care unit (ICU) admission to death[53]. In another retrospective cohort of 201 Chinese patients with COVID-19, the mortality was 52% among those who developed ARDS[61]. Among those who received MV, 66% died, 21% were discharged, and 13% remained hospitalized. In an Italian cohort of 1591 patients, the ICU mortality was 26%, but a significant proportion remained in the ICU at the time of the publication, which may have underestimated the true mortality[57].

Across countries, the consistent major risk factor associated with death in critically ill patients with COVID-19 is older age[15,53,54]. In Chinese retrospective cohorts, death from ARDS was more likely to occur in those of older age, i.e., ≥64 years (hazard ratio [HR] 6.17; 95% 3.26-11.67)[53,61]. Preliminary reports from Italy and the United States are reporting similar outcomes[57,61,62]. Despite, the most reported predictors of severe prognosis in patients with COVID-19 included age, sex, features derived from computed tomography scans, C-reactive protein, lactic dehydrogenase, and lymphocyte count[63]. The speed of symptom progression does not appear to predict a worse outcome[53]. Other risk factors associated with death among critically ill patients include the following[38,55,57,62]:

- The development of ARDS, particularly severe ARDS, and the need for mechanical ventilation;
- Comorbidities (e.g., chronic heart and pulmonary conditions, hypertension, diabetes, chronic kidney disease);
- Markers of inflammation or coagulation (e.g., D-dimer level >1 microg/mL admission, elevated fibrin degradation products, prolonged activated partial thromboplastin and prothrombin times);
- Select laboratory studies (e.g., worsening lymphopenia, neutrophilia).

CONCLUSION

Patients with COVID-19 who need to be transferred to the ICU are complex and have a high mortality rate. Many studies are being conducted with the purpose of finding one or more treatments capable of eliminating the disease and providing a cure. Until then, treatment is multidisciplinary and essentially supportive.

RESUMO

Há uma nova pandemia global que surgiu na China em 2019 e está ameaçando diferentes populações com insuficiência respiratória aguda grave. A doença tem um enorme potencial de transmissibilidade e requer medidas governamentais drásticas, orientadas para o distanciamento social e pelo uso de dispositivos de proteção (luvas, máscaras e escudos faciais). Uma vez caracterizada a necessidade de admissão na UTI, um conjunto de terapias essencialmente de suporte é adotado para oferecer suporte multiorgânico e permitir tempo para a cura. Normalmente, os pacientes que necessitam de suporte ventilatório apresentam infiltrados bilaterais na radiografia de tórax e na tomografia computadorizada de tórax, mostrando opacidades pulmonares em vidro fosco e consolidações subsegmentares. O suporte ventilatório invasivo não deve ser adiado em um cenário de intenso sofrimento ventilatório. O tratamento é essencialmente de suporte orgânico.

PALAVRAS-CHAVES: *Infecções por Coronavirus. Betacoronavirus. Pandemia. Critical Care.*

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COMMENTARY

Comment on "Plasmatic adipocyte biomarkers and foot pain associated with flatfoot in schoolchildren with obesity"

Sami A. Gabr[1,2]

1. Professor of biochemistry and molecular biology, Rehabilitation Research Chair, College of Applied Medical Sciences, King Saud University, Riyadh, KSA.
2. Department of Anatomy, Faculty of Medicine, Mansoura University, Egypt.

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KEYWORDS: *Biomarkers. Adipocytes. Flatfoot. Obesity. Child. Pain/etiology.*

Obesity presents a major impact on the quality of lifestyle and essential physical activities such as walking ability, aerobic capacity, and muscle strength[1]. In younger individuals, as well as in adults, obesity has been associated with a range of bone and musculoskeletal disorders, particularly the development and progression of knee osteoarthritis (OA) and foot deformations[1-3]. Maximum loads on the knee and hip joints can create considerable variation in peak loads and significant effects on the function and structure of the foot[4-6]. It has been reported that obesity in children negatively impacts foot structure and function; the feet are broader, flatter, rounder, and may have a deformity in ligamentous laxity within the foot that exerts a position of extreme pronation, known as flat foot, which is extended even in adolescent or adult feet[6-9]. In addition, the abnormality in foot structure, such as flat foot, makes the foot supple (or prone to collapse) and lacks the ability to supinate in order to form a rigid lever during push-off in gait[10-12]. In young obese children and adolescents, photography, computed tomography scans, and other anthropometric measurements have shown that excessive increase in weight-bearing forces may cause micro-trauma to the ligaments and muscular structures, damaging soft tissue and increasing the risk of joint collapse and flat feet[9-15].

Finally, supporting comments on plasmatic adipocyte biomarkers and foot pain associated with flatfoot in schoolchildren with obesity[12], many research works have reported that adipocytokines, particularly adiponectin, leptin, and resistin, have a significant association as physiological biomarkers of childhood obesity, with foot pain and flat foot among young and older ages with obesity[16-20]. These markers along with pro-inflammatory ones (TNF-α, IL-6) showed a significant association with bone mineral density (BMD) and bone mineral content (BMC)[20-23]. Thus, early identification of any structural abnormalities in the biomechanics of bone and its related biomarkers including adipocytokines is required to minimize the risk of future functional complications across a lifespan, particularly in childhood obesity. In addition, controlled exercise training programs of moderate-intensity are advised to minimize the potential risks of childhood obesity on the musculoskeletal system[19-23].

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CORRESPONDING AUTHOR: Sami A. Gabr
P.O. Box 10219 Riyadh 11433, KSA
Tel.: +966562060018; Fax: +96614698541
E-mail: nadalab2009@hotmail.com, dr.samigabr@gmail.com, sgabr@ksu.edu.sa

PALAVRAS-CHAVE: *Biomarcadores. Adipócitos. Pé chato. Obesidade. Criança. Dor/etiologia.*

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COMMENTARY

Comment on: "Evaluation of Neutrophil to Lymphocyte Ratio, Platelet to Lymphocyte Ratio, and Lymphocyte to Monocyte Ratio in Patients with Cellulitis"

Hasan Tahsin Gozdas[1]

1. Department of Infectious Diseases and Clinical Microbiology, Abant Izzet Baysal University Faculty of Medicine, Bolu, Turkey

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Easily accessible hemogram-derived parameters have gained the attention of physicians recently. These are simple, practical, and economical parameters that were found useful in the prediction of many clinical problems. They are often used as a sign of chronic inflammation. In chronic inflammatory conditions, their levels are elevated. Chronic inflammation has been linked with the pathogenesis of many different diseases and their complications. Neutrophil to lymphocyte ratio (NLR) is one of the most important and popular hemogram biomarkers showing chronic inflammation.

Skin and soft tissue infections are common health problems in the community. Their symptoms may be similar to that of non-infective dermatological diseases. So, it is important to distinguish these infections from non-infective conditions to reach an early and accurate diagnosis and determine the treatment. Moreover, infection symptoms related to skin and soft tissue may be subtle in the geriatric population. So, it can be difficult to recognize these infections in this ever-increasing patient group.

In the article by Ince et al.[1], NLR elevation was found to be beneficial in differentiating cellulite patients from non-infective healthy individuals. The authors also highlighted that NLR elevation was more evident in cellulite patients older than 65 years. So, NLR is a simple, practical, and economical hemogram biomarker that can assist us in the management of cellulite patients. Since the higher NLR levels are observed in patients over 65 years, this may be used as a clue to reach a diagnosis in this patient group. Future studies will provide us with new perspectives about the clinical use of NLR.

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CORRESPONDING AUTHOR: Hasan Tahsin Gozdas
Department of Infectious Diseases and Clinical Microbiology, Abant Izzet Baysal University Faculty of Medicine, Bolu, Turkey
Tel: +90 374 254 10 00 - Fax: +90 374 253 45 06
E-mail: dr.htgozdas@yahoo.com.tr