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MANAGING EDITOR: *César Teixeira*
E-MAIL: *ramb@amb.org.br*
WEBSITE: www.ramb.org.br

Address: Rua São Carlos do Pinhal, 324
Bela Vista – São Paulo
Postal Code: 01333-903
Phone no.: (+55 11) 3178-6800 Ext. 177

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EDITORIAL

Critical imaging analysis of suspicious non-palpable breast lesions

Renato de Oliveira Pereira[1]
Benedito Borges da Silva[1]

1. Programa de Pós-Graduação, Rede Nordeste de Biotecnologia (RENORBIO), Área de Saúde, Universidade Federal do Piauí, Teresina, PI, Brasil.

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Breast cancer remains the most common malignancy in the female population worldwide, with an incidence estimated at 2,088,849 new cases for 2018. It is also the most common cause of cancer death in women, with a mortality estimated at 626,679 cases for 2018[1,2]. In Brasil, a developing country, breast cancer is still frequently diagnosed in advanced stages, most prevalent in postmenopausal women and associated with high mortality[3-5]. In contrast, around one-third of cases are diagnosed as non-palpable lesions. Early clinically nonpalpable breast cancer has a unique natural history and biology, in comparison to symptomatic breast cancer, with implications in the treatment and prognosis of these patients, and increased detection is related to the introduction of screening programs and the use of diagnostic imaging methods[6]. Mammography (MMG), ultrasonography (US), and magnetic resonance imaging (MRI) are the most widely used and available imaging methods in our setting, despite the application of other methods and new technologies in the last few years[7].

Screening programs using MMG seem to result in a general decline in breast cancer mortality and recommendations that women over the age of 40 should undergo annual screening mammography have led to an increasing number of diagnoses of non-palpable breast lesions[8]. The first sign of non-palpable breast cancer may be calcifications, and MMG is currently the best modality to detect microcalcifications. Furthermore, breast cancer cases that are manifested as focal asymmetric densities, also evaluated by this method, tend to be non-palpable lesions. On the other hand, excessive diagnosis of architectural distortions (distortions of the breast parenchyma without a defined mass) leads to lower mammographic specificity[9,10]. It is important to consolidate and disseminate screening programs, still deficient in underdeveloped and developing countries, technical improvements, and adequate training of radiologists to recognize the imaging characteristics and behavior of these lesions so that MMG can be used in the diagnosis[7].

US is another safe and available diagnostic modality, with potential precision.

It may be used in interventional procedures and is well-tolerated by patients[8,11]. Some authors have shown that there is not a high level of evidence to suggest the benefit of US as a supplemental screening modality, and further studies are needed. However, it is well-established that US is considered a complementary diagnostic method to MMG for the detection of non-palpable breast lesions, improving the specificity and increasing cancer detection rates, particularly in cases of asymptomatic women with dense breast tissue[8]. Although ultrasound screening

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CORRESPONDING AUTHOR: Benedito Borges da Silva
Avenida Elias Joao Tajra, 1260, Teresina, PI, Brasil – 64049-300
E-mail: beneditoborges@globo.com

may also increase false-positive diagnosis, added to a screening program it may improve cancer detection rates in patients with non-plapable lesions and dense breast tissue. In addition to its use in the identification of non-palpable lesions, US is also fundamental for locating suspicious preoperative lesions, allowing the localization and removal of these lesions with a higher safety margin and lower number of complications[11,12].

MRI is another diagnostic modality for the detection of invasive breast cancers that are mammographically and clinically occult. It has a high sensitivity (94-100%) and low specificity (37-97%) rate for cancer detection. MRI is recommended only in high-risk populations, as a supplemental screening test[13]. Studies have shown that MRI identified the disease at an earlier stage than MMG and MRI combined with MMG is associated with higher survival rates, including non-palpable lesions. However, it is worth mentioning that the literature on breast-cancer screening with MRI is focused on high-risk women, in whom there is a higher prevalence of cancer and lower sensitivity to mammography[13,14]. Risk factors include genetic mutations, family history, and personal risk history. The Tyrer-Cuzick model (International Breast Cancer Intervention Study, or IBIS) is considered the most widely and frequently used model to determine whether an MRI of the breast should be done. The limiting factor in the use of MRI to trace non-palpable lesions is its high cost, low availability in several locations, and the need for contrast medium, along with insufficient data to recommend its use for screening patients with dense breast tissues, without other factors. Therefore, according to the ACR (Appropriateness Criteria) and the American Cancer Society (ACS), it is currently determined that MRI as adjuvant screening should be used only in women at high risk for breast cancer[14,15].

Regarding new imaging modalities, digital breast tomosynthesis has been popularized as an "improved mammography" and is a valuable resource in screening for breast cancer. Its capacity to reduce the juxtaposition of breast tissue has reduced recall rates and increased the number of cancers detected, particularly in patients younger than 50 years. Nevertheless, some cancers are not detected by tomosynthesis, since it remains a modality of anatomical study, that does not add physiological information which can be furnished by contrast-enhanced imaging modalities, such as MRI[16]. Nuclear medicine methods use gamma cameras to obtain images of the physiologic uptake of a radio drug in the breast, typically Tc ^{99m} sestamibi, and showed a capacity to detect occult tumors, as small as 2 mm, by MMG in screening programs, particularly in women with dense breasts. However, there is a discussion on the clinical relevance of this additional detection in reducing mortality and concern over the use of ionizing radiation[17]. Intravenous iodinated contrast-enhanced digital MMG demonstrated a significant increase in sensitivity for breast cancer detection in comparison to standard digital MMG in studies by Sorin et al.[18]. Furthermore, the use of automated breast US (ABUS) was approved in the United States in 2018. A retrospective study showed that breast cancer detection and agreement between readers were significantly increased in dense breasts when US was combined with MMG in comparison to the use of MMG alone[19].

In conclusion, we highlight the importance of imaging modalities in screening programs for the diagnosis of non-palpable breast lesions. MMG, due to its features described, is the main method. Despite not being the only method, MMG requires complementary tests in some situations, particularly in patients with dense breasts. US, due to its broad availability and low cost, is a good option as a complementary method. Screening programs with patients stratified into groups with similar characteristics such as breast density, age, and risk factors for breast cancer could facilitate access to methods such as MRI and tomosynthesis in developing countries.

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Sexual behavior in men during COVID-19

Fernando Nestor Facio Junior[1]
Maria Fernanda Warick Facio[2]
Ana Clara Nagle Spessoto[3]
Luis Cesar Fava Spessoto[1]

1. Professor de Urologia, Departamento de Urologia. Faculdade de Medicina de São José do Rio Preto (FAMERP/FUNFARME), São José do Rio Preto, SP, Brasil.
2. Médica, Faculdade de Medicina (FACERES), São José do Rio Preto, SP, Brasil.
3. Graduanda, Faculdade de Medicina de Catanduva (UNIFIPA), Catanduva, SP, Brasil.

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The rapid changes that have occurred during this pandemic have influenced people in various ways, affecting stress levels, finances, and health. As the world faces this situation, it is adjusting to a new reality involving the need for social distancing and a change in habits[1]. We do not know what the lasting effects of the COVID-19 pandemic will be on patterns of sexual behavior. Social distancing affects all aspects of daily life and it is not clear whether it affects the sexual habits of married partners.

As the period of quarantine is prolonged, understanding changes in sexual behavior may provide information regarding potentially unhealthy sexual attitudes. This crisis is contributing to the experimentation of addictive sexual behaviors, such as those practiced via the internet[2]. A pornography sharing website observed an increase in the use of free pornography during the quarantine, especially in Spain (61%) and Italy (57%). Similar patterns have been observed in the United States and regions of Asia, especially with regard to morning viewing[3]. Besides the consumption of this content by the adult population, the pandemic is exerting a negative impact on the physical, intellectual, emotional, and sexual activities of adolescents. When emotions are high and adolescents are more socially isolated, there can be an increase in the use of online pornography, the effects of which have implications in terms of emotional and relational problems in this generation[4]. Online content is active and portable, enabling fast and easy access through a variety of electronic devices, in which it is difficult for parents to monitor online media[5].

Although the majority of adolescents in the cognitive and volitional age may not experience consequences, some cases could generate a reduction in desire, an increase in masturbatory practices, and a reduced interest in real sex due to the gap between what is possible and what is being fantasized, which could lead to potential health problems in terms of dependence, paraphilias, and sexual disorders[5,6].

Another vulnerable population in this pandemic is composed of men older than 60 years of age, 66.2% of whom could develop more severe forms of the disease[7,8]. Androgen deficiency in this age group can exert a negative impact on multiple organs and quality of life[9]. Therefore, the maintenance of testosterone

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CORRESPONDING AUTHOR: Fernando Nestor Facio Junior
Av. Fernando Correa Pires, 3600, Departamento de Urologia, Faculdade de Medicina (FAMERP/FUNFARME), S. José do Rio Preto, SP, Brasil
Tel: +55 17 98164-5376 / Fax: +55 17 3232-0199
E-mail: fnfacio@yahoo.com.br

therapy is necessary. Low levels of this hormone may play a physiopathological role associated with SARS-CoV-2, as the worsening of hypogonadism with androgen depletion can contribute to the severe course of the disease and even death[10].

Besides these complications amidst the pandemic, low levels of testosterone in men older than 60 years of age can further aggravate the lack of libido and low energy, generating profound suffering due to the negative impact on sexual behavior. Thus, specialized, multidisciplinary care is needed, with online medical appointments aimed at the continuation of hormone replacement therapy. Considering the relationship between mental and sexual health, these patients need close follow-up, as they may be at greater risk of manifesting preexisting sexual dsyfunctions[11].

Conflicts of interest

The authors have nothing to disclose

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All authors have contributed equally to the work.

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Urinary lithiasis - conventional open surgery

Participants:
Ernesto Reggio[1]
Alexandre Danilovic[1]
Antonio Silvinato[1]
Wanderley Marques Bernardo[2]

1. Sociedade Brasileira de Urologia, São Paulo, SP, Brasil
2. Coordenador do Programa Diretrizes da Associação Medica Brasileira, São Paulo, SP, Brasil

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Contact: 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.

Open surgery for urolithiasis has been replaced by minimally invasive alternative techniques. The purpose of this Guideline is to present doctors, specialists, and healthcare institutions with recommendations that may assist in the decision-making regarding patients whose one of the treatment options for Urolithiasis is open surgery. A systematic review of the literature was performed, without time restrictions, in the Medline database, retrieving 4,457 papers, of which 19 were selected to answer the clinical question: What are the main indications for open surgery in urolithiasis? The details about our methodology and results are presented in Annex I.

INTRODUCTION

Open surgery for urolithiasis, once considered the gold standard for most symptomatic calculi, has been overwhelmingly replaced by minimally invasive alternative techniques, including extracorporeal shock wave lithotripsy (ECWL), ureterorenoscopy (URS), and percutaneous nephrolithotomy (PNL).

ECWL, described for the first time in 1980, quickly became one of the most common alternatives to open surgery for calculi[1,2]. Although the success rates vary, depending on specific factors of the calculi and the patient, the stone-free rates can exceed 90% in appropriately selected patients[3].

Ureterorenoscopy has also been increasingly used to treat calculi and is the fastest-growing endourological procedure for this purpose[4]. The success rates for treating ureteral calculi are very high, with calculus-free rates >94% when ureteroscopes are used to treat distal ureteral calculi and >95% when flexible endoscopes are used for treating proximal ureteral calculi[5].

PNL is the most appropriate minimally invasive technique for larger and more complex calculi. Soucey et al. (2009) obtained an average calculus-free rate of 78% immediately after the PNL in >500 patients with

complete or partial staghorn calculi, but at the three-month follow-up, the calculus-free rate improved to 91%[6].

RESULTS

Open surgery in adult patients

Laparoscopic and robotic procedures are conceptually more similar to the open surgical methods than to the minimally invasive techniques but can achieve their goals with smaller incisions, less tissue manipulation, as well as with a faster recovery time than open surgery. However, they require the availability of equipment and surgeons trained in robotic laparoscopy or robotics[3](D).

The Guidelines of the American Urological Association (AUA) consider the indications for laparoscopic/open/robotic surgeries to be rare and limited and deem these techniques more effective for removing large or complex calculi, particularly in patients with anatomic abnormalities of the gastrointestinal tract, especially those that require reconstruction, as in the case of ureteropelvic junction (UPJ) or concomitant ureteral stenosis[4](D). Previous guidelines (2005) by the AUA considered excessive morbid obesity or an extremely poor function of the affected renal unit indications for open surgery [5](D).

In the guidelines of the European Association of Urology (UAE), there is a consensus that most complex calculi, including partial and complete staghorn calculi, should be addressed primarily using PNL or a combination of PNL and ECWL. However, if a reasonable number of percutaneous approaches is not successful, or if multiple endourological approaches were tried without success, laparoscopic or open surgery can be a valid option for primary treatment[6](D).

Previous guidelines by the UAE (2012) indicated that open surgery for infundibular stenosis, calculi in calyceal diverticula (particularly in an anterior calyx), skeleton deformities, fixed hip and leg contractures and deformities, associated comorbidities, concomitant open surgery, kidney inferior pole renal (partial nephrectomy), non-functional kidney (nephrectomy), and ectopic kidney calculi, in which percutaneous access and ECWL may be difficult or impossible[7](D).

For large and complex intrarenal calculi, the kidney can be opened in order to access the complete collecting system by means of anatrophic nephrolithotomy. This is currently an unusual technique but a valid approach for managing large staghorn calculi resistant to minimally invasive approaches and in patients with comorbidities such as chronic obstructive pulmonary disease[8](C). Anatrophic nephrolithotomy in cases of complex and large volume calculi can present a rate of calculus-free patients, in a single session, better than that obtained from percutaneous kidney lithotripsy, with a lower total treatment cost, but with greater loss of kidney function([9]). With AN, it is possible to achieve a calculus-free rate between 80% and 100%, without many secondary interventions[10](C). The use of laparoscopy[8,11](C) and robotic assistance[12](C) allows a minimal postoperative hospital stay, with faster recovery and results comparable to those from the gold-standard open surgery.

In some patients with voluminous or complex calculi, the endoscopic treatment by percutaneous antegrade ureterorenoscopy can allow for faster elimination of the calculi since larger and more efficient instruments can be used([13]). The benefits and increased invasiveness with a risk of complications that accompany the use of percutaneous access must be taken into account in the decision-making. Ureterolithotomy can also be considered as an alternative therapy in these rare clinical scenarios. Both laparoscopic [14](A) and robot-assisted ureterolithotomy provide equivalent results to open surgery, but with reduced morbidity[15](D).

Nephrectomy may be necessary in patients with renal calculi and severe infection, such as in cases of xanthogranulomatous pyelonephritis. Although the laparoscopic access route is preferred, conversion to open surgery may be necessary in approximately 7% of the cases due to intense perirenal adherence[16](B).

There are several options for treating urinary lithiasis, with the following calculus-free rates described: extracorporeal shock wave lithotripsy - 75-100%; transureteroscopic cystolithotripsy - 63-100%, percutaneous cystolithotripsy - 89-100%; and open surgery - 100%. The use of percutaneous access presents lower morbidity, with similar results to those from transurethral treatment, while the extracorporeal lithotripsy provides the lowest rates of calculi elimination and is reserved for patients at high surgical risk([17](A). Open cystolithotomy may be indicated in cases of large-volume or hard calculi resistant to the endoscopic approach, abnormal anatomy to allow safe access, concomitant open procedure (such as prostatectomy or diverticulectomy), or in cases in which it is impossible to transpose the urethra[18-20](C).

Open surgery in pediatric patients

In children, most calculi can be controlled by using the ECWL and endoscopic techniques[21](C). The indications for open surgery include: failed primary therapy for the removal of the calculi; very young children with complex calculi; congenital obstruction that requires simultaneous surgical correction; severe orthopedic deformities that limit the positioning for endoscopic procedures; and abnormal position of the kidney[22](D). Open surgery can be replaced by laparoscopic procedures[23.24](C). Open surgery was considered the gold-standard treatment for pediatric bladder lithiasis for a long time, providing excellent rates and success. The development of smaller equipment, associated with the increased experience of endourologists with minimally invasive procedures, has led to greater use of endoscopic approaches for treating bladder calculi in pediatric patients.

Recommendation

In adult patients:

• Laparoscopic or open surgery is indicated in the rare cases in which lithotripsy by shock waves, ureterorenoscopy (flexible), and percutaneous nephrolithotomy fail or are unlikely to succeed (D).

• Both laparoscopic (A) and robot-assisted ureterolithotomy provide equivalent results to those of open surgery, but with reduced morbidity(D).

• Open cystolithotomy may be indicated in cases of large-volume or hard calculi resistant to the endoscopic approach, abnormal anatomy to allow safe access, concomitant open procedure (such as prostatectomy or diverticulectomy), or in cases in which it is impossible to transpose the urethra (C).

• In bladder lithiasis, the use of percutaneous access presents lower morbidity, with similar results to those from transurethral treatment, while the extracorporeal lithotripsy provides the lowest rates of calculi elimination and is reserved for patients at high surgical risk (A).

In pediatric patients:

• The indications for open surgery include: failed primary endoscopic therapy for the removal of the calculi; very young children with complex calculi; congenital obstruction that requires simultaneous surgical correction; severe orthopedic deformities that limit the positioning for endoscopic procedures; and abnormal position of the kidney (D).

• Open surgery can be replaced by laparoscopic procedures in pediatric patients (C).

ANNEX I

Clinical question

What are the main indications for open surgery in urolithiasis?

Eligibility criteria

The main reasons for exclusion were: not an answer to PICO and intermediary outcomes.

Narrative reviews, case studies, series of cases, studies with presentations of preliminary results were, initially, excluded.

Since we are discussing treatment options, we used a controlled randomized clinical trial.

Search for papers

Database

The scientific information databases consulted were Medline (via PubMed), Central (Cochrane), and manual search.

Identification of descriptors

P	Patients in whom the only option for urolithiasis treatment is open surgery
I	Open surgery for urolithiasis
C	Failure with other procedures in therapy
O	-

Search strategy

Searches were conducted until March 30, 2018.

• #1 (urolithiasis OR urinary lithiasis OR nephrolithiasis OR kidney calculi OR ureterolithiasis OR ureteral calculi OR urinary calculi) AND (lithotripsies OR lithotripsy OR litholapaxy OR litholapaxies OR percutaneous ultrasonic lithotripsy OR extracorporeal shockwave lithotripsy OR ESWL OR noninvasive litholapaxy OR ureteroscopy OR ureteroscopic OR open surgery) AND therapy/broad[filter]

• #2 (urolithiasis OR urinary lithiasis OR nephrolithiasis OR kidney calculi OR ureterolithiasis OR ureteral calculi OR urinary calculi) AND (anatrophic nephrolithotomy) AND therapy/broad[filter]

• #3 (urinary bladder calculi OR Bladder Stones OR Urinary Bladder Stones OR Vesical Calculi OR vesical calculus OR Bladder Calculi OR Bladder Calculus OR Cystolith) AND therapy/broad[filter]

• Manual search - References of references, reviews and guidelines.

Critical evaluation

Relevance - Clinical importance

This Guideline was prepared by means of a clinically relevant question in order to gather information in medicine to standardize approaches and assist in decision-making.

Reliability - Internal validity

The selection of the studies and the evaluation of the titles and abstracts obtained from the search strategy in the databases consulted were independently and blindly conducted, in total accordance with the inclusion and exclusion criteria. Finally, studies with potential relevance were separated. When the title and the summary were not enlightening, we sought for the full article. Only studies with texts available in its entirety were considered for critical evaluation.

We included studies available in Portuguese, English, Spanish, French, or Italian.

Results application - External validity

The level of scientific evidence was classified by type of study according to Oxford[25] (Table 1).

TABLE 01. GRADES FOR RECOMMENDATION AND LEVELS 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.

The selected evidence was defined as a randomized controlled clinical trial (RCT) and submitted to an appropriate critical evaluation checklist (Table 2). The critical evaluation of RCTs allows to classify them according to the Jadad score[26], considering Jadad trials < three (3) as inconsistent (grade B) and those with score ≥ three (3) consistent (grade A), and according to the Grade[27] score (strong or moderate evidence).

When the evidence selected was defined as a comparative study (observational cohorts, or non-randomized clinical trial), it was subjected to an adequate critical assessment checklist (Table 3), allowing for the classification of the study, according to the Newcastle-Ottawa Scale [28], which considered consistent cohort studies with scores ≥ 6, and inconsistent <6.

TABLE 2. PROCESS FOR CRITICAL EVALUATION OF RANDOMIZED CONTROLLED CLINICAL TRIALS

Study data Reference, study design, JADAD, level of evidence	Sample size calculation Estimated differences, power, significance level, the total number of patients
Patient selection Inclusion and exclusion criteria	Patients recruited, randomized, prognostic differences
Randomization Description and blinded allocation	Patient follow-up, time, losses, migration
Treatment protocol Intervention, control and blinding	Analysis Intention to treat, analyzed intervention and control
Outcomes considered Primary, secondary, measurement instrument for the outcome of interest	Results Benefits or harmful effects in absolute data, benefits or harmful effects on average

Method of extraction and result analysis

For results with available evidence, the population, intervention, outcomes, presence or absence of benefits and/or harmful events, and controversy must be specifically defined whenever possible.

The results will be presented preferably in absolute data, absolute risk, the number needed to treat (NNT) or number needed to harm (NNH) and, eventually, in mean and standard deviation values (Table 4).

TABLE 4. SPREADSHEET USED FOR DESCRIBING AND PRESENTING THE RESULTS OF EACH STUDY

Evidence included
Study design
Selected population
Follow-up time
Outcomes considered
Expression of results: percentage, risk, odds, hazard ratio, mean

TABLE 3. PROCESS FOR CRITICAL EVALUATION OF COHORT STUDIES

Representativeness of the exposed and selection of non-exposed (max. 2 points)	Definition of exposure (max. 1 point)	Demonstrating that the outcome of Interest was not present at the beginning of the study (max. 1 point	Comparability on the basis of the design or analysis (max. 2 points)	Outcome assessment (max. 1 point)	Adequate follow-up time (max. 2 points)	Score and level of evidence

Results

TABLE 5. NUMBER OF PAPERS RETRIEVED BY USING THE SEARCH STRATEGY IN EACH OF THE SCIENTIFIC DATABASES

DATABASE	NUMBER OF PAPERS
Primary	
PubMed/Medline	4457

Application of evidence - Recommendation

The recommendations will be elaborated by the authors of the review, with the initial characteristic of synthesis of evidence, being subject to validation by all authors who participated in creating the Guideline.

The global synthesis will be based on the evidence described. Its strength will be estimated (Oxford[25]/ Grade[27]) as 1b and 1c (grade A) or strong, and as 2a, 2b and 2c (grade B) or moderate weak, or very weak.

Conflict of interest

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

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Amyloidosis: the use of the Daratumumab

Adriano Anzai[1]
Armelin Utino[1]
Haroldo Katayama[1]
Ighor A. Z. Spir[1]
Marcio A. Lemos[1]
Mauricio Anhesini[1]
Oswaldo S. Tiezzi[1]
Patricia R. N. Spir[1]
Pericles Otani[1]
Wanderley M. Bernardo[2]

1. Grupo de M.B. E – Presidente Prudente, Presidente Prudente, SP, Brasil.
2. Coordenador do Programa Diretrizes da Associacao Medica Brasileira, Sao Paulo, SP, Brasil.

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QUESTION: What is the role of daratumumab in the treatment of Amyloidosis?

ANSWER: The use of daratumumab in patients with clinical symptoms of amyloidosis currently should not be recommended due to the weakness of the evidence available to support it.[1]

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Probable vertical transmission identified within six hours of life

Fabrício Silva Pessoa[1]
Marynéa Silva do Vale [2]
Patrícia Franco Marques[2]
Susana da Silva Figueira[2]
Izabel Athayde da Silva Cruz Salgado[1]
Roberta de Sousa Wernz Cancian Mochel[3]

1. Infectologista Pediátrico. Serviço de Infectologia Pediátrica do Hospital Universitário da Universidade Federal do Maranhão, São Luis, MA, Brasil.
2. Neonatologista. Unidade de Cuidados Intensivos Perinatais do Hospital Universitário da Universidade Federal do Maranhão, São Luis, MA, Brasil.
3. Pediatra. Unidade de Cuidados Intensivos Perinatais do Hospital Universitário da Universidade Federal do Maranhão, São Luis, MA, Brasil.

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SUMMARY

We present the case of 33 weeks + weeks pregnant patient (G1P0), with proven COVID-19 infection by RT-PCR and, at admission, she presented with a dry cough and "tiredness when talking,". Chest computed tomography was performed, which showed the presence of attenuations with ground glass opacification and bilateral consolidations. She then had a cesarean section because of maternal respiratory decompensation. She was transferred to the ICU of the same hospital with an O2 catheter. The newborn was transferred to the neonatal ICU of the same hospital in ambient air and maintained in respiratory and contact isolation. RT-PCR was collected for SARS-COV-2 at 6 h of life, which was positive. Faced with the knowledge gap on vertical transmission, RT-PCR for SARS-COV-2 at 6 h of life gives cause for concern, thus representing the possibility of vertical transmission by SARS-COV-2, although additional investigations are required.

KEYWORDS: *Betacoronavirus. Coronavirus infections. Infant, newborn. Infectious disease transmission, vertical.*

INTRODUCTION

A major concern is raised by COVID-19 during pregnancy and the potential transmission of infection from mother to child before, during, and after childbirth. Generally, transmitting respiratory viruses to newborns mostly occurs via the birth canal and during breastfeeding or close contact among healthcare providers or family members[1]. The possibility of vertical transmission through breastfeeding or the consumption of human milk is important. In light of current scientific data, the breast milk of a COVID-19-positive mother is not yet considered a transmission vehicle. Although the detection of SARS-COV-2 in human milk may be possible, additional research is required to assess its potential risk for viral transmission and whether this will be sufficient to cause COVID-19 disease in the newborn. It has been speculated that specific SARS-COV-2 antibodies pass through breast milk from a COVID-19-positive mother to the neonate within a few days after the onset of the disease, thus possibly modulating the clinical expression of the infant's infection. When mothers with COVID-19 are extremely sick to care for the newborns, the newborn is separately managed and fed freshly extracted breast milk without the requirement to pasteurize

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CORRESPONDING AUTHOR: Fabrício Silva Pessoa
Prevenção e Controle de Infecções Hospitaleres, Segurança do Paciente, Hospital Universitário
Universidade Federal do Maranhão, Rua Silva Jardim, s/nº, São Luís, MA, Brasil
Tel: +55 98 98158-3800
E-mail: fabriciosilvapessoa@hotmail.com

it. Moreover, preventive procedures such as hand hygiene and the use of a face mask during feedings are recommended[2,3]. In this context, we report the case of neonatal infection by COVID-19 in a neonatal unit in which the mother was confirmed as having COVID-19 and the newborn was RT-PCR-positive at 6h of life, thus suggesting the possibility of vertical transmission of SARS-COV-2. The clinical manifestations of the mother were severe while those of the newborn were mild with good clinical evolution.

CASE

A mother, 34 years old, of mixed race, a nurse, primiparous (G1P0A0), with a gestational age of 33 weeks and 6 days, identified by ultrasound at a few weeks of gestational age, was admitted to the obstetric center of a university hospital in the northeast region of Brasil on April 21, 2020, at 01:24 h, with flu-like symptoms (rhinorrhea, myalgia, fever > 38°C and odynophagia), which started seven days prior and progressed with gradual worsening of the condition and dyspnea. During admission, the patient had a dry cough and "tiredness when talking," and she was already using medication such as hydroxychloroquine and azithromycin. She had five prenatal appointments, Blood Typing B+, denied hypertension, reported gestational diabetes with dietary control, with negative TORCH serology. She received two doses of antenatal corticosteroid. Chest computed tomography was performed, showing the presence of attenuations with ground glass opacification and bilateral consolidation. Cesarean section was performed because of maternal respiratory decompensation (suspected of having COVID-19) on April 22, 2020, at 01:44 h, and the mother was transferred to the ICU of the same hospital, with an O_2 catheter. All respiratory isolation and protective equipment measures and care were adopted by the team of health professionals during the cesarean section. The newborn presented good vitality at birth, with Apgar 9/9, and without the requirement for neonatal resuscitation maneuvers; the birth weight was 2370 g, head circumference of 33 cm, and a length of 43 cm. The newborn was transferred to the neonatal unit, maintained in ambient air, kept in respiratory and contact isolation, and the RT-PCR was collected for SARS-COV-2 at 6h of life, which came back positive. During hospitalization, the newborn presented episodes of regurgitation, which satisfactorily progressed, as well as jaundice of prematurity, and was put on phototherapy. Chest CT was performed with normal results and a new RT-PCR swab was collected for SARS-COV-2, which was negative. The infant was discharged at 10 days of life, clinically well, with verbal and written guidance to maintain isolation until 14 days and on other hygiene measures to prevent transmission of the virus.

DISCUSSION

The literature published to date has not yet confirmed vertical transmission of *SARS-COV-2*, although this possibility is considered. Similarly, there have been no reports of viral RNA identified in vaginal secretions and amniotic fluid; however, it has recently been found in breast milk[4,5]. Because IgM immunoglobulin is a macromolecule and therefore does not cross the placental barrier, its detection in the first seven days of life suggests intra-uterine infection, similar to the case reported herein. However, Chen et al.[6] questioned the absence of morphological changes related to infection in the placenta. Li et al.[7] reported the case of a neonate with a positive RT-PCR nasopharyngeal swab for SARS-COV-2 at three days of life and questioned whether the newborn was not infected after birth, either by the mother or other family members or by professional caregivers in the hospital. Wang et al.[8] reported a case of neonatal infection by COVID-19 in China, in which the laboratory results were negative (including research for *Legionella pneumophila*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, Q rickettsia fever, adenovirus, respiratory syncytial virus, influenza A virus influenza B virus, and parainfluenza virus 1/2/3) with favorable clinical outcomes. In this reported case, the result of the pharyngeal swab for SARS-COV-2 was positive 36h after birth. As in our case, strict measures were considered to reduce the risk of infection. Although COVID-19 was detected by RT-PCR in the maternal peritoneal fluid collected during cesarean section in 28 patients with SARS, the researchers could not rule out the possibility of another contact transmission of the newborn. They concluded that clinical data on COVID-19 infection in newborns is still very limited, and it is still unclear whether SARS-COV-2 can be vertically transmitted through the placenta or what are the short- and long-term damage it causes[8]. Infection by SARS-COV-2 may occur as Late Neonatal Sepsis, probably through nosocomial transmission in the nursery or Neonatal Intensive Care Unit[9]. Late neonatal sepsis occurs

after 48h, and it is essential to collect neonatal samples in the first hours of life to signal the possibility of vertical transmission of newborns to mothers with suspected or confirmed COVID-19[10]. Dong et al.[11] reported high levels of IgM antibodies in the newborn of a mother with COVID-19, thus suggesting that infection occurred in the uterus since IgM antibodies do not cross the placental barrier. However, the RT-PCR for SARS COV-2 with samples of nasopharyngeal swabs from the newborn were repeatedly negative, which did not demonstrate the presence of the virus. Zeng et al.[12] reported six newborns with negative RT-PCR for SARS-COV-19 born to mothers with confirmed COVID-19, thus demonstrating the diagnostic limitation of vertical transmission because no RNA particles of the virus were identified. Zhu et al.[1] retrospectively analyzed the clinical characteristics of ten newborns from nine mothers with confirmed COVID-19 infection in five hospitals from January 20 to February 5, 2020, in Wuhan, a city in the province of Hubei. Of the studied newborns, four were born full-term and six were premature. Clinical findings demonstrated that the first symptom in newborns was shortness of breath, as well as initial symptoms such as fever, thrombocytopenia accompanied by impaired liver function, tachycardia, vomiting, and pneumothorax. Samples of pharyngeal swabs were collected for nucleic acid amplification tests for SARS-COV-2, nine days after birth, all with negative results. The conclusion of the research in these newborns was that 2019-nCoV perinatal infection may have adverse effects in newborns and cause problems during pregnancy, such as fetal distress, premature labor, respiratory distress syndrome, and thrombocytopenia, together with changes in liver function and even death. However, the vertical transmission of 2019-NCOV has not yet been confirmed[1]. Moreover, cases of neonatal infection by SARS-COV-2 were reported in China, the youngest being 30h after birth, longer than the case reported herein, which had a positive result at 6h of life. Hong et al.[13] reported that the newborns probably acquired SARS-COV-2 infection through close contact with infected mothers. In their publication, they described the criteria adopted in their hospital for the clinical diagnosis of SARS-COV-2 in the neonatal population such as at least one clinical symptom, including body temperature instability, hypoactivity, refusal of food or dyspnoea; chest X-rays showing abnormalities, including unilateral or bilateral ground-glass opacities; (3) diagnosis of SARS-COV-2 in relatives or caregivers; and close contact with persons suspected or confirmed for SARS-COV-2 infection, patients with unexplained pneumonia[13]. In a cohort study conducted by Zeng et al.[14], with 33 newborns of mothers with COVID-19 in Wuhan, China, 3 out of 33 children (9%) had early onset of SARS-COV-2 infection, and the authors reported that the sources of SARS-COV-2 contamination in the newborns were probably of material origin, bearing in mind the rigorous preventive procedures implemented during childbirth, as reported in our case.

CONCLUSION

In this case report, we described a neonate, with strong evidence that this is a case of intrauterine vertical transmission in a neonatal unit in the Northeast of Brasil, which was demonstrated by the presence of the virus at only 6h of life. Thus, it is essential to perform RT-PCR collection for COVID-19 in the first few hours of life. The maternal severity of the disease and the enormous viral replication that occurred in the mother may have led to probable in-utero transmission to the newborn.

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Author's Contribution

All authors have made a significant contribution to the findings and methods in the paper. All authors have read and approved the final draft. All authors have certified that the manuscript is original and that no portion is under consideration elsewhere or has been previously published in any form other than as an abstract. All authors have no conflict of interest.

RESUMO

Apresentamos o caso de uma paciente grávida de 33 semanas + (G1P0), com infecção de COVID-19 comprovada por RT-PCR que, na admissão, apresentava tosse seca e "cansaço ao falar". Foi realizada tomografia computadorizada do tórax, que mostrou a presença de atenuações com opacidade em vidro fosco e consolidações bilaterais. Ela então passou por uma cesariana devido a descompensação respiratória materna. Em seguida, foi transferida para a UTI do mesmo hospital com um cateter de O2. O recém-nascido foi transferido para a UTI neonatal do mesmo hospital, em ar ambiente, e mantido em isolamento respiratório e de contato. Material para o RT-PCR para SARS-COV-2 foi coletado às 6h de vida, e o resultado do teste foi positivo. Perante a lacuna de conhecimento sobre a transmissão vertical, o resultado positivo do RT-PCR para SARS-COV-2 às 6h de vida é motivo de preocupação, pois representa uma possível transmissão vertical do SARS-COV-2, embora investigações adicionais sejam necessárias.

PALAVRAS-CHAVE: *Betacoronavirus. Infecções por coronavirus. Recém-nascido. Transmissão vertical de doença infecciosa.*

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Lithium, an old friend and a forgotten enemy

Cátia Figueiredo[1]
Joana Lemos[1]

1. Department of Internal Medicine, Centro Hospitalar Tondela-Viseu, E.P.E., Viseu, Portugal.

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SUMMARY

INTRODUCTION: *Nephrogenic diabetes insipidus (DI) is a polyuric and polydipsic syndrome and can have multiple causing factors.*

CASE DESCRIPTION: *A 69-year-old woman with bipolar disorder medicated with lithium 400mg for 12 years on a daily basis. The patient was admitted, after psychiatric decompensation, with hypernatremia unresponsive to hypotonic iv fluids. The diagnosis of DI was made with high plasmatic osmolality measurement, low urine osmolality, and high levels of antidiuretic hormone. Full clinical recovery was possible with lithium suspension, hydration, and chlorthalidone.*

DISCUSSION: *Although frequently used in the past, Lithium (Li) is nowadays rarely used in clinical practice for prolonged treatments because of its potentially devastating side effects. Clinicians must be aware of those side effects in order to prevent organ damage, mainly in patients with severe bipolar disease and precarious response to alternative treatments.*

KEYWORDS: *Bipolar disorder. Lithium. Diabetes insipidus, nephrogenic.*

INTRODUCTION

Diabetes insipidus (DI) is a polyuric polydipsic syndrome that can be of central or nephrogenic origin. Patients with DI have a urinary output of more than 50mL per Kg in 24 hours and usually consume more than 3L of water per day[1]. Nephrogenic DI is associated with the inability of the kidney to respond to the neuropeptide vasopressin. Vasopressin is secreted in the posterior hypothalamus (paraventricular and supraoptic nuclei) in response to hypovolemia or osmolality shifts and acts in the receptor V2 of the principal cells of the conducting duct, allowing the reabsorption of water through the regulation of the aquaporin 2 water channel (AQP2)[2].

Nephrogenic DI can be congenital, detected in the early stages of life, or acquired, associated with drugs, systemic disease, or electrolyte abnormalities[2].

Lithium was once one of the most used and efficacious treatments of type 1 bipolar disease, but the emergence of serious side effects concerning nephrotoxicity led to its obliviation. Nephrogenic DI associated with Li usually appears after longer periods and with higher cumulative doses. Other factors include female gender, age, and other risk factors for chronic kidney disease[3]. Renal side effects are mostly associated with the action in the principal cells of the distal tubules and collecting ducts. Li enters through the epithelial sodium channel in the cell and accumulates there, interfering with the traffic of AQP2 and allowing the loss of a great quantity of water through dilute urine and leading to the clinical picture of polyuria, polydipsia, and hypernatremia[4].

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CORRESPONDING AUTHOR: Cátia Castanheira Figueiredo
Centro Hospitalar Tondela-Viseu – Rua Senhora da Graça nº 1, RE esq, Repeses, Viseu, Portugal
Tel: +351 32 96414-7134
E-mail: cati.castanheira@gmail.com

DESCRIPTION

A 69-year-old woman with a history of bipolar disorder with 12 years of evolution, chronically medicated with clozapine 100mg (1/2id), carbamazepine 200 mg (2id), lithium 400mg (2id), lorazepam 1mg (3id), memantine 20mg (id), and haloperidol (20 to 30 drops in SOS). Irrelevant family history. Admitted to the psychiatric department due to behavioral decompensation. Polyuria and polydipsia had been reported in the previous 6 months, with normoglycemia. Due to maniac behavior, risperidone 25mg IM was initiated every other week. The routine analysis detected hypernatremia (153mEq/L) and she initiated iv fluids with hypotonic NaCl at 0,45%, 1L per day. The paranoid behavior remained, and risperidone was raised to 50mg IM. Despite hypotonic fluid infusion, hypernatremia became more severe (172mEq/L), with associated hyperchloremia (135mEq/L). At the examination, she had altered mental status although easily aroused, dehydration, diuresis of 4L per day, without focal neurological deficits. No other remarkable points. A head CT scan did not show any relevant changes and a MRI was not performed. Analytically, there was an elevated plasma osmolarity of 331 mOsm/L (280-301 mOsm/L), with a low urinary osmolarity 139.7 mOsm/L (300-900 mOsm/L), and a low urinary sodium 44.4 mEq/L (54-150 mEq/L). The antidiuretic hormone in the blood was 11, 3 pg/mL (normal <6.7pg/mL), and urine osmolality did not increase with the administration of desmopressin, confirming the diagnosis of nephrogenic diabetes insipidus. Considering a probable relationship with lithium, its chronic intake was suspended, and neuroleptics were reduced. Hydratation was performed by nasogastric tube until recovery of mental status and thereafter with a liberal intake of fluids, chlorthalidone 25mg id was introduced. There was a normalization of the ionogram and full recovery of previous functional status with physical rehabilitation. After six months of therapy, chlorthalidone was suspended with no DI relapse.

DISCUSSION

The diagnosis of DI is made through a laboratory evaluation that includes serum and urine osmolality as well as sodium and serum levels of vasopressin. The water deprivation test is still used to diagnose DI in cases when the serum osmolality is above 300mosm/Kg. The response to the administration of D-amino D-arginine vasopressin allows differentiating the nephrogenic type from the central DI[2]. The diagnostic algorithm accuracy is only around 70%[1], which led to the proposal of a direct test that includes the measure of serum vasopressin levels after osmotic stimulation failed to enter clinical practice. There is increasing evidence for new markers such as copeptin that likely increase the accuracy of the etiology of the polyuric polydipsic syndromes[1,5]. Imaging tests like MRI do not appear to have a role in the diagnosis of DI[1].

Nephrogenic DI associated with lithium is seen rarely nowadays since its use as a mood stabilizer decreased dramatically. The treatment requires stopping the therapy, hydration, and sometimes thiazidic diuretics to restore the hydric balance by increasing the amount of sodium and water reabsorbed in the proximal tubules[2,3]. Other possibilities are the prostaglandin synthesis inhibitors and acetazolamide, with the last not approved yet[2].

The prognosis is usually good when the cessation of the Li therapy is tolerated by the patient, and nephrogenic DI can sometimes completely resolve[2,3].

CONCLUSION

The diagnosis of polyuric polydipsic syndromes is still a challenge in clinical practice, although it seems to be evolving to more accurate techniques. Although Li is now rarely used in clinical practice, clinicians must be aware of the possible side effects of prolonged treatments and ideally anticipate their appearance in order to prevent organ damage.

Author's Contribution

All authors have contributed equally to the work.

Learning points

- Lithium therapy, despite its great effectiveness, is now rarely used in clinical practice due to its side effects;
- Whenever there is a suspicion of a polyuric polydipsic syndrome, all causes must be excluded since nephrogenic DI can have many causes;
- Early recognition and management of this syndrome can prevent life-threatening complications.

RESUMO

INTRODUÇÃO: *O diabetes insípido nefrogênico faz parte das síndromes poliúricas polidipsicas e pode ter múltiplos fatores causais.*

CASO CLÍNICO: *Mulher de 69 anos, com doença bipolar medicada com lítio 400 mg por dia durante 12 anos. A doente foi internada, após descompensação da doença bipolar, por hipernatremia não responsiva a fluidoterapia hipotônica endovenosa. O diagnóstico de DI foi realizado com base na elevação da osmolaridade plasmática, baixa osmolaridade urinária e níveis elevados de hormona antidiurética. Verificou-se recuperação clínica completa com suspensão do lítio, hidratação e clorotalidona.*

DISCUSSÃO: *Apesar do seu uso frequente no passado, o lítio (Li) é hoje em dia raramente utilizado na prática clínica por períodos prolongados pelos seus efeitos potencialmente devastadores. Os médicos devem ter em conta os potenciais efeitos secundários de forma a prevenir lesão de órgão em doentes com doença bipolar de difícil controle com outra terapêutica.*

PALAVRAS-CHAVE: *Transtorno bipolar. Lítio. Diabetes insípido nefrogênico.*

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ORIGINAL ARTICLE

Prevalence of hot flashes in women of 40 to 65 years of age with metabolic syndrome

Helena Proni Fonseca Saú[1]
Ana Carolina Basso Schmitt[2]
Maria Regina Alves Cardoso[3]
José Mendes Aldrighi[4]

1. Mestre, Departamento de Saúde Materno-Infantil, Faculdade de Saúde Pública, Universidade de São Paulo, SP, Brasil.
2. Ph.D, Departamento de Fisioterapia Fonoaudiologia e Terapia Ocupacional, Faculdade de Medicina, Universidade de São Paulo, SP, Brasil.
3. Ph.D, Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, SP, Brasil.
4. Ph.D., Departamento de Saúde Materno-Infantil, Faculdade de Saúde Pública, Universidade de São Paulo, SP, Brasil;

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SUMMARY

OBJECTIVE: *Hot flashes have a negative impact on the quality of life of women during the menopausal transition and thereafter. The progressive reduction in gonadal estrogen levels associated with aging promotes an accumulation of abdominal fat, dyslipidemia, and arterial hypertension, all of which are components of metabolic syndrome (MetS). The objective of the present study was to estimate the prevalence of hot flashes and evaluate their relationship with MetS in women ≥ 40 years of age.*

METHODS: *This was a cross-sectional study involving women aged between 40 and 65 years. We used the Kupperman index to quantify the climacteric symptoms and the National Cholesterol Education Program Adult Treatment Panel III criteria for the diagnosis of MetS.*

RESULTS: *1,435 women were initially selected, and we obtained information from 647. The mean age at menopause was 45.99 years (SD 6.61 years) and the prevalence of hot flashes and MetS were 55.83% (95% CI: 52.35-59.25%) and 46.29% (95% CI: 44.75-52.53%), respectively. We identified a positive association between MetS and hot flashes (OR 1.16; 95% CI: 1.01-1.33).*

CONCLUSIONS: *In women ≥ 40 years of age, hot flashes are highly prevalent and appear to be associated with MetS.*

KEYWORDS: *Climacteric. Perimenopause. Hot flashes. Metabolic syndrome.*

INTRODUCTION

Metabolic syndrome (MetS) is highly prevalent, representing a major public health problem because it increases cardiovascular risk and results in significant costs to the health system[1]. Observational studies have suggested that MetS is three times more common in women ≥ 40 years of age than in younger women[2].

Hot flashes affect 50-80% of the female population during the menopausal transition and thereafter, reaching a peak between two years before and two years after menopause[3,4]. They are often associated with sleep disorders, changes in mood, and fatigue, which can impair the quality of life and generate high costs for health systems[5,6]. Because of fluctuations in ovarian function, many women ≥ 40 years of age experience hot flashes and other manifestations such as accumulation of abdominal fat, obesity, and dyslipidemia, which are major components of MetS[2-4,7].

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CORRESPONDING AUTHOR: Helena Proni Fonseca Saú
Universidade de São Paulo, Faculdade de Saúde Pública – Av. Dr. Arnaldo, 715, São Paulo, SP, Brasil – 01246-904
Tel: +55 11 98540-9153
E-mail: lila_xlv@hotmail.com

Recent studies suggest that hot flashes signal a higher risk of cardiovascular disease (CVD), possibly due to activation of the sympathetic nervous system, which can alter blood pressure, the lipid profile, and insulin resistance[8-10]. Those factors, together with waist circumference, which is also often increased in women with hot flashes, constitute the diagnostic criteria for MetS[11,12].

It is possible that the prevalence of hot flashes is higher in women with MetS, given that each of the constituents of the syndrome can, in isolation, be responsible for the onset of the vasomotor symptoms. Despite the isolated impact of the components of MetS on the triggering of hot flashes, some studies have shown that the relationship between vasomotor symptoms and MetS itself has been poorly investigated and that the few available findings are conflicting[12-15]. Therefore, the objective of the present study was to determine whether there is an association between hot flashes and MetS[16].

METHODS

This was a cross-sectional study, based on information obtained from 7,212 women followed-up in 2014 via the Family Health Program in the city of Pindamonhangaba, Brasil, and stored in the Pindamonhangaba Health Care Project database. We selected the participants by probability sampling, using a systematic sampling procedure. The calculation of the ideal sample size, considering a 50% prevalence of hot flashes[3] and a maximum error of 5% in 95% of the possible samples, resulted in 384 individuals. We included women between 40 and 65 years of age. Women who did not complete the questionnaire employed for calculation of the Kupperman index (used in the investigation of hot flashes) were excluded, as were those who did not undergo laboratory tests, those who moved away from the study area, those for whom the data were incomplete or inconsistent, those who had cognitive deficits, and those who were using any oral contraceptives or hormone replacement therapy.

The variables studied were age; the level of education; religion; body weight; height; waist circumference; hip circumference; serum triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein; fasting glycemia; and blood pressure. For blood pressure measurements, we use a calibrated, digital, automated sphygmomanometer with an arm cuff (BP 3AG1; MicroLife AG, Widnau, Switzerland) that has been tested and validated by the British Hypertension Society, with the patient in a seated position and following the recommendations for the cuff size selection. We performed three blood pressure measurements on the left arm, at 1-min intervals, considering the mean of the last two measurements in our analysis. Hypertension was defined as a systolic blood pressure ≥ 130 mmHg and a diastolic blood pressure ≥ 85 mmHg[17,18]. We calculated the body mass index (BMI) by dividing the weight (in kilograms) by the height (in meters) squared (kg/m^2), subsequently stratifying the patient status in accordance with the guidelines of the Brazilian Association for the Study of Obesity and Metabolic Syndrome[17]: underweight (BMI < 18.5 kg/m^2); normal weight (BMI 18.5-24.9 kg/m^2); overweight (BMI ≥ 25.0 kg/m^2); pre-obesity (BMI 25.0-29.9 kg/m^2); class I obesity (30.0-34.9 kg/m^2); class II obesity (35.0-39.9 kg/m^2); and class III obesity (BMI ≥ 40 kg/m^2). We also determined the smoking status of the patients. Using the Kupperman index, we classified the intensity of the menopausal symptoms as mild, moderate, or severe. A diagnosis of MetS was made based on the National Cholesterol Education Program Adult Treatment Panel III criteria[18]; that is, when at least three of the five following alterations are present: serum concentration of high-density lipoprotein cholesterol < 50 mg/dL; waist circumference > 88 cm; serum triglyceride concentration ≥ 150 mg/dL; systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg; and fasting glycemia ≥ 110 mg/dL. The dependent variable was the occurrence of hot flashes, whereas all other variables were analyzed as independent variables.

The women were interviewed by a well-trained team at primary health care clinics, where they were also submitted to the collection of blood samples for standardized laboratory tests. Those who did not appear for their scheduled appointments were interviewed in their homes and referred for the collection of blood samples. The data collection was supervised by a coordinator, and 10% of the interviews were repeated by telephone to reduce the risk of bias.

All blood samples were collected in the morning (after an 8h fast), by the same nurse, using a 25 x 0.7 mm, silicone-coated, tri-beveled, sterile 22-G needle. The blood was collected into sterile, disposable, 16 x 100 mm, 8 mL polyethylene terephthalate vacuum collection tubes (Vacuette; Greiner Bio-One, Kremsmünster, Austria), containing a clot activator and separator gel. The biochemical analysis was performed

at the Pindamonhangaba Municipal Laboratory with commercial diagnostics kits (Roche Diagnostics, Mannheim, Germany) – that were submitted to automated analysis.

Statistical calculations were performed with the Stata SE statistical software package, version 12.0 (Stata Corp., College Station, TX, USA). Initially, we used descriptive statistics to analyze the data. We calculated the prevalence of hot flashes and that of MetS, with the respective 95% confidence intervals. We then calculated the odds ratio for MetS in relation to the dependent variable. Multiple logistic regression models were constructed to estimate the relative weight of each independent variable after adjustment for skin color, income, depression, anxiety, and smoking, all of which were considered potential confounders, and we calculated the adjusted odds ratios, together with the respective 95% confidence intervals.

In compliance with the requirements of Resolution 466/12 of the Brazilian National Ministry of Health[19], the study was approved by the Research Ethics Committee of the University of São Paulo School of Public Health (CAAE: 64011917.3.0000.5421). All participants gave written informed consent.

RESULTS

Of the 1,435 women initially recruited, 513 were excluded because they did not complete the questionnaire employed for calculation of the Kupperman index, had cognitive deficits, or moved away from the study area. Of the remaining 900 women, 264 were excluded: 196 because they did not undergo the laboratory tests or because the related data were incomplete; and 59 because they were under 40 years old. Therefore, the final sample was comprised of 647 women.

Table 1 shows the characteristics of the study population. The prevalence of hot flashes was 55.83% (95% CI: 52.35-59.25%), and the prevalence of MetS was 48.63% (95% CI: 44.75-52.53%). Among the women evaluated, the mean age at menopause was 45.99 years (SD 6.61). In addition, the mean values for blood pressure, fasting glycemia, waist circumference, and BMI were above the desired levels[19], translating to a high cardiovascular risk in the study population.

We found that a diagnosis of MetS increased the likelihood of the occurrence of hot flashes (crude OR 1.16; 95% CI: 1.01-1.33). The odds ratio was adjusted for the potential confounders (skin color, income, depression, anxiety, and stress). In other words, women with MetS were 16% more likely to report hot flashes than those without the syndrome, confirming a positive association between MetS and hot flashes.

DISCUSSION

The hypoestrogenism that occurs during the menopausal transition can result in uncomfortable symptoms, such as hot flashes, which significantly impair the quality of life[6,20]. Clinically, hot flashes present as sudden-onset episodes of perceived overheating, typically in the trunk and face, resulting from an imbalance in thermoregulatory processes at the level of the hypothalamus in response to the reduction in estrogen levels. That imbalance causes the body to interpret small temperature oscillations as major changes, triggering cooling mechanisms such as flushing and sweating[21]. Recent studies suggest that hot flashes not only have a significant effect on the quality of life but also indicate a higher risk of CVD, possibly due to activation of the sympathetic nervous system.

In addition to a high prevalence of hot flashes (55.83%), the present study showed high values for glycemia, blood pressure, waist circumference, and BMI, which indicate a higher risk of MetS and therefore of CVD. We also identified a clear association between complaints of hot flashes and MetS, a finding similar to those obtained in other studies[12-14]. That association can be explained by the increased sympathetic activity occurring in both conditions, as well as by obesity[22]. This major cardiovascular risk factor, i.e., obesity, acts as an insulator, reducing the metabolic heat loss capacity[12,23].

In a study involving 183 postmenopausal women, Lee et al.[12] found that hot flashes were more common in women with MetS. Ryu et al.[13] studied 1,906

TABLE 1. CHARACTERISTICS OF THE SAMPLE. SAÚ, HPF.

Characteristic	(*N* = 647)
Hot flashes (%)	55.83
Metabolic syndrome (%)	48.63
Age (years), mean ± SD	50.65 ± 8.49
Age at menopause (years), mean	45.99 ± 6.61
Systolic blood pressure (mmHg), mean ± SD	130 ± 22.96
Diastolic blood pressure (mmHg), mean ± SD	80.10 ± 14.31
Glycemia (mg/dL), mean ± SD	103.11 ± 41.87
Waist circumference (cm), mean ± SD	93.44 ± 12.48
Body mass index (kg/m²), mean ± SD	29.54 ± 5.51
Monthly income (R$), mean ± SD	1646.19 ± 1155.81

R$, Brazilian reals (exchange rate as of August 11, 2020: R$1.00 = US$0.19).

postmenopausal women and also found that those with MetS were more symptomatic. Our results are also similar to those obtained in a study of 183 postmenopausal women conducted by Lee et al.[12]. In overweight or obese women and in those with a waist circumference greater than 88 cm, we also observed a higher prevalence of hot flashes. That could be explained by the aforementioned insulating property of adipose tissue, which would trigger increases in body temperature. In our sample of younger women, we observed a higher frequency of hot flash complaints, especially in the two years surrounding the menopausal transition, which, in our sample, occurred at a mean age of approximately 46 years.

Since the present study had a cross-sectional design, we cannot make any inferences regarding causation or temporality among the variables studied. However, because we found an association between hot flashes and MetS, future studies could consider the occurrence of hot flashes as a risk marker for CVD and MetS. In fact, there have been numerous studies showing that hot flashes are associated with activation of the hypothalamic-pituitary-adrenal axis, with the release of cortisol, and with norepinephrine stimulation[24,25]. Therefore, the only way to establish causality among these factors in a more consistent manner is to conduct longitudinal cohort studies. There is evidence that sympathetic hyperactivity is a common denominator between MetS and the onset of hot flashes[23,26]. That is likely due to vascular phenomena resulting from the release of vasoactive substances (cortisol and norepinephrine), which is influenced not only by hypoestrogenism but also by the aging process[13]. Finally, given the possibility of a relationship between hot flashes and MetS, as well as the known cardiovascular risk in women with MetS, it is recommended that immediate interventions be instituted in all women with hot flashes, especially those with risk factors for MetS.

The limitations of our study include its cross-sectional nature, as well as the losses during the sample collection phase and the recruitment of subjects only from the public health system, which might not adequately represent the general population. Those limitations create an opportunity for further, more complex, studies, which could confirm the data presented here.

CONCLUSIONS

In women of 40 years of age or older, hot flashes appear to be highly prevalent. There also appears to be a positive association between the occurrence of hot flashes and MetS.

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Author's Contributions:

Helena Proni Fonseca Saú: data curation, formal analysis, methodology; Ana Carolina Basso Schmitt: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project management; Maria Regina Alves Cardoso: conceptualization, data curation, formal analysis, methodology, project management; José Mendes Aldrighi: conceptualization, data curation, investigation, methodology, project management.

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RESUMO

OBJETIVO: *As ondas de calor têm um impacto negativo na qualidade de vida das mulheres no climatério. A redução progressiva dos níveis de estrogênio gonadal associada ao envelhecimento promovem o acúmulo de gordura abdominal, dislipidemia e hipertensão arterial, componentes da síndrome metabólica (SM). O objetivo do presente estudo foi estimar a prevalência de ondas de calor e avaliar sua relação com SM em mulheres com idade ≥40 anos.*

MÉTODOS: *Estudo transversal envolvendo mulheres entre 40 e 65 anos de idade. Utilizamos o índice de Kupperman para quantificar os sintomas climatéricos e os critérios do National Cholesterol Education Program Adult Treatment Panel III para o diagnóstico de SM.*

RESULTADOS: *Mil, quatrocentas e trinta e cinco mulheres foram selecionadas inicialmente e obtivemos informações de 647. A idade média da menopausa foi de 45,99 anos (DP 6,61 anos) e a prevalência de ondas de calor e SM foi de 55,83% (95% CI: 52,35-59,25%) e 46,29% (95% CI: 44,75-52,53%), respectivamente. Identificamos uma associação positiva entre SM e ondas de calor (OR 1,16; IC95%: 1,01-1,33).*

CONCLUSÕES: *Em mulheres com idade ≥40 anos, as ondas de calor são altamente prevalentes e parecem estar associadas a SM.*

PALAVRAS-CHAVE: *Climatério. Perimenopausa. Fogachos. Síndrome metabólica.*

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ORIGINAL ARTICLE

Pain and anxiety in office histeroscopy

Mayanna Oliveira Rolim[1]
Ana Luiza Ramos Morais[1]
Camila Sampaio Nogueira[2]
Maria do Socorro Monte de Araujo[3]
Denise Vasconcelos de Moraes[1]
Raquel Autran Coelho[4]

1. Médica na Maternidade-Escola Assis Chateubriand, Universidade Federal do Ceará, Fortaleza, CE, Brasil.
2. Estudante de Medicina na Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, CE, Brasil.
3. Enfermeira na Maternidade-Escola Assis Chateubriand, Universidade Federal do Ceará, Fortaleza, CE, Brasil.
4. Professora Associada na Faculdade de Medicina, Universidade Federal do Ceará e Unichristus, Fortaleza, CE, Brasil.

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SUMMARY

BACKGROUND: *Anxiety is almost always present before medical interventions and may play a role in pain perception. We aim to evaluate factors associated with pain intensity reported by patients submitted to Office Hysteroscopy (OH).*

METHODS: *Cross-sectional observational study, with data from April to November 2015. It included patients attended at the Assis Chateaubriand Maternity School (MEAC/UFC) with an indication of office hysteroscopy. Before the examination, the patients answered a validated questionnaire about anxiety (STAI). After the examination, women answered the Visual Analogue Scale (VAS). The data were analyzed using the Statistical Package for the Social Sciences (SPSS) 15.0, with Spearman correlation, Mann-Whitney U-test, and analyses of variance.*

RESULTS: *252 patients were included, with a mean age of 45.7 years, of whom 29% were postmenopausal (mean pain 5.5) and 71% were in menacme (mean pain 5.1) (p = 0.258). The anxiety trait and state showed a significant influence on the pain scale (p <0.001 and p=0.001), but age or endometrial sample did not. 27% of the patients were nulliparous. Less pain was associated with the number (p=0.01) and vaginal (p=0.005) of deliveries. The main indication for the procedure was abnormal uterine bleeding (54.4%).*

CONCLUSION: *OH may be associated with moderate but tolerable discomfort. There was a significant correlation between higher scores on the pain scale and anxiety. There was evidence of reduced pain with parity and type of delivery, but not with reproductive age or endometrial biopsy.*

KEYWORDS: *Pain Measurement. Manifest Anxiety Scale. Hysteroscopy.*

INTRODUCTION

Hysteroscopy represents the gold standard for the evaluation of the uterine cavity and adequate endometrial sampling due to its minimal invasiveness and high diagnostic success rate[1]. Hysteroscopy can be performed in the outpatient department, without anesthesia. Office hysteroscopy (OH) is becoming increasingly popular, with the use of the minihysteroscope with a vaginoscopic and no-touch approach, leading to less painful and better-tolerated examinations and even operations[2,3].

In general, this procedure is very safe and well-tolerated by patients. Nevertheless, thinner

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CORRESPONDING AUTHOR: Raquel Autran Coelho
Departamento de Saúde da Mulher, da Criança e do Adolescente (UFC)
Rua Professor Costa Mendes, 1608, 5o andar, Fortaleza, CE, Brasil – 60430-140
Tel/Fax: +55 85 3366-8041
E-mail: raquelautrancp@gmail.com

scopes do not guarantee a painless procedure, as some women still report significant suffering. Yang and Vollenhoven[4] reported that pain is the most common reason for failing to perform OH. Potential factors associated with pain perception during this procedure include the diameter of the scope, medical experience, age, and anxiety of the patient. Therefore, some factors may contribute to the selection of women candidates for analgesia, due to the greater susceptibility to severe pain during the outpatient procedure[5].

Anxiety is almost always present before medical interventions and may play a role in pain perception. There seems to be a positive association between the anxiety level and visual analog scale (VAS) pain reporting, and in some cases, nervousness may lead to catastrophizing (exaggerated negative orientation toward pain stimuli). There are some validated scales widely used to measure the general anxiety level. Nonetheless, the effect of anxiety on pain perception during OH has not been well-defined yet[3,6,7].

In view of the lack of regional studies on the tolerance of OH, we sought to evaluate factors associated with the level of pain reported by patients submitted to OH in a tertiary hospital in the northeast of Brasil, as well as to observe the influence of women's anxiety during the examination.

METHODS

This is an observational, cross-sectional, descriptive study based on data collected from April 2015 to November 2017. The study population consecutively included all women who attended the gynecology department of the Assis Chateaubriand Maternity School (MEAC), Federal University of Ceará (UFC), with an indication for OH. Patients with contraindications for the examination (gestation, cervicitis, genital bleeding, and acute pelvic inflammatory disease) were excluded, in addition to those who had previously had analgesics or any other cervical preparation, were under 18 years of age, were intellectually incapable of answering the questionnaire, or voluntarily manifested the desire not to participate in the study.

Clinical and demographic information were obtained from each participant during a medical interview. Immediately before the examination, the patients answered a validated self-assessment questionnaire about anxiety (STAI)[8], through the reduced Portuguese version of Spielberguer STAI-Static Anxiety Inventory (STAI)[9,10]. The STAI was introduced in 1970 and revised in 1983, with questions on a 1 to 4 Likert scale, and is among the most widely researched measure of general anxiety, available in many different languages. It gives a score of 20-80, and the higher the score, the greater the anxiety. One of its particularities is to differentiate the state anxiety (STAI-S), which evaluates the current state of anxiety, asking how women feel "right now," from a patient's trait anxiety (STAI-T), which evaluates relatively stable aspects of propensity to be anxious. The short-form version consists of 6 self-reported items with high internal consistency alpha coefficients.

All the examinations were carried out in an ambulatory setting without analgesia or anesthesia, during in the first phase of the menstrual cycle, for women in the menacme, by surgeons with different experiences in OH. Participants were positioned in the gynecological position. A 2.9mm rigid hysteroscope was introduced under direct vision into the uterine cavity (Karl Storz, Tuttlingen, Germany). The distension medium used was 0.9% saline solution with a continuous flow and intrauterine pressure of 75mmHg, controlled by an electronic pump (Karl Storz Endoskope®, Hysteromat, Germany). The image was transmitted in real-time to a monitor, allowing the patient to watch the examination. Endometrial biopsies were performed with a Novak curette, if necessary. OH lasted between 15 to 30 minutes.

After the procedure, women answered the VAS, a one-dimensional instrument for the evaluation of pain intensity. Pain rating according to a 0–10 cm VAS (0–3 mild pain, 4–7 moderate pain, 8–10 severe pain) is recommended by World Health Organization[11].

Quantitative data are presented as mean and standard deviation. Spearman's correlation, Mann-Whitney U-test, and analyses of variance were used to verify the relationship between the intensity of pain perception and other variables in the sample. p-values <0.05 were considered significant. All statistical analyses were performed with the software Statistical Package for Social Sciences (SSPS) 22.

This work was approved by the Research Ethics Committee - CEP of the Assis Chateaubriand Teaching Maternity Hospital under protocol 934.442 of 1/14/2015. The patients signed a Free and Informed Consent Form to participate in the study.

RESULTS

The study included 252 patients, with a mean age of 45.7 ± 10.8 years old, and a mean pain of 5.6 ± 3.2, according to the VAS. 29% of women were post-menopausal. Age and menopausal status were not associated with mean pain (p= 0.99 and 0.26, respectively). 27% of the patients were nulliparous. Pain was associated with the number (p=0.01) of deliveries. Regarding the type of delivery, 51.1% of women had at least 1 vaginal delivery and reported less pain (p=0.005) (Table 1)

TABLE 1. MEAN PAIN SCORE AFTER OH, ACCORDING TO PATIENT CHARACTERISTICS.

Characteristics	Pain (VAS 0 - 10)	P-value
Menopause		
Yes	5.5 ± 3.0	0.258
No	5.1 ± 2.9	
Previous vaginal delivery		
Yes	4.3 ± 2.8	0.005*
No	5.9 ± 2.9	
Endometrial biopsy		
Yes	5.1 ± 2.8	0.374
No	5.5 ± 3.3	

Note: Data expressed as mean ± standard deviation; VAS: Visual Analogic Scale Mann-Whitney U test;*Significance considered p 0.05

The main indications for the procedure were abnormal uterine bleeding (54.4%) and endometrial thickening (31.4%). 66.9% of patients underwent an endometrial biopsy with a mean pain of 5.1 ± 2.8. There was no correlation between the biopsy procedure and the VAS (p= 0.37). There were no cases of carcinoma in the samples.

Pain perception during hysteroscopy was categorized into three groups according to VAS (slight, moderate or intense pain): (1) <4, with 105 cases (41.7%); (2) ≥4 and ≤7, with 75 cases (29.8%); and (3) >7, with 72 cases (28.6%).

Mean state anxiety (STAI-S) was 47.6 ± 0, while mean trait anxiety (STAI-T) was 43 ± 15, on a 0 to 80 scale. The pain reported by VAS was correlated to both scales of anxiety (Table 2).

There was a positive correlation between the VAS and STAI-S (r=0.21, p=0.01) and between the VAS and STAI-T (r=0.28, p<0.001).

DISCUSSION

The mean pain reported by the patients in this study, according to the VAS, was 4.5, thus considered mild to moderate, but with tolerable discomfort. The main complaint of patients submitted to OH is pain, a factor that limits the success of the examination[4]. The pain scale used is quick to apply and easily understood by the patient. For the VAS evaluation, scores of 2.5 to 3 were considered the upper limit score for mild pain, and scores above the upper limit of the VAS of 6.5 to 7 defined pain as severe[12]. However, as a one-dimensional instrument, it only analyzes the intensity of pain. The mean pain index values shown for OH have conflicting results in different populations and regions of the world, ranging from 1.8 to 6.02[7,8,13-18]. This may be due to different service experiences combined with the higher prevalence of normal births in some countries in relation to the Brazilian population.

In previous studies with mixed populations and different indications of hysteroscopy, a wide range of women referring to a VAS ≥4 was observed, varying from 21% to 88%[17,19]. We found 58.4% of women with a VAS ≥4. Several factors can influence these results, including reproductive status, distension medium, and surgeon experience. Such findings confirm that hysteroscopy is a painful examination in a considerable number of cases. Patients may experience pain as a result of physical stimuli such as intrauterine pressure and manipulation. However, pain is subjective; its perception is modulated by states of mood and emotion[13]. In fact, patient support has been linked to less pain and anxiety[6,20,21].

TABLE 2. COMPARISON OF PAIN IN GROUPS OF PATIENTS UNDERGOING HYSTEROSCOPY AFTER STRATIFICATION OF PAIN INTENSITY LEVELS IN SUBGROUPS: SLIGHT, MODERATE, OR INTENSE

	Pain intensity			
Variables	Slight pain (0-3) N=105	Moderate pain (4-7) N=75	Intense pain (8-10) N=72	p
Age (years)	46.1 ± 9.9	44.8 ± 11.2	45.9 ± 10.8	0.857
STAI-S	43.8 ± 15.5	45.9 ± 12.6	51.2 ± 14.0	0.009*
STAI-T	38.6 ± 14.6	43.8 ± 13.6	48.9 ± 15.5	<0.001*

Note: Data expressed as mean ± standard deviation ANOVA;*Significance considered p 0.05

In this study, the anxiety trait and state showed a significant correlation with the pain scale completed after the procedure. The mean STAI-S and STAI-T were above 40, which is higher than the score of the Brazilian female population (mean: 35.7) and suggests a moderate level of anxiety before the examination, similar to other studies. It has been suggested that scores of 39 to 55 are indicative of clinically significant anxiety[7,12,20,22].

Patients' anxiety about medical appointments may influence the perception of pain, the success rate, and satisfaction[13,23]. The effect of the anxiety state on pain has been suggested in some previous studies[7,21], but not in others[20,22]. It is difficult to explain these conflicting results due to the heterogeneity of the studies and population, but it is important to notice that there is a high prevalence of women presenting moderate levels of anxiety before hysteroscopy.

Based on this context, efforts should be made to identify predictors or interventions that may assist in identifying individuals at greater risk of anxiety and preventing/limiting anxiety and its consequences. Providing a greater degree of comfort during the examination, such as by offering the patient a detailed explanation of the method, for example, can be an important pain reduction strategy.

Parity and previous vaginal delivery were associated with less pain in OH in this study. There is no consensus on the history of normal delivery as a variable capable of determining pain reduction. It has shown a great impact on pain reduction in a clinical trial with multivariate analysis[17]. In Brasil, a country with a high cesarean rate in the health system, the use of strategies capable of reducing pain regardless of the obstetric history has a good acceptance[20].

Although there is evidence that genital atrophy may make it difficult to perform the examination, the findings of this study do not support such claims[17]. Age did not influence the pain level among the women analyzed. The main indications for hysteroscopy in the present study were abnormal uterine bleeding, endometrial thickening, and myomatosis, and most of the women were pre-menopausal.

The performance of a guided biopsy can be as painful as or even more uncomfortable than the exam itself[4,5]. The endometrial biopsy was performed with Novak and the material was collected in a non-directed way, which could be a contributing factor for pain. There was no association, however, between the biopsy and the pain scale among the women studied.

The examination was carried out in a University Hospital by resident physicians in gynecology with a varied learning curve, guided by supervising physicians, which inherently leads to a variation in the performance. Other studies found that experienced surgeons are a protective factor for pain perception during diagnostic hysteroscopy[20,24]. Factors such as technical ability may be correlated with the degree of anxiety and pain during hysteroscopy[3]. Another possible limitation is the lack of information about women's history of chronic pelvic pain and dysmenorrhea as a predictive factor of pain during hysteroscopy. Besides that, the questionnaire applied could lead to bias in the study when associated with the low cognitive level of some patients.

Office Hysteroscopy was associated with moderate but tolerable discomfort. There was a significant correlation between anxiety and higher scores on the pain scale. There was evidence of a relationship between reduced pain and parity and type of delivery, but not with reproductive age or endometrial biopsy procedure. The early identification of possible predictors of pain could trigger interventions to improve analgesia and reduce anxiety in patients who will undergo OH.

Conflicts of interest

The authors have no conflicts of interest, be it political, economic, regarding resources for carrying out the research, or intellectual property.

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Author's Contribution

Mayanna Rolim; Raquel Coelho; Camila Nogueira 1. Substantial contributions to the study concept and design, data collection or analysis and interpretation; 2. Writing of the article or relevant critical review of the intellectual content and 3. Final approval of the version to be published.

Ana Luiza Morais; Maria do Socorro Araujo; Denise de Moraes 1. Substantial contributions to the study concept and design, data collection or analysis and interpretation; 2. Final approval of the version to be published.

RESUMO

INTRODUÇÃO: *A ansiedade está quase sempre presente antes de intervenções médicas e pode desempenhar um papel importante na percepção da dor. Buscou-se avaliar os fatores associados à intensidade da dor relatados pelos pacientes submetidos a histeroscopia ambulatorial (HA).*

MÉTODOS: *Estudo observacional transversal, com dados de abril a novembro de 2015. Foram incluídas pacientes atendidas na Maternidade Escola Assis Chateaubriand (Meac/UFC) com indicação de HA. Antes do exame, as pacientes responderam a um questionário validado sobre ansiedade (IAM). Após o exame, as mulheres responderam à Escala Visual Analógica (EVA). Os dados foram analisados no Statistical Package for the Social Sciences (SPSS) 15.0, com correlação de Spearman, teste U de Mann-Whitney e Anova.*

RESULTADOS: *Foram incluídas 252 pacientes, com idade média de 45,7 anos, das quais 29% estavam na pós-menopausa (dor média 5,5) e 71% eram menacme (dor média 5,1) (p = 0,258). O traço e o estado de ansiedade mostraram influência significativa na escala de dor (p<0,001 e p=0,001). Vinte e sete por cento das pacientes eram nulíparas. Menor dor foi associada ao número (p=0,01) e tipo vaginal (p=0,005) de partos. A principal indicação para o procedimento foi sangramento uterino anormal (54,4%); 66,1% necessitaram de amostra endometrial.*

CONCLUSÕES: *A HA pode estar associada a um desconforto moderado, mas tolerável. Houve correlação significativa entre escores mais altos na escala de dor e ansiedade, menor paridade, mas não com idade reprodutiva ou procedimento de biópsia endometrial.*

PALAVRAS-CHAVE: *Medição da dor. Escala de ansiedade manifesta. Histeroscopia.*

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ORIGINAL ARTICLE

Protective effect of dexmedetomidine on perioperative myocardial injury in patients with Stanford type-A aortic dissection

Dalong Wang[1*]
Quan Lin[2*]
Meiqing Du[1]
Guanrong Zheng[1]
Weimin Xu[1]
Haishan Zhang[1]
Ke Liu[1]

1. Department of Anesthesiology, Shengli Oilfield Central Hospital, Dongying 257000, China.
2. Department of Administration, Shengli Oilfield Central Hospital, Dongying 257000, China.
* Contributed equally.

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SUMMARY

OBJECTIVE: *To investigate the protective effect and mechanism of dexmedetomidine (Dex) on perioperative myocardial injury in patients with Stanford type-A aortic dissection (AD).*

METHODS: *Eighty-six patients with Stanford type-A AD were randomly divided into Dex and control groups, with 43 cases in each group. During the surgery, the control group received the routine anesthesia, and the Dex group received Dex treatment based on routine anesthesia. The heart rate (HR) and mean arterial pressure (MAP) were recorded before Dex loading (t0), 10 min after Dex loading (t1), at the skin incision (t2), sternum sawing (t3), before cardiopulmonary bypass (t4), at the extubation (t5), and at end of surgery (t6). The blood indexes were determined before anesthesia induction (T0) and postoperatively after 12h (T1), 24h (T2), 48h (T3), and 72h (T4).*

RESULTS: *At t2 and t3, the HR and MAP in the Dex group were lower than in the control group ($P < 0.05$). Compared with the control group, in the Dex group at T1, T2, and T3, the serum creatine kinase-MB, cardiac troponin-I, C-reactive protein, and tumor necrosis factor-α levels were decreased, and the interleukin-10 level, the serum total superoxide dismutase, and total anti-oxidant capability increased, while the myeloperoxidase and malondialdehyde levels decreased (all $P < 0.05$).*

CONCLUSIONS: *Dex treatment may alleviate perioperative myocardial injury in patients with Stanford type-A AD by resisting inflammatory response and oxidative stress.*

KEYWORDS: *Dexmedetomidine. Aneurysm, dissecting. Myocardium. Oxidative stress. Inflammation/metabolism. Inflammation mediators/metabolism.*

INTRODUCTION

Aortic dissection (AD) refers to the tearing of the aortic intima under the action of certain pathogenic factors. In this disease, the blood flows into the middle layer of the aortic wall through the torn intimal orifice, which causes the intima to exfoliate along the aortic cavity to form a hematoma, i.e., pseudolumen.

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CORRESPONDING AUTHOR: Ke Liu
Department of Anesthesiology, Shengli Oilfield Central Hospital, 31, Jinan Road, Dongying, China – 257000
Tel: +86 546 877-9266
E-mail: liukesddy@163.com

AD is a rare but fatal disease that is dangerous and progresses rapidly. Patients with AD often die from hematoma rupture, massive hemorrhage, and pericardial tamponade[1]. The high-risk factors of AD include hypertension, Marfan's syndrome, aortic degeneration, cocaine abuse, atherosclerosis, and so on, and hypertension and Marfan's syndrome are the most common[2,3]. According to the location of the primary rupture and the treatment methods, AD is classified as Standford type A and B. Standford type-A AD is more common, which involves the ascending aorta. In Standford type-A AD, the period from the onset to the 14th day is the acute phase, during which the active drug treatment to control blood pressure and appropriate analgesia and sedation should be performed[4]. At present, surgery is the most effective method to treat Stanford type-A AD, but the operation process is complicated, with great trauma and bleeding, and the incidences of postoperative complications and mortality rate are high[5]. The acute myocardial infarction, pericardial tamponade, hypotension, shock, and old age are the main risk factors for early death after surgery in AD patients[6]. Therefore, strengthening the perioperative myocardial protection and blood pressure management are of great significance to improve the success rate of surgery and prognosis of patients. Dexmedetomidine (Dex) is an imidazole derivative that selectively activates the α2 adrenergic receptors. It is widely used in clinical anesthesia assistance due to its sedative, analgesic, and sympathetic blockade effects[7]. Dex can reduce the number of other drugs to achieve the best intoxication effect while minimizing side effects. At present, there are many animal experiments on the protective effect of Dex on myocardium[8,9]. Clinical studies mainly focus on cardio-protection of Dex during coronary artery bypass grafting[10,11], but the application of Dex for Standford type-A AD is rarely reported. The aim of this study was to investigate the protective effect and mechanism of Dex on perioperative myocardial injury in patients with Stanford type-A AD, so as to provide a reference for its further clinical application.

SUBJECTS AND METHODS

Subjects

The clinical data of 86 patients with Stanford type-A AD receiving surgery in the Shengli Oilfield Central Hospital (Dongying, China) from July 2014 to December 2017 were selected. There were 56 males and 30 females, aged 28-69 years, with an average age of 47.52±8.31 years. The body mass index (BMI) was 23.11±4.92 kg/m^2. There were 19 cases of smoking history, 50 cases of hypertension history, and 11 cases of diabetes mellitus history. The American Society of Anesthesiologists (ASA) grade was III (49 cases) or IV (37 cases). The anesthesia duration was 254.48±55.04 min. The cardiopulmonary bypass (CPB) duration was 138.98±28.12 min. The surgery duration was 275.28±59.28 min. Eighty-six patients were randomly divided into Dex and control groups, 43 cases in each group. There was no significant difference in gender, age, BMI, smoking history, hypertension history, diabetes mellitus history, ASA grade, anesthesia duration, CPB duration, or surgery duration between Dex and control groups ($P > 0.05$). This study was in line with the medical ethics standards and was approved by the ethics committee of the Shengli Oilfield Central Hospital. Informed consent was obtained from all subjects.

Inclusion and exclusion criteria

The inclusion criteria were as follows: 18-70 years old; Stanford type-A AD confirmed by clinical and imaging examinations, within 14 days of onset; normal liver and kidney function. The exclusion criteria were as follows: complicated with other cardiopulmonary diseases; neurological dysfunction; obvious severe tissue perfusion defects; a long-term history of sedative use.

Anesthesia and intraoperative monitoring

In the two groups, after admission, oxygen was inhaled by mask, and ECG and pulse oxygen saturation were monitored routinely. At the same time, a bispectral index monitor was used to monitor the depth of anesthesia. The peripheral venous access of one upper limb was opened and a compound sodium lactate Ringer solution was intravenously dripped. Left-radial-artery puncture catheterization was performed to monitor the arterial blood pressure. The induction and maintenance of anesthesia were the same in the two groups. An intravenous injection of midazolam (0.05-0.1 mg/kg), etomidate (0.10-0.30 mg/kg), rocuronium (1.00 mg/kg) and sufentanil (1.00-2.00 μg/kg) was used for the induction of anesthesia. After endotracheal intubation, mechanical ventilation was performed. The oxygen concentration was 60%, with an oxygen flow rate of 0.5-1.0 L/min, a tidal volume of 8-10 ml/kg, I/E ratio of 1: 2, and a respiratory rate of 10-14 times/min. The end-expiratory partial pressure

of carbon dioxide was maintained at 35-45 mmHg. The right internal jugular vein was punctured, and a three-chamber 7F central venous catheter (depth 13-15 cm) was placed for infusion and monitoring of the central venous pressure. Intravenous infusion of sufentanil [0.5-1.5 μg/(kg·h)] and propofol [4-6 mg/(kg·h)] was used for anesthesia maintenance. Intermittent intravenous injection of pancuronium was performed to maintain muscle relaxation. The intraoperative bispectral index was maintained at 40-60. In the Dex group, after anesthesia induction, Dex was intravenously dripped (1 ug/kg) for 10 min, followed by intravenously dripping at a rate of 5 μg/(kg·h) to the end of surgery. The control group received an intravenous drip of 0.9% sodium chloride after anesthesia, using the same volume and methods as in the Dex group. All surgical operations were performed under general anesthesia, deep hypothermic circulatory arrest, and selective cerebral perfusion, and were completed by the same group of surgeons.

Hemodynamic monitoring

The heart rate (HR) and mean arterial pressure (MAP) were recorded before Dex loading (t0), 10 min after Dex loading (t1), at the skin incision (t2), sternum sawing (t3), before cardiopulmonary bypass (t4), at the extubation (t5), and at the end of surgery (t6).

Determination of blood indexes

The peripheral venous blood samples were collected before anesthesia induction (T0), and postoperatively after 12h (T1), 24h (T2), 48h (T3), and 72h (T4). After centrifuging at 3000 rpm for 10 min, the serum was stored in a refrigerator at -70 °C. The serum levels of myocardial injury indexes, including creatine kinase-MB (CK-MB) and cardiac troponin-I (cTnI), inflammatory cytokines, including C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-10 and oxidative stress indexes, including total superoxide dismutase (TSOD), total anti-oxidant capability (TAOC), myeloperoxidase (MPO) and malondialdehyde (MDA), were determined by enzyme-linked immunosorbent assay. The operations were carried out strictly according to the instructions of the kits (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

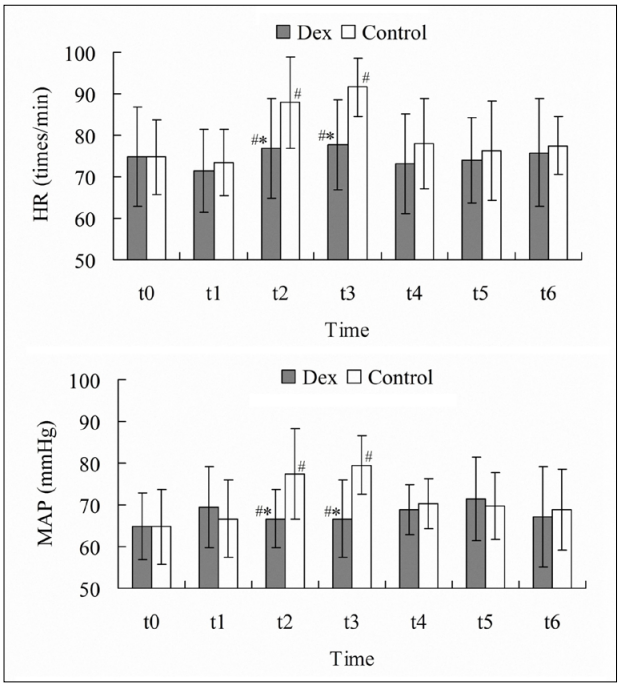
Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, USA). The enumeration data were presented as number and rate, and the comparison between the two groups was performed using the χ^2 test. The measurement data were presented as mean±standard deviation, and the comparison between the two groups was performed using the t-test. Statistical significance was accepted at two-sided $P < 0.05$.

RESULTS

Comparison of HR and MAP between two groups

As shown in Figure 1, from t0 to t6, the HR and MAP in the control and Dex groups gradually increased, followed by a decrease. At t2 and t3, the HR and MAP in the Dex group were significantly lower than those in the control group ($P < 0.05$). At all the other time points, there was no significant difference in the indexes between the two groups ($P > 0.05$).

FIGURE 1. COMPARISON OF HR AND MAP BETWEEN THE DEX AND CONTROL GROUPS.



#P < 0.05 compared with t0;*P < 0.05 compared with the control group. HR, heart rate; MAP, mean arterial pressure; Dex, dexmedetomidine.

Comparison of serum CK-MB and cTnI levels between two groups

At T0, T1, T2, T3, and T4, the serum CK-MB level in the Dex group was 2.92±0.41, 24.5±3.43, 16.83±2.36, 15.04±2.10, and 7.21±0.98 ng/ml, respectively; in the control group, it was 2.82±0.39, 29.7±4.16, 27.7±3.88, 17.21±2.38, and 8.03±1.12 ng/ml, respectively. At T0,

T1, T2, T3, and T4, the cTnI level in the Dex group was 0.33±0.05 2.21±0.31, 1.63±0.22, 1.42±0.20, and 0.89±0.12 ng/ml, respectively; in the control group, it was 0.31±0.04, 3.27±0.45, 2.35±0.32, 2.52±0.35, and 1.31±0.18 ng/ml, respectively. From T0 to T4, the serum CK-MB and cTnI levels in the Dex and control groups increased, followed by a decrease. Each of them was the highest at T1. In addition, at T1, T2, and T3, the serum CK-MB level in the Dex group was significantly lower than that in the control group ($P < 0.05$). At T1, T2, T3, and T4, the serum cTnI level in the Dex group was significantly lower than that in the control group, respectively ($P < 0.05$).

Comparison of serum CRP, TNF-α, IL-6, and IL-10 levels between the two groups

With the time prolonging from T0 to T4, the serum CRP, TNF-α, and IL-6 levels in each group increased, followed by a decrease. Over time, the serum IL-10 level in each group decreased, followed by an increase. In each group, the serum CRP level at T2, TNF-α level at T2, and IL-6 level at T1 were the highest among the 5 time points, and the IL-10 level at T1 was the lowest among the 5 time points. In addition, compared with the control group, in the Dex group, the CRP level at T1, T2, and T3 and TNF-α and IL-6 levels at T1, T2, T3, and T4 were significantly decreased ($P < 0.05$), while the IL-10 level at T1, T2, T3, and T4 was significantly increased ($P < 0.05$) (Figure 2).

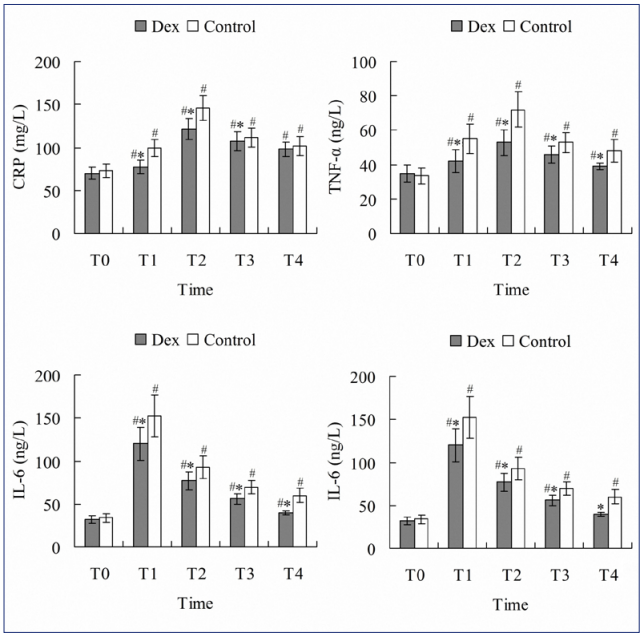
Comparison of serum TSOD, TAOC, MPO, and MDA levels between the two groups

Figure 3 shows that, from T0 to T4, the serum TSOD and TAOC levels in each group decreased, followed by a gradual increase, and the serum MPO and MDA levels in each group increased, followed by a gradual decrease. In each group, the TSOD and TAOC levels at T1 were the highest among the 5 time points, and the MPO and MDA levels at T1 were the lowest among the 5 time points. In addition, compared with the control group, in the Dex group, the TSOD and TAOC levels at T1, T2, and T3 were significantly increased ($P < 0.05$), and the MPO level at T1, T2, and T3 and MDA level at T1, T2, T3, and T4 were significantly decreased ($P < 0.05$).

DISCUSSION

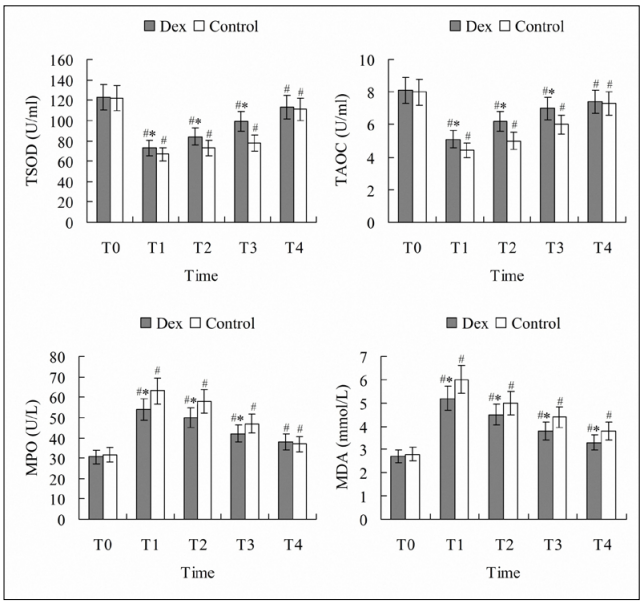
In AD, as the blood continuously flows into the middle layer through the torn intimal orifice, the aortic intima exfoliates to form a hematoma, which leads to the weakness of the aortic wall. The increase of blood pressure and tachycardia caused by any reason during the onset of AD may lead to an increase of shear force in the aortic wall and increase the risk of intimal rupture[12,13]. Therefore, active sedation and

FIGURE 2. COMPARISON OF SERUM CRP, TNF-α, IL-6, AND IL-10 LEVELS BETWEEN THE DEX AND CONTROL GROUPS.



#P < 0.05 compared with T0;*P < 0.05 compared with the control group. CRP, C-reactive protein; TNF-α, tumor necrosis factor-α; IL, interleukin; Dex, dexmedetomidine.

FIGURE 3. COMPARISON OF SERUM CRP, TNF-α, IL-6, AND IL-10 LEVELS BETWEEN THE DEX AND CONTROL GROUPS.



#P < 0.05 compared with T0;*P < 0.05 compared with the control group. TSOD, total superoxide dismutase; TAOC, total anti-oxidant capability; MPO, myeloperoxidase; MDA, malondialdehyde; Dex, dexmedetomidine.

analgesia, strict control of blood pressure, maintenance of hemodynamics, and reduction of shear force in the aortic wall are the basic principles for the perioperative management of patients with acute AD. This study investigated the protective effect of Dex on patients with Stanford type-A AD. Results showed that, compared with the patients using routine anesthesia, in patients additionally using Dex, the HR and MAP at t2 and t3 were significantly decreased. This indicates that the Dex treatment can further maintain hemodynamic stability during surgery in patients with Stanford type-A AD.

CK-MB is a specific myocardial isozyme, which is abundant in myocardial cells. Under normal circumstances, the serum CK-MB level is very low. When the myocardial cells are damaged, CK-MB is released into the blood in large quantities. It has high sensitivity in judging the myocardial damage and is often used in the clinical diagnosis of myocarditis and for evaluating its severity[14]. The cardiac troponin (cTn) molecule is spherical and consists of three subunits including cTnI, cTnT, and cTnC. cTnI in the myocardium is different from that in other muscle tissues. Meanwhile, the molecular weight of cTnI is small. In the early stage of myocardial injury, it can be rapidly released into the blood to increase its blood concentration. cTnI can be used as an early diagnostic index for myocardial injury[15]. Results of this study showed that, at T1, T2, and T3, the serum CK-MB level in the Dex group was significantly lower than that in the control group. At T1, T2, T3, and T4, the serum cTnI levels in the Dex group were significantly lower than those in the control group. This indicates that the Dex treatment can alleviate perioperative myocardial injury in patients with Stanford type-A AD.

The occurrence, development, and clinical manifestations of AD are significantly correlated with the inflammatory response[16]. CRP is a non-specific marker of inflammation. It is the most powerful predictor and risk factor of cardiovascular disease. Its level is increased rapidly in inflammation, injury, and infectious diseases. A previous study has shown that the serum level of CRP is increased significantly in patients with AD[17]. TNF-α is produced by mononuclear macrophages and plays a central role in the inflammatory response. It can promote the inflammatory response by injuring vascular endothelial cells, increasing the expression of endothelial cell-adhesion molecules and other inflammatory factors[18]. IL-6 is also a key component of inflammatory response. It can induce hepatocytes to synthesize acute-phase proteins and regulate macrophages to increase the TNF-α level[19]. IL-10 is an endogenous anti-inflammatory factor, which can inhibit the expression of inflammatory mediators such as TNF-α and IL-6[20]. In the present study, compared with the control group, in the Dex group, the CRP level at T1, T2, and T3 and TNF-α and IL-6 levels at T1, T2, T3, and T4 were significantly decreased, and the IL-10 levels at T1, T2, T3, and T4 were significantly increased. This suggests that the Dex treatment can reduce the inflammatory response in patients with Stanford type A AD, which may be related to its protective effect on myocardial injury.

During the formation of AD, the blockage of aortic branch vessels affects the blood flow, leads to ischemia and poor perfusion of corresponding tissues. The recanalization of vessels can lead to ischemia-reperfusion injury, which can cause a large number of neutrophils to accumulate in the lung, heart, and other organs. They activate degranulation and produce and release a large amount of oxygen free radicals, which cause oxidative stress damage[21]. Lipids in cells have a high affinity with oxygen free radicals. Oxygen free radicals attack lipids, which can damage cell structure and function and produce MDA[22]. MPO is the key enzyme catalyzing the generation of oxygen free radicals, and its content can also reflect the production of oxygen free radicals[23]. SOD and other antioxidant catalytic enzymes can scavenge oxygen free radicals to a certain extent. However, when oxygen free radicals are generated in large quantities and exceed the body's antioxidant capacity, SOD will be continuously consumed and TAOC will be reduced[24]. Results of this study showed that, compared with the control group, in the Dex group, the TSOD and TAOC levels at T1, T2, and T3 were significantly increased, and the MPO levels at T1, T2, and T3 and MDA levels at T1, T2, T3, and T4 were significantly decreased. This indicates that the Dex treatment can reduce oxidative stress, thus alleviating myocardial injury in patients with Stanford type-A AD.

CONCLUSION

The Dex treatment can alleviate perioperative myocardial injury in patients with Stanford type-A AD. Its mechanism may be related to resistance to inflammatory response and oxidative stress. This study has

provided a reference for further clinical application of Dex in AD surgery. However, the results of this study are obtained only from clinical manifestations and laboratory detections, and no myocardial pathological result has been found. In addition, the correlations among different indexes are not analyzed. These are the limitations of this study, which need to be solved in further studies.

Author's Contribution

Ke Liu and Quan Lin designed the study. Dalong Wang and Meiqing Du participated in data collection. Guanrong Zheng and Weimin Xu performed the statistical analyses. Dalong Wang drafted the manuscript. Ke Liu and Haishan Zhang critically revised the manuscript. All authors read and approved the final manuscript.

RESUMO

OBJETIVO: *Investigar o efeito protetor e o mecanismo da dexmedetomidina (Dex) na lesão perioperativa do miocárdio em doentes com dissecação aórtica Tipo A de Stanford (AD).*

MÉTODOS: *Oitenta e seis pacientes com o Tipo A de Stanford foram aleatoriamente divididos em Dex e grupos de controle, 43 casos em cada grupo. Durante a cirurgia, o grupo de controle recebeu a anestesia de rotina, e o grupo Dex recebeu tratamento Dex baseado na anestesia de rotina. A frequência cardíaca (AR) e a pressão arterial média (MAP) foram registradas no momento anterior ao Dex carregar (t0), 10 minutos após o Dex carregar (t1), incisão cutânea (t2), serragem de esterno (t3), antes do bypass cardiopulmonar (t4), extubação (t5) e fim da cirurgia (t6). Os índices de sangue foram determinados no momento antes da indução da anestesia (T0) e no pós-operatório 12 horas (T1), 24 horas (T2), 48 horas (T3) e 72 horas (T4).*

RESULTADOS: *Em T2 e t3, o RH e o MAP do grupo Dex foram inferiores ao grupo de controle (p<0,05). Em comparação com o grupo de controle, no grupo Dex em T1, T2 e T3, os níveis séricos de creatina quinase-MB, troponina-I, proteína C-reativa e necrose do fator-α do tumor diminuíram, o nível interleucina-10 aumentou, o desalinhamento total do superóxido sérico e a capacidade antioxidante total aumentaram e os níveis de mielopeperóxido e malondialdeído diminuíram (todos p<0,05).*

CONCLUSÃO: *O tratamento com Dex pode aliviar a lesão do miocárdio perioperativo em doentes com o Tipo A de Stanford por resistência à resposta inflamatória e ao estresse oxidativo.*

PALAVRAS-CHAVE: *Dexmedetomidina. Aneurisma dissecante. Miocárdio. Estresse oxidativo. Inflamação/metabolismo. Mediadores da inflamação/metabolismo.*

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ORIGINAL ARTICLE

Predictive value of plasma copeptin level for diagnosis and mortality of pulmonary embolism

Caglar Ozmen[1]
Onur Sinan Deveci[1]
Muhammet Bugra Karaaslan[2]
Oya Baydar[3]
Anil Akray[1]
Ali Deniz[1]
Caglar Emre Cagliyan[1]
Ismail Hanta[3]
Ayhan Usal[1]

1. Cukurova University, Faculty of Medicine, Department of Cardiology, Adana, Turkey.
2. Osmancik State Hospital, Department of Cardiology, Osmancık/Çorum, Turkey.
3. Cukurova University Faculty of Medicine, Department of Chest Disease, Adana, Turkey.

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SUMMARY

OBJECTIVE: *Early diagnosis and risk stratification may provide a better prognosis in pulmonary embolism (PE). Copeptin has emerged as a valuable predictive biomarker in various cardiovascular diseases. The aim of this study was to determine the levels of copeptin in patients with acute PE and to evaluate its relationship with disease severity and PE-related death.*

METHODS: *Fifty-four patients and 60 healthy individuals were included in this study. Copeptin concentrations and right ventricular dysfunction were analyzed. The correlation between copeptin levels and hemodynamic and echocardiographic parameters was examined. After these first measurements, patients were evaluated with PE-related mortality at the one-year follow-up.*

RESULTS: *The copeptin levels were higher in PE patients than in the control group (8.3 ng/mL vs 3.8 ng/mL, p<0.001). Copeptin levels were found to be significantly higher in patients with PE-related death and right ventricular dysfunction (10.2 vs 7.5 ng/ml, p=0.001; 10.5 vs 7.5 ng/ml, p=0.002, respectively). When the cut-off value of copeptin was ≥5.85, its sensitivity and specificity for predicting PE were 71.9% and 85.0%, respectively (AUC=0.762, 95% CI=0.635-0.889, p<0.001).*

CONCLUSIONS: *The copeptin measurement had moderate sensitivity and specificity in predicting the diagnosis of PE, and the copeptin level was significantly higher in patients with PE-related death at the one-year follow-up. Copeptin may be a useful new biomarker in predicting diagnosis, risk stratification, and prognosis of PE.*

KEYWORDS: *Biomarkers. Death. Pulmonary embolism/diagnosis. Pulmonary embolism/mortality. Arginine vasopressin/metabolism.*

INTRODUCTION

Acute pulmonary embolism (PE) is an obstructive disease of the pulmonary arterial system associated with significant mortality rates, i.e., up to 30%, if not correctly diagnosed and treated[1]. Early diagnosis and risk stratification may provide better prognosis; however, there is not yet a particular biochemical

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CORRESPONDING AUTHOR: Caglar Ozmen
Cukurova University, Faculty of Medicine, Department of Cardiology, Seyhan, Adana, Turkey – 01140
Tel: +90 532 583-9441
E-mail: caglarozm@hotmail.com

marker useful for early diagnosis or for providing prognostic information. In clinical practice, scoring systems and some biomarkers with proven efficacy, such as D-Dimer, B-type natriuretic peptide (BNP) and troponin I, are used in the diagnosis and risk stratification of acute PE. Since D-Dimer levels may increase in many clinical conditions (cardiac arrest, trauma, hemorrhage, malignancies, shock, disseminated intravascular coagulation, and systemic inflammatory response syndrome), some difficulties arise in the diagnosis of PE[2]. The increase of BNP is highly sensitive but poorly specific for detecting PE patients at risk for severe adverse events, such as cardiac arrest, shock, need for intensive care units, need for thrombolysis, or vasopressors or mechanical ventilation[3]. It has been shown that troponin assays alone cannot be used to diagnose PE. Therefore, for clinicians, the heterogeneity of troponin assays and their lack of harmonization result in interpretative challenges[4]. Due to the many factors that can affect D-Dimer, BNP, and troponin levels in PE, results should be interpreted carefully.

Copeptin, the C-terminal part of pro-arginine vasopressin (AVP), is a glycosylated polypeptide consisting of 39 amino acids. Unlike AVP, copeptin is stable for a long time in plasma and is easily measured[5,6]. The clinical use of AVP is limited due to its particularly short half-life and its instability in frozen plasma[7]. In addition, more than 99% of circulating AVP is bound to platelets, and the small molecular size of AVP does not make it suitable for conventional immunoassays[7]. For these reasons, copeptin is now considered a well-defined surrogate biomarker for AVP. Due to its rapid release kinetics, copeptin has emerged as a valuable predictive and prognostic biomarker in several clinical conditions, including acute coronary syndrome, stroke, shock, and left heart failure[8-11].

In acute PE, acute anatomical obstruction and vasoconstriction of the pulmonary artery (PA) result in increased pulmonary vascular resistance and right ventricular (RV) dilatation. At the same time, neurohumoral activation causes inotropic and chronotropic stimulation and, as a result, the AVP system is activated[12]. Thus, copeptin may show a new pathophysiological axis of PE by reflecting a systemic reaction to impaired hemodynamics due to RV dysfunction in acute PE. Some studies have reported that copeptin may be a highly significant prognostic marker in early mortality and risk stratification in patients with acute PE[13-15]. In this study, we investigated the copeptin levels and their relationship with disease severity and PE-related death in acute PE.

METHODS

Study design

This single-center prospective study was performed in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Cukurova University (Nr. 94). Written informed consent was obtained from all participants before enrollment. We included 54 patients diagnosed with acute PE who were referred to the Cukurova University Medical Faculty of Cardiology and Chest Disease Department (Adana, Turkey) between May 2018 and December 2018. The control group consisted of 60 healthy, age- and gender-matched volunteers who had no medical history, were on no medication, and came to the cardiology and chest disease outpatient clinic. When selecting the control group, careful attention was paid to ensure they were completely healthy volunteers. Candidates in the normal control group were excluded if they had a history of chronic diseases and their biochemical examination results, electrocardiogram, and echocardiography were abnormal. The exclusion criteria of the study population were as follows: acute or chronic inflammatory diseases, pulmonary hypertension (diagnosed by echocardiography), acute or chronic infectious diseases, history of venous thromboembolism, post-op or bed rest within the past 30 days, renal failure, and acute coronary syndromes. The severity of acute PE was defined according to the principles of the European Society of Cardiology (ESC) Guidelines[16]. Acute PE patients were evaluated regarding PE-related death and RV dysfunction at the one-year follow-up. The patients' clinical statuses were evaluated with face-to-face assessments and telephone interviews during the follow-up.

The echocardiographic examination was performed using the Vivid S5 cardiovascular ultrasound system with a 3S 1.5 - 3.6 MHz transthoracic probe (GE Medical Systems, Buckinghamshire, UK) to evaluate RV dimensions and function. The assessment of RV size was performed by measuring RV end-diastolic mid-cavity diameters from the apical four-chamber view. Tricuspid annular plane systolic excursion (TAPSE) was measured as the displacement of the lateral tricuspid annulus toward the apex during the systolic movement of the RV. Individually, the RV dysfunction was

evaluated for the presence or absence of the following signs: TAPSE < 15 mm and RV/LV end-diastolic ratio > 1 or mid-cavity RVEDD (right ventricular end-diastolic diameter) > 35 mm from the apical four-chamber view. If two or more of these criteria were present, RV dysfunction was diagnosed.

Biochemical analyses

Blood samples were collected from patients and the control group within a maximum of six hours after the diagnosis of acute PE to evaluate the acute phase. Plasma BNP levels were analyzed using an immunoassay kit (Biosite Diagnostics, La Jolla, CA). The measurable range of the BNP assays was 5.0 to 5000.0 pg/mL. Plasma D-dimer levels were assessed using the Liatest D-Di immuno-turbidimetric assay (Diagnostica Stago), with ≥ 0.5 µg/mL as the cut-off value. Copeptin was measured using a commercially available ELISA kit (EASTBIOPHARM, Hangzhou Eastbiopharm Co. Ltd., China) with a detection limit of 0.024 ng/mL and an inter-assay coefficient of 12%. The tubes were immediately placed on ice and centrifuged at 2,000 x g for 15 min at 4°C to collect the plasma, which was divided into aliquots and stored frozen at −80°C for further analyses.

Statistical analysis

Descriptive data were shown as n and % values in categorical data and mean ± standard deviation and median interquartile range values in continuous data. The chi-square test was used for the comparison of categorical data. The measurement data were tested for normal distribution by the Kolmogorov-Smirnov test. The Mann-Whitney U test and Kruskal-Wallis test were used, wherever appropriate, for the comparison of non-normally distributed measurement data. The Independent Samples t-test was used in independent groups for normally distributed data. Relationships between copeptin and baseline variables were assessed by Spearman rank correlation coefficients. The threshold value for copeptin in disease prediction was determined by the ROC curve analysis. P<0.05 was considered statistically significant for all analyses. The analyses were performed using IBM© SPSS version 20.

RESULTS

The study was conducted with a total of 114 participants. Of the participants in the study, 54 (44.4%) were in the patient group and 60 (55.6%) were in the control group. The mean age of the patient group was 56.81±11.3 years, and the mean age of the control group was 53.73±15.0 years. Gender, age, and body mass index distributions were similar between the groups.

The distribution of the characteristics and clinical features of the patients is shown in Table 1. Intermediate-high risk PE was diagnosed in 16 patients (29.6%), intermediate-low risk in 23 (42.6%), and low-risk in 15 (27.8%). Twelve patients (22.2%) were taking thrombolytic treatment, and 42 (77.8%) patients were treated with heparin according to the current guideline[17]. Three patients (5.6%) died during in-hospital or 30-day observation. PE-related death occurred in 12 patients (22.2%) at the one-year follow-up.

TABLE 1. THE BASELINE CHARACTERISTICS, CLINICAL SYMPTOMS, AND COMORBIDITIES OF THE STUDY COHORT

		n	(%)
Subjects	Control	60	(55.6)
	Patient	54	(44.4)
Symptoms	Chest pain	10	(18.5)
	Dyspnea	17	(31.5)
	Syncope	9	(16.7)
	Signs/symptoms of DVT	18	(33.3)
Risk factors for VTE	Previous trauma/surgery	12	(22.2)
	Lower extremity DVT	19	(35.2)
	Travel/immobilisation	10	(18.5)
	Unprovoked	13	(24.1)
Risk Categories	Low-risk	15	(27.8)
	Intermediate-low risk	23	(42.6)
	Intermediate-high risk	16	(29.6)
Comorbidities	Chronic compensated heart failure	14	(25.9)
	Chronic pulmonary disease	11	(20.4)
	Obesity	11	(20.4)
	Diabetes mellitus	10	(18.5)
	Anemia	8	(14.8)
Hemodynamic status at presentation	Mild hypotension	14	(25.9)
	Tachycardia	24	(44.5)
	Hypoxia	16	(29.6)
PE-related death		12	(22.2)
Rv Dysfunction		13	(24.1)

DVT: deep vein thrombosis, RV: Right ventricle, VTE: Venous thromboembolism

The levels of troponin I (0.31±0.44 vs 0.16±0.22 ng/mL; p<0.014), BNP (524±361 vs 168±247 pg/mL; p<0.001) and D-Dimer (4.125±3.154 vs 1.327±2.345 ng/mL; p<0.001) were higher in acute PE patients compared to the control group. Copeptin was higher in PE patients than in the control group (8.3 vs 3.8 ng/

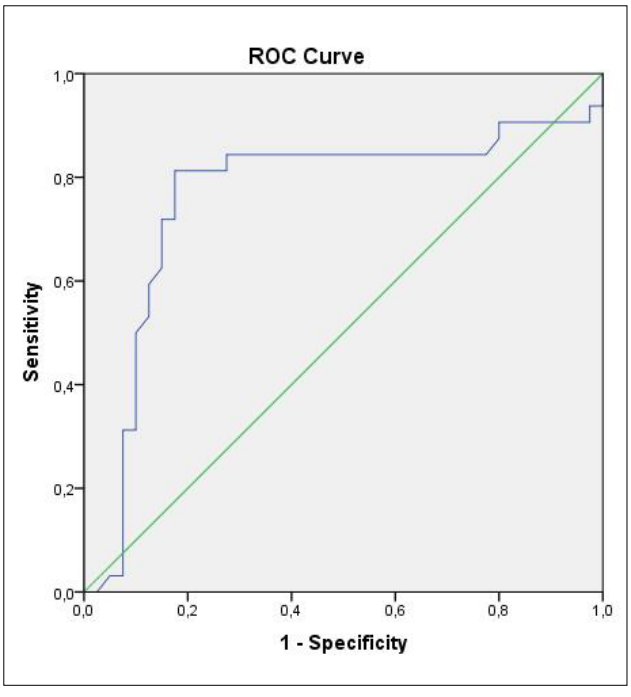
mL; p<0.001). In addition, copeptin was found to be significantly higher in patients with PE-related death and RV dysfunction at the one-year follow-up (10.2 vs 7.5 ng/ml, p=0.001 for PE-related death; 10.5 vs 7.5 ng/ml, p=0.002 for RV dysfunction) (Table 2). When the cut-off value of copeptin was taken as ≥5.85, its sensitivity and specificity for predicting PE were 71.9% and 85.0%, respectively (AUC=0.762, 95% CI=0.635-0.889, p<0.001) (Figure 1). Their negative and positive predictive values were 83.4% and 80.2%, respectively.

TABLE 2. EXAMINATION OF COPEPTIN LEVELS ACCORDING TO DEMOGRAPHIC AND CLINICAL FEATURES

		Copeptin		p
		Median	(Interquartile Range)	
Subjects	Control	3.8	(3.0-4.6)	<0.001
	Patient	8.3	(7.0-9.5)	
Gender	Female	5.2	(4.3-8.2)	0.304
	Male	4.7	(3.3-7.5)	
Risk Categories	Low-risk	6.1	(5.6-6.7)	<0.001
	Intermediate-low risk	8.3	(7.4-9.2)	
	Intermediate-high risk	10.5	(9.4-11.2)	
PE-related death	No	7.5	(6.7-8.4)	0.001
	Yes	10.2	(9.5-11.9)	
Rv Dysfunction	No	7.5	(6.8-8.8)	0.002
	Yes	10.5	(8.7-11.2)	

RV: Right ventricle

FIGURE 1



Copeptin levels showed a linear increase as the risk category increased according to post-hoc analysis results. Copeptin concentration in low-risk PE was 6.1 ng/mL, in intermediate-low-risk PE it was 8.3 ng/mL, and in intermediate-high risk PE it was 10.5 ng/mL (p<0.001 for each). Moderate and positive correlations were found between copeptin and pulse, RVEDD, and RV/LV ratio (r=0.571, p<0.001; r=0.588, p<0.001; r=0.504, p=0.003, respectively). There were strong and positive correlations between copeptin and the respiratory rate (r=0.705, p<0.001). There was a strong and negative correlation between copeptin and arterial oxygen saturation (r=-0.726, p<0.001), and a moderate and negative correlation between copeptin and TAPSE (r=-0.461, p=0.008).

DISCUSSION

The main findings of this study are as follows: 1) The copeptin measurement had moderate sensitivity and specificity for predicting acute PE; 2) Copeptin was significantly higher in patients with PE-related death and in patients who developed acute RV failure at the one-year follow-up; 3) Copeptin levels increased as the risk class increased in patients with acute PE.

Copeptin is stoichiometrically secreted with AVP from the neurohypophysis and is more stable than AVP, thus overcoming the limitations and difficulties of AVP measurement[6]. Copeptin is released into the blood circulation in life-threatening stress conditions. These include heart failure[11], acute coronary syndrome[8], sepsis[18], pulmonary hypertension[19], and acute PE[13-15].

Copeptin appears to be particularly useful for the prognostic assessment of acute diseases due to its rapid release kinetics[20]. In their study, Kalkan et al.[21] reported that the group including patients with acute PE had higher copeptin levels compared to those without acute PE. The cut-off value of copeptin was 4.84 ng/dL for the diagnostic predictor of acute PE and had a sensitivity of 68.1% and specificity of 83.7% in this study. In our study, when the cut-off value of copeptin was ≥5.85 ng/mL, its sensitivity and specificity were 71.9% and 85.0%, respectively, for the diagnostic predictor of acute PE (AUC=0.762, 95% CI=0.635-0.889, p<0.001).

Acute PE is associated with a high mortality rate. In their study, Hellenkamp et al.[22] found a 7.6-fold higher risk of PE-related death in the group with high copeptin levels. In our study, it was found that patients

with PE-related death at the one-year follow-up had significantly higher levels of copeptin. Limited biomarkers have been identified in the diagnosis and risk stratification of PE and for predicting acute RV failure, which is the most common cause of death in acute PE[14]. Vasopressin is engaged in myocardial remodeling through its V1 receptor on cardiomyocytes, causing increased ventricular hypertrophy, decreased contractility, and the process of myocardial fibrosis[23]. Even though AVP is mainly derived from the LV, these data increase the probability that elevated AVP levels in PE patients play a role in RV remodeling. In our study, copeptin was significantly higher in patients with PE in whom RV dysfunction developed. In addition, we found a significant positive correlation between copeptin and the RV/LV ratio and RVEDD and a significant negative correlation between copeptin and TAPSE. The activation of the AVP system — calculated by copeptin levels — might be an early indication of neurohumoral stimulation in patients with RV dysfunction.

In this study, copeptin levels reflected PE severity. Risk assessment using the algorithm proposed by the 2019 ESC guideline requires three steps (assessment of hemodynamic stability, laboratory test/imaging methods, and calculation of the pulmonary embolism severity index score), resulting in a complicated and costly approach[17]. Wyzgał et al.[14] reported a relationship between the severity of the disease and increased copeptin levels in PE. Hellenkamp et al.[13] performed a study including 268 PE patients, and it was concluded that copeptin could be useful for risk stratification. For these reasons, there is an increasing need for rapid alternative biomarker-based strategies in predicting acute PE diagnosis and risk assessment. Our findings suggest that copeptin may be an essential marker in predicting the diagnosis and PE-related deaths.

We recognize some limitations of this study. It was a single-center study with a relatively small sample size. To eliminate the contributing effect of comorbidities on the deaths of patients with PE, the study had many exclusion criteria that limit the generalizability of our study.

CONCLUSION

Copeptin levels are elevated in patients with PE, and the measurement of copeptin concentration may help in predicting diagnosis, risk stratification, and prognosis of acute PE patients. Multicenter and more extensive studies are necessary to determine its role in predicting acute normotensive PE patients and confirm the prognostic value of copeptin.

Conflict of interest statement

All authors confirmed that there are no conflicts of interest associated with this publication.

Ethics

This study was performed in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the Medical Ethics Committee of Cukurova University (Nr. 94).

Author's Contribution

The concept for research or article/hypothesis generation: Caglar Ozmen, Onur Sinan Deveci; Planning the methods to generate hypothesis: Anil Akray, Caglar Emre Cagliyan; Supervision and responsibility for the organization and course of the project and manuscript preparation: Ayhan Usal, Caglar Ozmen, Oya Baydar; Supplying equipment, space, and personnel vital to the Project: Onur Sinan Deveci, Ismail Hanta, Caglar Emre Cagliyan; Biological materials, reagents, referred patients: Ali Deniz, Oya Baydar, Muhammet Bugra Karaaslan; Discussion of the results, approval of the final version of the work: Muhammet Bugra Karaaslan, Onur Sinan Deveci, Ali Deniz.

RESUMO

OBJETIVO: *O diagnóstico precoce e a estratificação de risco podem proporcionar um melhor prognóstico em casos de embolia pulmonar (EP). A copeptina surgiu como um valioso biomarcador preditivo de várias doenças cardiovasculares. O objetivo deste estudo é determinar os níveis de copeptina em pacientes com EP aguda e avaliar a sua relação com a severidade da doença e mortes relacionadas à EP.*

MÉTODOS: *Um total de 54 pacientes e 60 indivíduos saudáveis foram incluídos neste estudo. As concentrações de copeptina e disfunções ventriculares direitas foram analisadas. A correlação entre os níveis de copeptina e parâmetros ecocardiográficos e hemodinâmicos foi examinada. Após essas primeiras medições, os pacientes foram avaliados em relação à mortalidade relacionada à EP após um ano.*

RESULTADOS: *Os níveis de copeptina foram maiores em pacientes com EP do que no grupo de controle (8,3 ng/mL vs 3,8 ng/mL, p<0,001). Os níveis de copeptina eram significativamente maiores em pacientes com mortes relacionadas à EP e disfunção ventricular direita (10,2 vs 7,5 ng/ml, p=0,001; 10,5 vs 7,5 ng/ml, p=0,002, respectivamente). Com um valor de corte ≥5,85 para a copeptina, sua sensibilidade e especificidade preditivas para EP foram 71,9% e 85,0%, respectivamente (AUC=0,762, 95% IC=0,635 - 0,889, p<0,001).*

CONCLUSÃO: *A medição da copeptina teve sensibilidade e especificidade preditivas moderadas para o diagnóstico de EP, e o nível de copeptina foi significativamente maior em pacientes com mortes relacionadas à EP após um ano. A copeptina pode ser um novo biomarcador preditivo útil para o diagnóstico, a estratificação de risco e o prognóstico de PE.*

PALAVRAS-CHAVE: *Biomarcadores. Morte. Embolia pulmonar/diagnóstico. Embolia pulmonar/mortalidade. Arginina vasopressina/metabolism.*

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ORIGINAL ARTICLE

Analytical methods for assessing changes induced by gamma exposure in an animal model

Matheus F. Soares Mingote[1]
Tarcísio P.R. Campos[2]
Rodinei Augusti[3]
Geovanni D. Cassali[4]

1. Departamento de Engenharia Nuclear, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
2. Professor Titular em Ciência da Radiação Aplicada à Saúde. Departamento de Engenharia Nuclear, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
3. Professor titular em Química Analítica Avançada - Espectrometria de massa, Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
4. Professor titular do Departamento de Patologia Geral – ICB. Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

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SUMMARY

OBJECTIVE: *Ionizing radiation can cause radio-induced changes in the cellular metabolome due to the breakdown of DNA bonds. Our goal was to find the early tissue response to radiation exposure supported by distinct analytical methods.*

METHODS: *Histological analyses were performed on the organs extracted from rats to search for microscopic changes. The histological slides stained with hematoxyline-eosin (HE) were analyzed in magnification (40x). Subsequently, the tissues were subjected to mass spectrometry that allowed molecular analysis and DESI-MSI that generated the molecular image of lipids, assessing changes in intensities, especially in the brain.*

RESULTS: *The histological analysis found nonspecific inflammatory changes; no areas of fibrosis, necrosis, or apoptosis were identified, suggesting non-morphological tissue alterations. However, the DESI-MSI images of brain lipids allowed the observation of many radio-induced changes in the lipid's intensities.*

CONCLUSIONS: *No early radio induced histological or mass weight changes in the radiation exposed rats could be observed at 5 Gy. However, early changes in the molecular level were observed in the DESI-MSI images of the brain lipids. The DESI-MSI method proved to be efficient and relevant, allowing a regional molecular analysis of the tissues, expanding a new field of study that is still in its infancy: radiometabolomics.*

KEYWORDS: *Metabolome. Radiation. Gamma rays. Mass spectrometry. Lipids. Spectrometry, mass, matrix-assisted laser desorption-ionization. Brain.*

INTRODUCTION

The International Cancer Research Agency of the World Health Organization (IARC/WHO) has shown that cancer is the leading cause of morbidity and mortality worldwide[1].

The absence of repair mechanisms and control of the cell cycle allows the emergence of neoplastic lesions that characterize cancer[2]. One of the therapeutic modalities in cancer is radiotherapy, in which a large portion or the whole organ infiltrated by the tumor is exposed to radiation. Gamma radiation is a

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CORRESPONDING AUTHOR: Matheus Mingote
Av. Antonio Carlos 6627 – Belo Horizonte, Minas Gerais – Brasil – 31270-901
E-mail: matheus.soares@gmail.com

type of ionizing radiation (IR) that can cause various cellular damages due to direct and/or indirect structural changes resulting from the rupture of molecular bonds in the DNA of the neoplastic cells, resulting in their loss of clonogenicity[3].

In addition, healthy tissues are also affected by radiation exposure, resulting in several adverse effects of the radiation therapy, such as cerebral edema, radiation necrosis, cerebral atrophy, neurocognitive deficits[4], thickened bronchial wall, dilated alveolar space[5], pneumonitis, pulmonary fibrosis[6], cardiomyopathy, reduction of myofibrils with fibrosis[7], hepatocytes with focal necrosis, nephritis with glomerular capillary necrosis, or degeneration of the proximal cell lineage.

DESI-MSI is an analytical imaging method used to obtain lipid profiles of normal and neoplastic tissues, identify metabolites in microbiology, and study brain neurotransmitters[8-11]. Radio-induced brain changes can cause several adverse effects such as cognitive losses and radionecrosis[12]. DESI-MSI allows assessing changes in the tissue metabolic profile so that it can be used in a review of radiation therapy protocols in order to minimize the deleterious effects of ionizing radiation.

The goal of the present research was to find the early tissue response to the radiation exposure of total-body-irradiation (TBI) at 5 Gy supported by distinct analytical methods.

METHODS

Group selection and Irradiation Protocol

Statistical analyzes were performed with the body and organ mass weights of 40 male albino rats, 90 days old, from the Rattus norvegicus, Wistar line, weighing approximately 300 ± 15g. The animals were euthanized, the organs removed, weighed, and stored. The tissues were prepared and histological and molecular analyses were performed. The research goals and protocols were previously submitted; the study was approved by the Ethics Committee on Animal Use (CEUA-UFMG), protocol 339/2014, after standardization for animal care; euthanasia. The animals of the IR group had their total body irradiated in the Co-60 Gamma-LIG/CDTN radiator, all together in 4 boxes, receiving 5 Gy absorbed dose, at the Laboratory of Irradiation Gamma-LIG of the Center of Development of the Nuclear Technology (CDTN). The irradiated animals were sacrificed according to time kinetics corresponding to 12h, 48h, 96h, and 01 week after exposure.

Histological methods

The organs were fixed at 10% buffered formalin and included in paraffin blocks. The 4 μm sections were obtained and stained with hematoxylin and eosin (HE). The slides were evaluated by a pathologist, and the images were captured by a camera connected to an optical microscope (Olympus BX-40; Olympus, Tokyo, Japan). The histological analysis was performed on the tissue's images.

DESI-MSI

The solvents were acetonitrile and N, N-dimethyl formaldehyde ACN:DMF (1:1) since these chemical products do not cause morphological changes. The infusion pump was adjusted with a flow rate of 3.5 $\mu L.min^{-1}$. The nitrogen nebulizer gas was turned on at 160 psi pressure. The high voltage source was connected to the ion source and a voltage of 5 kV applied. The mass spectra were acquired in the 200-1000 mass range, with an incidence angle of ~ 54° between the source and the sample surface. The analyses were made on a ThermoFisher Scientific Q executive Orbitrap MS instrument. The entire process is represented in Figure 1.

The ions were selected based on the changes in the intensity of the ions in the acquired images. In order to compare the control and experimental brain tissue with tissue responses in the respective time kinetics (24h, 48h, 96h, and 1 week), the ion intensities were properly equalized for comparison purposes.

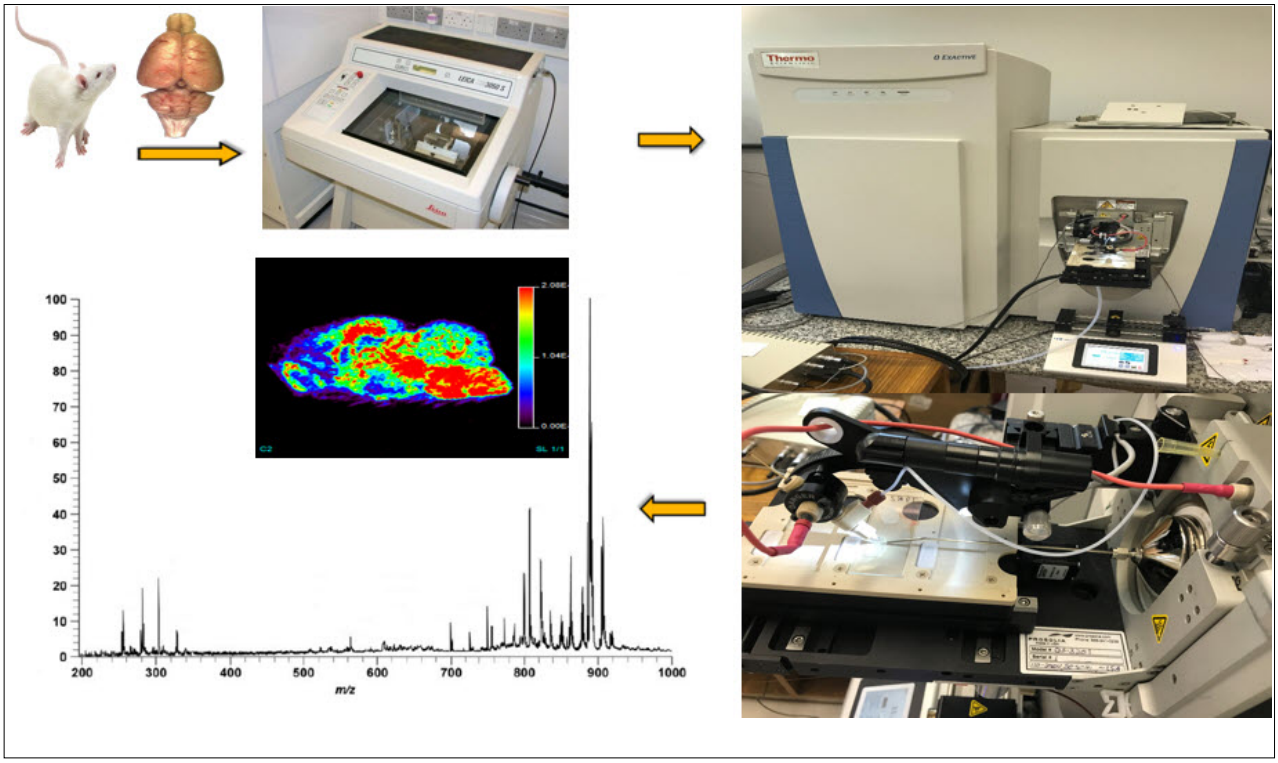
RESULTS

Histopathological analyses of organs

Histological slides stained with HE were analyzed in magnification (40x), and the major histological characteristics found in the control group and the irradiated group were observed.

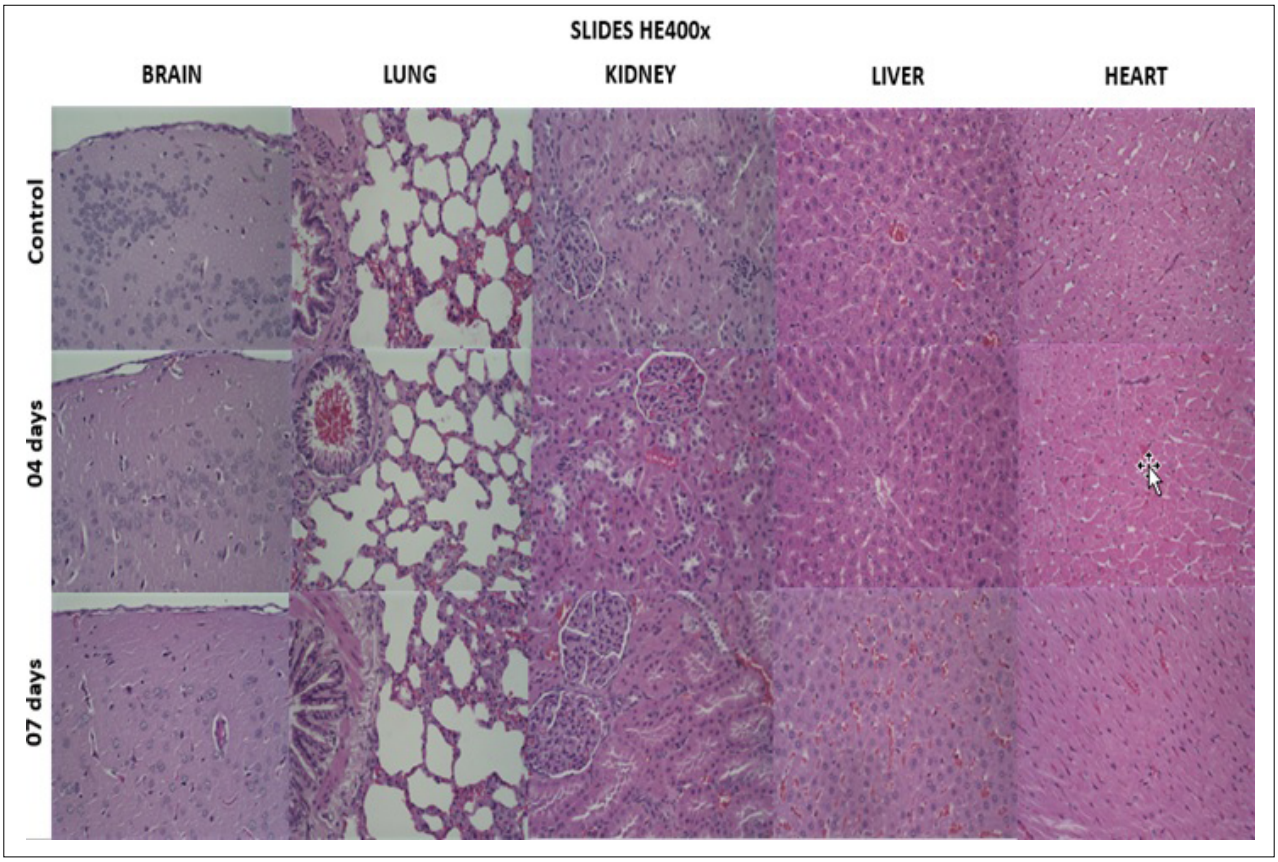
The histological brain slides from the irradiated group did not present changes such as inflammatory lymphomononuclear infiltrates, edemas, vascular degenerations, and areas of necrosis, as depicted in Figure 2. The lung plaques of the irradiated group did not present changes such as inflammatory lymphomononuclear infiltrates, exudates, edema, and thickening of the bronchial wall, as shown in Figure 2.

FIGURE 1. SCHEMATIC REPRESENTATION OF THE DESI-MSI APPARATUS USED TO OBTAIN EACH TISSUE IMAGE.



Source: Mingote[12]

FIGURE 2. BRAIN, LUNG, KIDNEY, LIVER, AND HEART 400X SLIDES



Source: Mingote[12]

The irradiated kidney slides showed no signs of focal or diffuse glomerulonephritis and no changes were observed such as inflammatory lymphomononuclear infiltrate, capillary necrosis, and cell degeneration in the proximal and distal tubules. The liver slides of the irradiated group had preserved architecture formed by hepatocyte cords, sinusoid capillaries, and centrilobular vein. No dilatation of sinusoidal capillaries, areas of focal necrosis, and cellular degeneration with vacuolated hepatocytes were observed. Figure 2 also shows the heart laminations of the irradiated group, demonstrating the preservation of the skeletal striated muscle structure without the presence of myofibrillar degeneration, areas of myofibrils necrosis, and areas of fibrosis.

DESI images

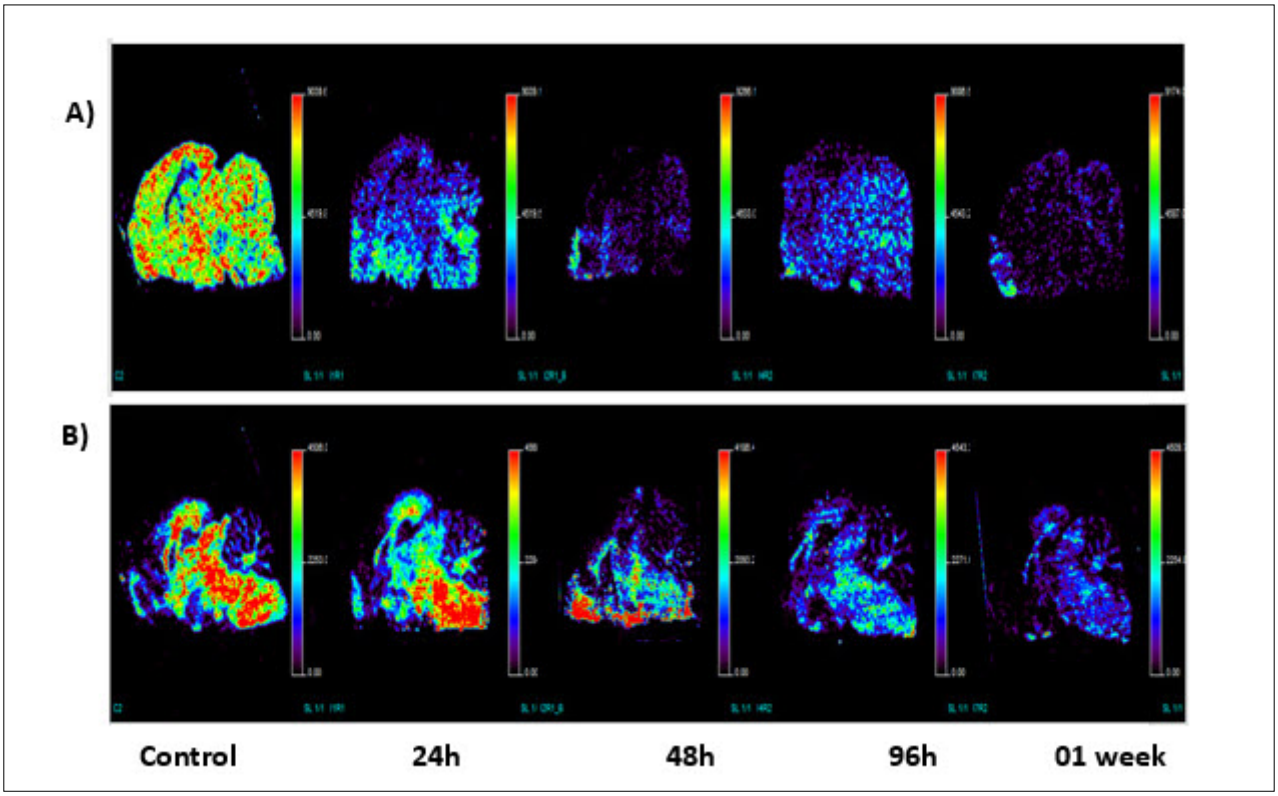
Pertinent changes were noticed in Ion images of the brain tissue of Wistar rats from samples of the control group and the irradiated group analyzed in DESI. The generated images referred to only one ion and were determined by its m/z ratio. Thus, the scan reading of each pixel allowed the ions present at each point of the sample to be quantified and evaluated by their tissue distribution. Some ions were selected, which showed substantial differences in intensity and expressed in each organ area. Figure 3 shows the images of two ions obtained and their profile in the different regions of the Central Nervous System (CNS). The main changes observed were an abrupt modulation in lipid intensity in various regions for both samples.

DISCUSSION

Our findings show that the histological method did not reveal relevant changes in the levels of microscopic tissue.

Ionizing radiation has enough energy to cause electronic excitation, i.e., ionization, producing the breakdown of chemical bonds in the biological molecules (DNA, lipids, proteins) of tissues[13,14]. Lipid peroxidation is a cytotoxic process defined by successive biochemical events triggered by free radicals that determine the extensive oxidation of unsaturated fatty acids and membranes. The by-products of those processes

FIGURE 3. ION A) M/Z 794.622 AND B) ION M/Z 914.693. THE IMAGES REPRESENT THE INTENSITIES OF THE LIPID'S IONS, IN COLOR LEVELS, TAKEN IN A DISTINCT ANIMAL BRAIN SECTION, WHOSE PIXELS REPRESENT A SPECIFIC AREA OF THE RAT'S BRAIN, TAKEN AT THE AFTER-RADIATION TIMES OF 24H, 48H, 96H, AND 01 WEEK.



Source: Mingote[12]

induce structural and functional changes in the cell membrane, causing changes in the flow of the ionic and molecular substrate, triggering several biochemical changes. As an example, metabolism and serum phospholipid levels (PLs) were altered after exposure to ionizing radiation.

DESI, however, proved to be extremely effective in detecting important radio induced molecular changes[15]. Polyunsaturated fatty acids, as well as structural lipids of the cell membrane, are largely susceptible to damage caused by gamma irradiation. The results showed that the two selected lipids revealed significant changes in the CNS. The lipid distribution of m/z 794,622 and m/z 914,693 from the control group to the irradiated groups was completely altered. The kinetics of time shows a change in lipid expression spatially in the brain, as well as an attempt to respond to minimize the damaging effects of radiation on tissues. After irradiation, an abrupt modulation in the intensity of this lipid was observed in all brain regions in the 24h samples, and in the 48hh samples especially. The 96h sample showed a slight diffuse increase; however, in the 01-week sample, there was a decrease in lipid intensity in all areas.

Changes in lipid expression lead to changes in signaling mechanisms for cellular and molecular repair. Molecular changes may be associated with the alteration and degeneration of myelin, present in numerous neurodegenerative diseases. It is still necessary to assess whether oxidative damage can lead to cell membrane dysfunction, causing changes in metabolism, selective change in cell permeability and signaling, which can lead to apoptosis[14,16-18].

Molecular analyses in the other organs are still being developed since all research is maintained through inter-institutional cooperation to obtain data and images.

CONCLUSIONS

The effects of ionizing radiation must be better identified and understood at the molecular level. Although macroscopic and microscopic changes in the tissue are not yet evident, it is necessary to further deepen the techniques applied to the metabolic molecular analysis to achieve a better understanding of the mechanisms that can alter cell structures and function. DESI-MSI proved to be efficient and relevant, allowing regional molecular analysis of the entire CNS, expanding a new field of study that is still incipient: radiometabolomics. Our studies may contribute further to the review of the protocols in dose planimetry to minimize the deleterious effects of ionizing radiation.

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Author's Contribution

Study concept and design: MFSM, TPRC, RA; data acquisition: MFSM; data analysis/interpretation: MFSM, RA, GDC; supervision or mentorship: TPRC, RA, GDC. Each author contributed with important intellectual content during the manuscript drafting or revision and accepted accountability for the overall work of ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. MFSM takes responsibility that this study has been reported honestly, accurately, and transparently and that no important aspects of the study have been omitted.

RESUMO

OBJETIVO: *Radiação ionizante pode causar alterações no metaboloma celular devido à quebra de ligações no DNA. O objetivo deste trabalho foi evidenciar a resposta aguda tecidual induzida pela exposição da radiação ionizante.*

MÉTODOS: *Análises histológicas foram realizadas nos órgãos extraídos de ratos para análise de alterações microscópicas. As lâminas histológicas coradas com hematoxilina eosina (HE) foram analisadas em aumento (40x). Posteriormente, os tecidos foram submetidos a espectrometria de massa, que permitiu análise molecular e o Desi-MSI que gerou imagem molecular de lipídios, identificando alterações na intensidade, principalmente no cérebro.*

RESULTADOS: *As análises histológicas encontraram alterações inflamatórias inespecíficas, nem áreas de fibrose, necrose ou apoptose, sugerindo ausência de alterações morfológicas. As imagens de lipídios cerebrais obtidas por Desi-MSI permitiram observar as inúmeras alterações na intensidade nas seções teciduais do encéfalo.*

CONCLUSÕES: *Alterações agudas radioinduzidas de massa do órgão e histológicas nos órgãos dos ratos expostos não puderam ser observadas a 5 Gy. Entretanto, mudanças em nível molecular foram observadas nas imagens de Desi-MSI dos lipídios cerebrais. O método Desi-MSI mostrou-se eficiente e relevante, permitindo a análise molecular regi-onal dos tecidos no SNC, expandindo um novo campo de estudo que ainda está em sua infância: a radiometaboloma.*

PALAVRAS-CHAVE: *Metaboloma. Radiação. Raios gama. Espectrometria de massa. Lipídios. Espectrometria de massas por ionização e dessorção a laser assistida por matriz. Encéfalo.*

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ORIGINAL ARTICLE

Index of cardiac-electrophysiological balance and the effects of thrombolytic therapy on the electrocardiogram of patients with pulmonary embolism

Yakup Alsancak[1]
Ahmet Taha Sahın[1]
Ahmet Seyfeddin Gurbuz[1]
Ahmet Lutfi Sertdemir[1]
Abdullah Icli[1]
Hakan Akilli[1]
Mehmet Akif Duzenli[1]

1. Necmettin Erbakan University, Meram Medical Faculty Department of Cardiology, Konya, Turkey.

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SUMMARY

OBJECTIVE: *Different parameters on electrocardiograms (ECG) have been investigated to predict arrhythmia and mortality in patients with acute pulmonary embolism (APE). The acute effect of thrombolytic therapy (TT) on these parameters has not been investigated yet.*

METHODS: *We examined the data of 83 patients who were evaluated as high-risk APE and discharged from the hospital after TT. First, the high-risk APE patients' ECGs were compared with healthy control subjects (n = 55). After their admission and 24 hours later, the ECGs of patients with APE were compared. Heart rate, P-wave morphology, QRS duration, QT distance, Tp-e, and the index of cardiac electrophysiological balance (iCEB) were analyzed.*

RESULTS: *Although P maximum was not different between the groups' ECGs, heart rate, QT, QTc (corrected QT) interval, Tp-e intervals, Tp-e/QT ratio, and P wave dispersion were significantly higher in the APE group (**P** values < 0.031). iCEB or iCEBc (corrected iCEB) values were lower in APE group (**P** < 0.001). After TT, we determined a decrease in heart rate, Tp-e interval, and Tp-e/QT ratio (**P** < 0.001). Although we detected a decrease in the QT and QTc interval and QT dispersion (QTd), QTd had no statistical significance (respectively **P**-value 0.013, 0.029, and 0.096). The iCEB and iCEBc levels were lower after TT (**P**-value was 0.035 and 0.044 respectively).*

CONCLUSION: *The QT, QTc, Tp-e interval, Tp-e/QTc ratio, iCEB, and iCEBc values significantly decreased after TT. It may be thought that effective TT causes partial improvement in ventricular repolarization in an early period.*

KEYWORDS: *Electrocardiography. Pulmonary embolism. Thrombolytic therapy.*

INTRODUCTION

Acute pulmonary embolism (APE) is still an important cause of cardiovascular mortality and morbidity. Therefore, the diagnosis should be made very quickly in suspicious clinical situations and treatment should be started. Since the condition may be asymptomatic, it has a wide range of clinical findings such as shortness of breath, chest pain, palpitations, syncope, and even sudden death, so diagnostic tests have a critical

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CORRESPONDING AUTHOR: Yakup Alsancak,
Necmettin Erbakan University, Meram Medical Faculty, Department of Cardiology Meram/Konya, Turkey – 042065 - Tel: 90 506 910 14 04
E-mail: dryakupalsancak@gmail.com

role in determining the presence or absence of APE. For this reason, computed tomographic pulmonary angiography, echocardiography, ventilation/perfusion scan (lung scintigraphy) and, rarely, pulmonary angiography are widely use to diagnoses[1]. However, it is not always possible to access adequate technical equipment or personnel; in this case, an old friend that is an easy-to-access, non-invasive, and inexpensive technique, i.e., the electrocardiography, attracts attention once again.

Despite many non-specific electrocardiographical (ECG) findings during pulmonary embolism, ST-segment depressions in several leads, complete or incomplete right bundle branch block (RBBB), T-wave inversion, and S1Q3T3 pattern are the best-known changes[2-4]. In addition to these, some studies have investigated different parameters on surface ECG to predict arrhythmia and mortality in patients with APE over the last decade. QT-interval prolongation, which is a well-known ECG finding that is a marker for electrical instability and sudden cardiac death, was observed in patients with pulmonary embolism[5]. Also, a QTc (corrected QT) difference higher than 20 ms between V1 and V6 was shown to identify acute APE[6]. Moreover, QT dispersion (QTd), which demonstrates the difference in re-polarization, is significantly increased in patients with APE[7]. Another ECG parameter, P-wave dispersion, which is a marker of atrial depolarization heterogenity and known to predict the development of atrial fibrillation, was increased in patients with APE[8]. Novel pro-arrhythmogenic markers, the T-wave peak-to-end (Tp-e) interval and Tp-e/QT ratio, which suggest transmural dispersion of the repolarization ventricle, were also increased in patients with APE[5,9-11]. More recently, the index of cardiac electrophysiological balance (iCEB), calculated by dividing the QT interval by the QRS duration, has been shown to be a predictor of ventricular arrhythmias, but its relationship with APE has not been investigated yet[12,13].

When considering high-risk APE, improvement of these ECG changes may be observed when a treatment method (mechanical embolectomy, thrombolytic therapy, etc.) is applied, eliminating the thrombus and providing pulmonary blood flow. Therefore, the aim of the study was to investigate the relationship between the iCEB and diagnoses of APE and evaluate the status of ECG changes previously observed in patients with APE after thrombolytic therapy, which has never been investigated in the literature.

METHODS

Study population and patient selection

In this retrospective study, we analyzed patients who were older than 18 years and had been diagnosed with APE and received thrombolytic therapy in our university hospital department of cardiology between January 2017 and January 2020. Particularly, patients were included whose clinical diagnosis had been confirmed by computed tomography angiography. So, we obtained the data of 83 patients who were evaluated as high-risk APE and discharged from hospital after thrombolytic therapy.

Patient's ages, co-morbidities, hemodynamic conditions, and laboratory findings were recorded. The ECGs that had been obtained during the admission and 24 hours after the thrombolytic regimen were evaluated. Unsuitable or excessive noises in the ECGs, presence of documented atrial fibrillation or cardiac pacemaker rhythm, or left and right bundle branch block on the ECG; the presence of preexcitation syndromes; the presence of left ventricular hypertrophy criteria; known use of any drugs affecting the cardiac conduction system during admission, chronic renal failure (creatinine > 1.5 mg/dl); the presence of electrolyte abnormalities; suspected active infection or malignancy during admission; chronic obstructive pulmonary diseases; and a history of percutaneous coronary intervention or coronary artery bypass surgery for coronary artery disease were accepted as the exclusion criteria for screened patients. When these exclusion criteria were applied to the study population, of the 22 patients excluded from the study, 6 had missing echocardiography or laboratory data and excessive "noise" in the ECG; 5 had a history of coronary artery disease (percutaneous coronary intervention = 3, coronary artery bypass grafting = 2); 3 had left or right branch bundle block; 4 had a chronic renal failure with increased creatinine levels; 4 had documented atrial fibrillation. Eventually, 61 patients' pre- and post-treatment records were evaluated. In addition, 55 healthy control subjects were enrolled in the study to investigate the effect of massive APE on surface ECG. So, a total of 116 patients' data were analyzed. The flowchart of the study design is shown in Figure 1.

The study was approved by the Institutional Review Board and was conducted in accordance with the Helsinki declaration. The study was approved by the local Ethics Committee of our faculty under 13.09.2019/93/2048 ID number.

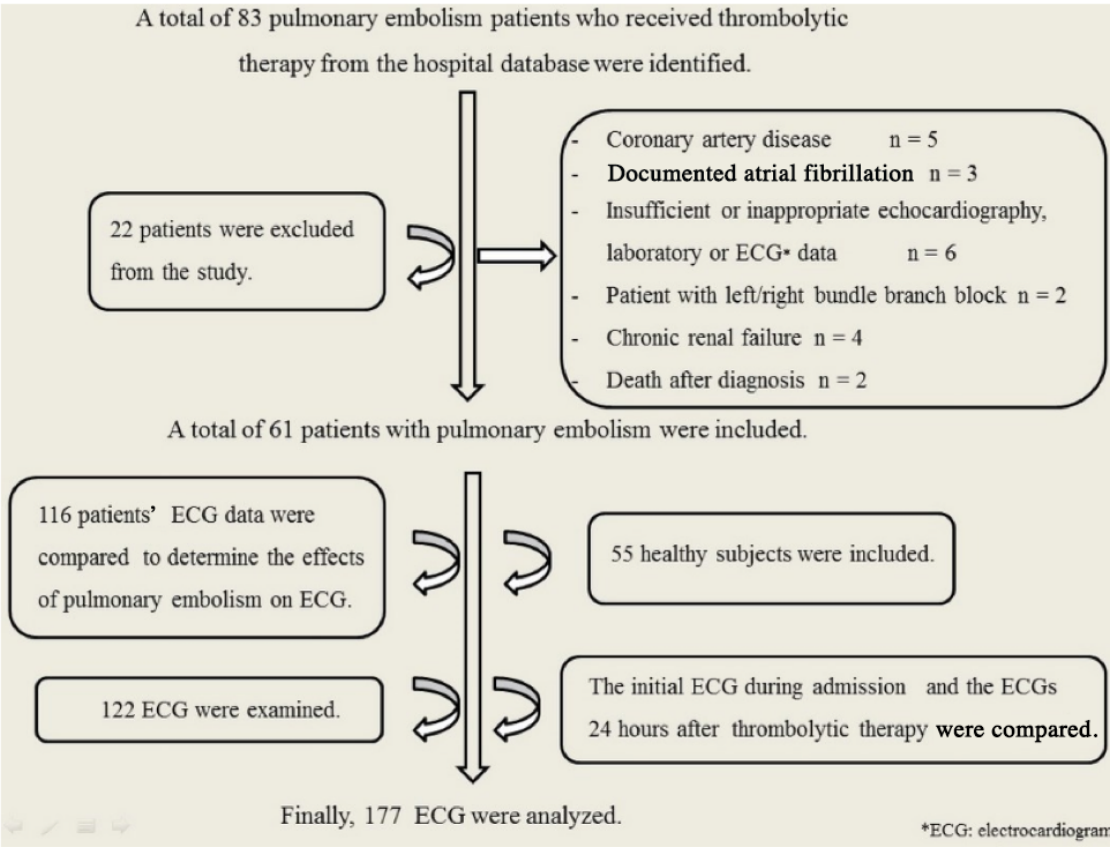

FIGURE 1. FLOWCHART OF THE STUDY DESIGN

Electrocardiographic examinations

Heart rate, P-wave morphology, QRS duration, QT distance, Tp-e, and iCEB were analyzed. All ECG samples were examined on a digital platform and measurements were then taken using special software (Adobe Photoshop) to provide necessary magnification. An average value of three measurements was taken for each lead.

The beginning point of the P-wave was described as the first upward positive or downward negative deflection between the isoelectric line, and the end of the P-wave was characterized as the point where the last deflection of the P-wave met the isoelectric line. Maximum (P_{max}) and minimum P (P_{min}) wave durations were recorded. P-wave dispersion (PWD) was defined as the difference between the maximum and minimum P-wave durations[14]. The QT interval was conventionally obtained by manually measuring from the onset of the QRS complex to the crossing point of the T wave and isoelectric line. The heart-rate-corrected QT interval was calculated using Bazett's formula [cQT = QT$\sqrt{}$ (R – R interval)]. QT dispersion (QTd) was obtained by measuring the longest QT interval (QT_{max}) and the shortest QT interval (QT_{min}) in any lead[15]. QT interval measurements were taken by examining recordings from leads D2 and precordial V5, and the longer lead was recorded for statistical analysis.[16] The distance from the peak of the T-wave (T_{peak}) to the endpoint of the T-wave (T end) ($T_{peak-end}$ or Tp-e) was obtained from the chest leads. The Tp-e/QT ratio was obtained by dividing the Tp-e duration by the QT interval in the precordial V5 lead[16,17]. The index of cardiac electrophysiological balance (iCEB) was obtained by dividing the QT interval by the QRS duration in the same lead (D2 or V5)[12,13]. The iCEBc was accepted as QTc/QRS ratio.

Statistical Analysis

SPSS® version 16.0 statistical package software (SPSS Inc., Chicago, IL, United States) was used for statistical analyses. Descriptive statistics were shown as mean±standard deviation or median (interquartile range) for continuous variables, and nominal variables were shown as the number of cases (n) and percentages (%). The normality of distribution was evaluated using the Kolmogorov–Smirnov test. Mean values of continuous variables were compared between independent groups using the Student's T-test, one-way ANOVA test, or Kruskal-Wallis test as appropriate. The chi-square test was performed to compare the study groups in terms of categorical variables. The pre-treatment and post-treatment echocardiographic and ECG

parameters of patients with APE were compared with paired t-tests or *K*-related sample tests. A p-value below 0.05 was considered statistically significant.

RESULTS

A total of 116 patients with a mean age of 63.13 [11.95] years, 83 (71.6%) of whom were women, formed the study population. There were no statistically significant differences between the groups in terms of gender, age, diabetes mellitus, hypertension, and smoking status ($P > 0.05$). Biochemical and hematological laboratory parameters were similar except for white blood count, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), calcium, and high-density lipoprotein cholesterol. White blood count, SGOT, SGPT levels were higher in the APE group ($P < 0.045$), and troponin levels were found to be high in patients with APE, as expected. In computed tomographical findings of the APE group, 83% of the patients had a thrombus located in the main and lobar pulmonary arteries, 17% had a thrombus seen in the subsegmental branches. The study groups were comparable in terms of echocardiographic and ECG parameters. Accordingly, in patients with APE, pulmonary artery pressure, and left ventricular end-diastolic and systolic diameter were higher than in the control group ($P = 0.001$, $P = 0.022$, and $P = 0.023$, respectively). Moreover, the left atrial size and left ventricle ejection fraction were similar between groups. Although P maximum was not different between the groups on ECG, the heart rate, QT, QTc interval, Tp-e intervals, Tp-e/QT ratio, and PWD were significantly higher in the APE group (P values < 0.031). Also, iCEB or iCEBc values were lower in the APE group ($P < 0.001$). The demographic features, laboratory parameters, electrocardiographic and echocardiographic characteristics, and comparison between the groups are summarized in Tables 1 and 2.

In the analysis conducted to assess the effect of thrombolytic therapy, based on the echocardiographic evaluation, we detected only that the pulmonary artery pressure was significantly decreased (56.73 [13.31] mmHg to 41.64 [12.24] mmHg) after thrombolytic therapy, as expected ($P = 0.001$). We detected that the systolic blood pressure of patients with APE increased from 84.08 [12.35] to 111.04 [13.62] mmHg and diastolic blood pressures from 53.89 [15.11] to 68.12 [14.45] mmHg after 24 hours ($P < 0.001$). Furthermore, we determined a significant decrease in heart rate, Tp-e interval, and Tp-e/QT ratio ($P < 0.001$). Although we detected a decrease of the QT, QTc interval, and QTd, QTd had no statistical significance (respectively, P-value 0.013, 0.029, and 0.096). In addition, the iCEB and iCEBc levels were lower after thrombolytic therapy (*P*-value 0.035 and 0.044 respectively). Table 3 and Figure 2 demonstrate the changes in ECG parameters after the treatment.

FIGURE 2. CHANGES IN ELECTROCARDIOGRAPHIC PARAMETERS AFTER THE TREATMENT

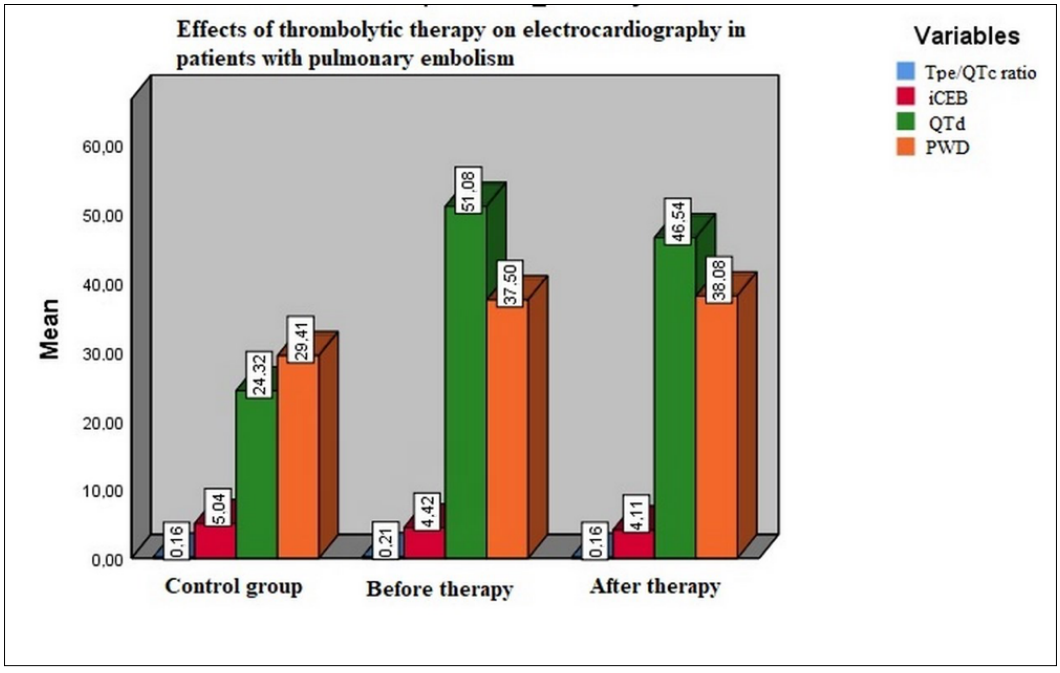

TABLE 1. BASAL CHARACTERISTICS, LABORATORY AND ECHOCARDIOGRAPHICAL FEATURES OF THE STUDY POPULATION

Variables	Control group (n = 55)	Patients with pulmonary embolism (n = 61)	P-value
Demographic characteristics			
Age (years, mean±std)	61.67± 8.82	64.4± 14.16	0.214
Gender (female, n/%)	42 (76)	41 (67)	0.275
Diabetes mellitus (n/%)	18 (33)	13 (22)	0.165
Hypertension (n/%)	22 (40)	28 (46)	0.109
Smoking (n/%)	9 (16)	16 (26)	0.061
Laboratory parameters			
Hemoglobin (g/dL, mean±std)	13.25±3.35	12.73± 2.04	0.312
WBC (103/ uL, mean±std)	7.88±1.66	11.35±4.26	0.001
Platelet (103/ uL, mean±std)	268±78.29	257±117	0.573
Creatinine (mg/dL, mean±std)	0.78±0.19	0.84± 0.23	0.114
Potassium (mmol/L, mean±std)	4.48±0.57	4.49±0.56	0.978
SGOT (U/L, median, IQR)	22.33 (18-29)	63.93 (24-65)	0.011
SGPT (U/L, median, IQR)	20.35 (16-40)	48.56 (35-75)	0.029
Calcium (mg/dL, mean±std)	9.03±1.54	8.52±0.75	0.045
Magnesium (mg/dL, mean±std)	1.86±0.17	2.16±1.26	0.085
LDL (mg/dL, mean±std)	123±36.93	108±20.24	0.124
HDL (mg/dL, mean±std)	47.5±12.9	35.38±15.83	0.005
Triglycerides (mg/dL, mean±std)	149±80.11	146±92.71	0.860
Troponin (ng/mL, mean±std)	-	3.22±2.07	-
Echocardiographic features			
LVEDD (cm, mean±std)	4.19±2.48	4.41±0.52	0.022
LVESD (cm, mean±std)	2.42±1.82	2.68±0.49	0.023
Ejection fraction (%,mean±std)	54.72±7.4	55.78±4.84	0.359
Left atrial size (cm, mean±std)	2.61±2.02	4.41±2.25	0.076
PAB (mmHg, mean±std)	29.12±4.37	56.73±13.31	0.001

*HDL: high-density lipoprotein cholesterol; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; LDL: low-density lipoprotein cholesterol; PAB: pulmonary artery pressure; WBC: white blood cell count.

TABLE 2. ELECTROCARDIOGRAPHIC COMPARISON BETWEEN PULMONARY EMBOLISM AND THE CONTROL GROUP

Variables (mean±std)	Control group (n = 55)	Patients with pulmonary embolism (n = 61)	P-value
Heart rate (pulse/min)	71.96±12.09	114.59±17.5	0.001
P maximum (msn)	79.61±25.75	77.83±15.98	0.665
P minimum (msn)	50.18±19.62	40.32±12.51	0.002
PWD (msn)	29.41±14.01	37.5±14.49	0.003
QRS interval (msn)	68.23±21.91	87.86±17.23	0.001
QT interval (msn)	343.87±57.7	386.52±39.88	0.041
QTc interval (msn)	383.96±39.44	424.39±44.1	0.031
QTd (msn, mean (std)	24.32±16.72	51.08±20.75	0.001
QT/QRS ratio (İCEB)	5.04±1.51	4.42±0.82	0.011
QTc/QRS ratio (c-İCEB)	5.63±2.19	4.85±0.85	0.001
Tp-e interval (msn)	54.36±14.46	80.49±19.18	0.001
Tp-e/QT ratio	0.16±0.04	0.21±0.05	0.001
Tp-e/cQT ratio	0.13±0.03	0.18±0.04	0.001

*PWD: P-wave dispersion; QTd: QT dispersion; QTc: corrected QT interval; Tp-e: T peak to end interval. *Data are presented as mean (standard deviation).

TABLE 3. COMPARISON OF ELECTROCARDIOGRAPHIC PARAMETERS BEFORE AND AFTER THROMBOLYTIC THERAPY

Variables (mean±std)	Before thrombolytic therapy	After thrombolytic therapy	P-value
Heart rate (pulse/min)	114.59±17.5	90.98±19.62	0.001
P maximum (msn)	77.83±15.98	75.73±16.47	0.423
P minimum (msn)	40.32±12.51	37.21±9.68	0.097
PWD (msn)	37.5±14.49	38.08±15.24	0.837
QRS interval (msn)	87.86±17.23	85.55±13.13	0.217
QT interval (msn)	386.52±39.88	346.59±48.56	0.013
QTc interval (msn)	424.39±44.1	410.89±49.87	0.029
QTd (msn)	51.08±20.75	46.54±19.15	0.096
QT/QRS ratio (iCEB)	4.42±0.82	4.11±0.83	0.035
QTc/QRS ratio (iCEBc)	4.85±0.85	4.62±0.94	0.044
Tp-e interval (msn)	80.49±19.18	57.29±20.07	0.001
Tp-e/QT ratio	0.21±0.05	0.16 ±0.05	0.001
Tp-e/cQT ratio	0.18±0.04	0.13±0.04	0.001

*PWD: P-wave dispersion; QTd: QT dispersion; QTc: corrected QT interval; Tpe: T peak to end interval. *Data are presented as mean (standard deviation)

DISCUSSION

In the current study, our results indicate that PWD, QT, QTc, Tp-e intervals, and Tp-e/QT ratio on standard 12-lead surface ECG were higher in patients with APE. Moreover, the results of this study demonstrate that the index of cardiac electrophysiological balance (iCEB) was lower in this group of patients. Besides these findings, QT, QTc, Tp-e interval, Tp-e/QT ratio, and iCEB value showed significant decreases after thrombolytic therapy. We could not detect a significant change in QTd and PWD after the treatment.

Many ECG patterns that can be observed during APE have been described in detail, even an ECG scoring system has been developed to identify high-risk patients. Daniel et al.[18] developed a score using S1Q3T3, RBBB, T-wave inversion, and tachycardia on ECG, and they speculated that a higher score indicates severe pulmonary hypertension. Also, it was shown that scores may be a basic risk stratification tool for patients with acute PE[19]. Furthermore, the TwiST score (tachycardia, S-wave in lead I, and TWI in leads V1 through V3), which is a prognostic finding for adverse clinical events in APE, was suggested by Hariharan et al.[20]. In addition to these scoring systems, by adding different ECG findings (atrial fibrillation or ST elevation in AVR or Qr in lead V1, or right axis deviation, or P pulmonale), there have been attempts to demonstrate the relationship between APE and ECG[21,22]. However, there is a reality that must be noted: only 70% of patients with APE have abnormal ECG recordings[23].

The relationship between different ECG signs and APE still draws attention due to the wide range of findings on ECG. In this context, ECG repolarization markers have also been associated with APE. Although the underlying pathophysiology is not yet clear for this condition, several mechanisms have been suggested. Right ventricular enlargement and acute cor pulmonale due to rapid ventricular pressure overload, impairment of coronary and left ventricle perfusion, and reduced cardiac out-put due to right ventricle damage, increasing right ventricle wall tension and neurohumoral activity and, eventually, myocardial ischemia may be accepted as major causes of this situation[4,19,24,25]. Tp-e and Tp-e/QT ratio are thought to be pro-arrhythmogenic markers, which give an idea about transmural dispersion of left ventricular (LV) repolarization[9,10]. Icli et al.[5] demonstrated that prolonged cTp-e is a useful marker in early risk stratification and hospital mortality in patients with acute PE. In ours and another study, we also detected a higher Tp-e interval in patients with APE than in the control groups[11]. In addition, we showed a higher QTd, Tp-e/QT, and Tp-e/QTc ratio in high-risk APE. Unlike other studies, we found a significant reduction in Tp-e, Tp-e/QT, QT, QTc intervals after thrombolytic therapy in a short period. Although QTd values tend to decrease after the treatment, they could not

reach statistical significance. QTd may be expected to decrease in a longer follow-up period. The index of cardiac electrophysiological balance (iCEB), estimated as the QT interval divided by the QRS duration, is a novel risk indicator for predicting malignant ventricular arrhythmias[12]. It has been demonstrated that the iCEB is equal to the cardiac wavelength λ (λ = effective refractory period (ERP) x conduction velocity) and that an increased or decreased ratio of iCEB might potentially predict TdP or non-TdP mediated VT/VF, respectively. Authors have speculated that iCEB may reflect both of the depolarization and repolarization phases of the cardiac action potential[12,13]. There are limited data about iCEB according to the literature. Sivri et al.[26] demonstrated elevated iCEB levels after hemodialysis that indicate an increased risk of TdP-mediated ventricular arrhythmia. In another study, no significant relationship between the iCEB and the degree of coronary collateral circulation in patients with chronic total occlusion was shown[27]. In this study, we observed that the iCEB and iCEBc values were lower in patients with APE compared to the healthy controls. So, decreased levels of iCEB may predict non-torsades de pointes mediated ventricular arrhythmia in patients with acute APE. We found that the downward trend of the iCEB/iCEBc values continued significantly after thrombolytic therapy, unlike other ventricular arrhythmia indicators. Despite the significant decrease in the QT interval, the iCEB or iCEBc values were observed to be lower after the therapy due to the absence of a considerable narrowing in the QRS interval. Perhaps, these results may have emerged due to our strict exclusion criteria. If patients with QRS > 120 msn had been included in the study, our results might have been different for the iCEB. We think that a longer follow-up period is needed to provide more information about the iCEB. According to these results, we may conclude that effective thrombolytic therapy causes partial improvement in ventricular repolarization in the early period. Regarding the atrial status, we detected a higher PWD in patients with APE, but we could not demonstrate an improvement after thrombolytic therapy. PWD, which is known to predict the development of atrial fibrillation, also relatively reflects the abnormalities of atrial enlargement and structure[14,28]. Elevated right ventricle end-diastolic pressure and acute tricuspid valve insufficiency are the main causes of increasing right-atrial pressure and atrial-wall tension. Since the right atrium has an anatomically thinner structure, it may take a long time to return to the normal dimensions after acute enlargement. Therefore, improvement in the PWD value may not be observed after thrombolytic therapy in a short follow up period. Finally, hypocalcemia is a frequent occurrence in patients with APE, such as our study population, and it may cause ECG changes during APE and malignant arrhythmias[29]. We need further studies on this subject.

Limitations

Our study has a few limitations. Firstly, this is a single-center, retrospective study with a low number of participants. Secondly, the patients were not followed up for malignant ventricular arrhythmia, sudden cardiac death, and their ECG changes. Thirdly, the lack of 24-hour electrocardiographic Holter monitoring to detect arrhythmic conditions in these patients may be considered as a limitation. Although we excluded patients who were known to be using any drugs affecting the cardiac conduction system during admission, the lack of data on patients receiving beta-blockers, non-dihydropyridine calcium channel blockers or digital, and antibiotics with known efficacy on electrocardiography (erythromycin, azithromycin, etc.) may also be considered a limitation. Finally, we think that the absence of mortality analysis by ECG changes may be considered the main limitation because patients who died after thrombolytic therapy were not included in the study.

CONCLUSION

To the best of our knowledge, there is no study investigating the effects of thrombolytic therapy on ECG parameters and iCEB in high-risk APE in the literature. In our study, we observed higher QTc, Tp-e, Tp-e/QTc, PWD interval, and lower iCEB, iCEBc values in patients with acute pulmonary embolism compared to healthy subjects. Besides these findings, we demonstrated that QT, QTc, Tp-e interval, Tp-e/QTc ratio, and iCEB/iCEBc values showed significant decreases after thrombolytic therapy. According to these results, we may conclude that effective thrombolytic therapy causes partial improvement in ventricular repolarization in the early period. Larger studies on this subject are needed to confirm our results.

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None

Author's Contribution

Concept/design: Y.A, A.T.S, A.S.G, A.L.S, A.İ, H.A, M.A.D; Data analysis/interpretation: Y.A, A.T.S, A.S.G, A.L.S; Drafting of the article: Y.A, A.T.S, A.S.G, A.L.S; Critical revision of the article: Y.A, A.İ, H.A, M.A.D; Approval of the article: Y.A, A.S.G, M.A.D; Statistics: Y.A, ASG with a professional of statistical analysis; Data collection: Y.A, A.T.S, A.L.S.

RESUMO

OBJETIVO: *Diferentes parâmetros de eletrocardiograma (ECG) têm sido investigados para predizer mortalidade e arritmia em pacientes com embolia pulmonar aguda (EPA). O efeito agudo da terapia trombolítica (TT) nesses parâmetros ainda não foi investigado.*

MÉTODOS: *Examinamos os dados de 83 pacientes avaliados com EPA de alto risco e que receberam alta hospitalar após TT. Primeiramente, comparamos os ECGs dos pacientes com EPA de alto risco com os de indivíduos saudáveis (n = 55). Os ECGs dos pacientes com EPA foram comparados logo após a internação e 24 horas mais tarde. A frequência cardíaca, a morfologia da onda P, a duração do QRS, o intervalo QT, Tp-e e o índice de equilíbrio eletrofisiológico cardíaco (iCEB) foram analisados.*

RESULTADOS: *Embora o valor máximo de P não tenha sido diferente entre os grupos no ECG, a frequência cardíaca, QT, intervalo QTc (QT corrigido), intervalos Tpe, razão TP-e/QT e dispersão da onda P foram significativamente mais elevados no grupo de EPA (valores de P < 0,031). Os valores do iCEB ou iCEBc (iCEB corrigido) foram inferiores no grupo de APE (P < 0,001). Após a TT, observamos uma diminuição da frequência cardíaca, do intervalo TP-e e da razão TP-e/QT (**P** < 0,001). Apesar de termos observado uma diminuição do intervalo QT e QTc e da dispersão do QT (QTd), o valor de QTd não apresentou uma diferença estatisticamente significativa (respectivamente, valor de P 0,013, 0,029 e 0,096). Os níveis do iCEB e iCEBc foram menores após a TT (valor de P 0,035 e 0,044, respectivamente).*

CONCLUSÃO: *Os valores de QT, QTc, intervalo Tp-e, razão Tp-e/QTc, iCEB e iCEBc diminuíram significativamente após TT. Pode-se concluir que a TT eficaz causa uma melhora parcial da repolarização ventricular no período inicial.*

PALAVRAS-CHAVE: *Eletrocardiografia. Embolia pulmonar. Terapia trombolítica.*

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ORIGINAL ARTICLE

Comparative evaluation of memory T cells in COVID-19 patients and the predictive role of $CD4^+CD8^+$ double positive T lymphocytes as a new marker

Yasin Kalpakci[1]
Tuba Hacibekiroglu[2]
Gulay Trak[3]
Cengiz Karacaer[4]
Taner Demirci[5]
Havva Kocayigit[6]
Cenk Sunu[7]
Ceyhun Varim[8]
Mesude Falay[9]

1. Medical Doctor, Department of Hematology, Sakarya University Medicine Faculty, Sakarya, Turkey.
2. Associate Professor, Department of Hematology, Sakarya University Medicine Faculty, Sakarya, Turkey.
3. Medical Doctor, Department of Microbiology, Sakarya University Medicine Faculty, Sakarya, Turkey.
4. Medical Doctor, Department of Internal Medicine, Sakarya University Medicine Faculty, Sakarya, Turkey.
5. Assistant Professor, Department of Internal Medicine Division of Endocrinology, Sakarya University Medicine Faculty, Sakarya, Turkey.
6. Medical Doctor, Department of Anesthesiology, Sakarya University Medicine Faculty, Sakarya, Turkey.
7. Associate Professor, Department of Internal Medicine, Sakarya University Medicine Faculty, Sakarya, Turkey.
8. Medical Doctor, Department of Hematology, Duzen Laboratory Group, İstanbul, Turkey.
9. Hematology, Ankara Numune Training and Research Hospital, Ankara, Turkey

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SUMMARY

BACKGROUND: *The COVID-19 pandemic has affected the entire world, posing a serious threat to human health. T cells play a critical role in the cellular immune response against viral infections. We aimed to reveal the relationship between T cell subsets and disease severity.*

METHODS: *40 COVID-19 patients were randomly recruited in this cross-sectional study. All cases were confirmed by quantitative RT-PCR. Patients were divided into two equivalent groups, one severe and one nonsevere. Clinical, laboratory and flow cytometric data were obtained from both clinical groups and compared.*

RESULTS: *Lymphocyte subsets, CD4+ and CD8+ T cells, memory CD4+ T cells, memory CD8+ T cells, naive CD4+ T cells, effector memory CD4+ T cells, central memory CD4+ T cells, and CD3+CD4+ CD25+ T cells were significantly lower in severe patients. The naive T cell/CD4 + EM T cell ratio, which is an indicator of the differentiation from naive T cells to memory cells, was relatively reduced in severe disease. Peripheral CD4+CD8+ double-positive T cells were notably lower in severe presentations of the disease (median DP T cells 11.12 µL vs 1.95 µL; p< 0.001).*

CONCLUSIONS: *As disease severity increases in COVID-19 infection, the number of T cell subsets decreases significantly. Suppression of differentiation from naive T cells to effector memory T cells is the result of severe impairment in adaptive immune functions. Peripheral CD4+CD8+ double-positive T cells were significantly reduced in severe disease presentations and may be a useful marker to predict disease severity.*

KEYWORDS: *COVID-19, Lymphocyte Subsets, Adaptive Immunity.*

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CORRESPONDING AUTHOR: Yasin Kalpakci
Korucuk Mahallesi, Korucuk Caddesi, ADA:3334, No 2118, Adapazari, Sakarya, Turkey – 54260
Tel: +90 532 739-8495 / Fax: +90 264 255-2105
E-mail: dr.yasin@live.com

INTRODUCTION

Most patients with COVID-19 are asymptomatic or have only mild symptoms[1]. Symptomatic cases mostly suffer from mild fever, cough, shortness of breath, muscle pain, headache, and diarrhea[2,3]. COVID-19 can lead to severe pneumonia, acute respiratory distress syndrome (ARDS), multiple organ failure, and, eventually, death in patients with advanced age and severe comorbid diseases[2,4]. One of the most implicated mechanisms in severe disease is immune system alterations[5–7].

The physiopathology of COVID-19 and the underlying mechanisms in severe cases are the most important challenges of the ongoing studies. Several studies have highlighted that coronaviruses such as SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus), MERS-CoV (Middle East Respiratory Syndrome-Coronavirus), SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) leads to a severe reduction in T lymphocyte subsets, which are not common in other viral infections[8,9]. Studies have focused on revealing the immune system changes to explain pathogenesis. In severe cases of the disease, memory T cell and regulatory T cell changes may be responsible for the uncontrolled inflammatory response and the lack of specific immunity.

METHODS

Study Design and Participants

This study was designed as a cross-sectional study. The diagnosis was confirmed by quantative RT-PCR. A total of 40 patients over 18 years old were recruited randomly in the study. The patients were divided into two equivalent groups, according to clinical and laboratory findings, i.e., severe and nonsevere. Written informed consent was obtained from all participants. Patients using convalescent plasma, tocilizumab, and systemic steroids were excluded.

Definitions

The Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) (Released by the National Health Commission & State Administration of Traditional Chinese Medicine on March 3, 2020) was used to define the severity of the disease[10]. Patients were classified as mild/moderate/severe/critical according to this protocol. We defined mild/moderate cases as nonsevere and severe/critical cases as severe. Cases with respiratory distress (30 breaths/min), oxygen saturation ≤93% at rest, arterial partial pressure of oxygen (PaO2) fraction of inspired oxygen (FiO2) 300mmHg (l mmHg = 0.133kPa), and all cases requiring mechanical ventilation support or having any organ failure due to COVID-19 were included in the severe group; cases that did not meet these criteria were included in the nonsevere group.

Data Collection

Demographic data and laboratory findings including complete blood count, routine serum biochemical tests, acute phase and infection indicators, and coagulation parameters were collected from inpatient records. Lymphocyte subsets were analyzed from fresh blood samples by flow cytometry. We used the dual-platform flow cytometric method to measure (DP FCM) the lymphocyte subsets[11,12]. All other clinical and laboratory data were collected simultaneously with a flow cytometric analysis.

Flow Cytometry

On the same day of the analysis, 4-5 ml of peripheral blood samples were taken from the tubes containing EDTA and sent to the microbiology laboratory of our hospital without waiting. Peripheral blood samples in tubes containing EDTA were labeled using monoclonal antibodies. For this purpose, the number of cells was calculated to be $1x10^6$ cells per mL. Lymphocyte subsets were analyzed by flow cytometry as previously described in the literature.[13] Subsets were determined using antibodies as follows: CD3 (FITC) / CD4 (PeCY7) / CD8 (APC Cy7) / CD45ROPE / CD45RA (APC) / CD197 (PerCpCy5,5) / CD25 (APC Cy7) (BD Biosciences, AB). The tubes were incubated for 20 minutes at room temperature in the dark. At the end of the incubation, the red blood cells in the samples were removed by adding 2-3 mL of Lysing Solution (Becton Dickinson, San Jose, CA 95131 USA). Following washing with Lysing Solution, they were washed with 2 mL of PBS (Phosphate Buffer Saline), the cells were suspended with 500 µL of PBS containing 1% paraformaldehyde and kept in the dark at 2-8 °C until the time of analysis. The cells were analyzed with the FACSCantoII (Becton Dickinson, Immunocytometry Systems, San Jose, CA 95131 USA) model flow cytometry device using the BD FACSDiva program.

Statistical Analysis

Statistical analysis was performed with SPSS Statistics (IBM Corporation, Somers, NY) software,

version 22). The normality of the distribution of continuous variables was determined using the Kolmogorov–Smirnov test. The continuous variables were expressed as mean and standard deviation or as median and interquartile range, depending on the normality of their distribution. Categorical variables are interpreted by frequency tables. The Mann–Whitney U test was used to compare the variables that were not normally distributed. On the other hand, the Student's t-test was used to compare the variables with a normal distribution. Categorical features and relationships between the groups were assessed using an appropriate chi-square test. A p-value of <0.05 was accepted as statistically significant.

RESULTS

Demographic and Basic Clinical Features of Severe and Nonsevere COVID-19 Patients

The demographic features, basic clinical and laboratory characteristics of the 40 patients are presented in Table 1. While there was no significant difference in the women and men ratio, patients in the severe disease group were significantly older (mean age of severe patients: 71.9 ± 11.2; nonsevere patients: 55.4 ± 17.0, p<0.001). There were 3 patients with concomitant malignancy and 20 patients with a comorbid chronic metabolic disease, and the differences between the two groups were not significant, but the number of patients was insufficient for interpretation.

Biochemical and Inflammatory Markers, Blood Cell Counts Representing Disease Severity

The inflammatory and biochemical markers (C-reactive protein, erythrocyte sedimentation rate, procalcitonin, d-dimer, ferritin, fibrinogen, and lactate dehydrogenase) were found to be significantly elevated in the severe disease group (Table 1). In the severe disease group, the white blood cell count (WBC) and absolute neutrophil count (ANC) increased considerably, whereas a critical decrease in the absolute lymphocyte count (ALC) was noticed, with no distinct difference in platelet counts (WBC 8.86±3.51 vs 5.89±2.30 $10^3/mm^3$; p= 0.003, ANC 7.72±3.41 vs 3.67±2.01 $10^3/mm^3$; p <0.001, ALC 0.61 vs 1.39 $10^3/mm^3$; p <0.001, platelet count 221.6±123.8 vs 199.5±100.3 $10^3/mm^3$; p=0.538 respectively). The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were increased significantly in the severe disease group (NLR 10.55 vs 2.21; p <0.001, PLR 319.5 vs 119.5; p <0.001).

TABLE 1. DEMOGRAPHIC FEATURES OF PATIENTS AND COMPARISON OF INFLAMMATORY MARKERS AND BLOOD CELL COUNTS ACCORDING TO THE CLINICAL SEVERITY OF THE DISEASE.

	Nonsevere patients (n=20)	Severe patients (n=20)	P-value
Age, years	55.4 ± 17.0	71.9 ± 11.2	0.001
Gender, F/M (%)	13/7 (65/35)	7/13 (35/65)	0.056
Chronic diseases No (%) *Other chronic diseases(%) Malignancy (%)	 12 (60) 7 (35) 1 (5)	 5 (25) 13 (65) 2 (10)	0.081
C-reactive protein (CRP), mg/L	12.10 (3.31-38.80)	167.50 (85.55-190.0)	<0.001
Sedimentation, mm/1 hr	43.11 ± 29.08	79.15 ± 23.27	<0.001
Procalcitonin, ng/mL	0.062 (0.034-0.079)	0.726 (0.197-3.232)	<0.001
Albumin, gr/L	36.4 ± 3.5	23. 8 ± 3.6	<0.001
D-dimer, ng/mL	663.5 (346.5-1205)	2120 (1612.5-4300)	<0.001
Ferritin, ng/mL	120.9 (44.3-427.4)	864.5 (460.4-2000.0)	<0.001
Fibrinogen, mg/dL	348.0 (294.5-381.0)	477.5 (390.8-609.8)	<0.001
Lactate dehydrogenase (LDH), IU/L	223.5 (211-262.5)	395 (308-502.5)	<0.001
White blood cell count, 10^3 / mm^3	5.89±2.30	8.86±3.51	0.003
Absolute neutrophil count, 10^3 / mm^3	3.67±2.01	7.72±3.41	<0.001
Absolute lymphocyte count, 10^3 / mm^3	1.39 (1.16-2.25)	0.61 (0.38-0.97)	<0.001
Platelet count, 10^3 / mm^3	199.5±100.3	221.6±123.8	0.538
Neutrophil lymphocyte ratio (NLR)	2.21 (1.38-2.94)	10.55 (6.56-22.63)	<0.001
Platelet lymphocyte ratio (PLR)	119.5 (94.1-155.4)	319.5 (184.0-604.7)	<0.001

*Due to the low number of patients, patients with diabetes mellitus, hypertension, coronary heart disease, chronic kidney disease, and chronic inflammatory diseases were not demonstrated in the chronic metabolic disease group.

Comparison of Lymphocyte Subsets and Memory T cells According to Disease Severity

Lymphocyte subsets were compared according to disease severity and are presented in Table 2. CD8+ cytotoxic T cells and CD4+ helper T cells were significantly lower in patients with severe disease (CD8+ T cells 192.00 vs 504.15 µL; p <0.001, CD4+ T cells 395.45 vs 958.83 µL; p <0.001); however, there was no significant difference in the CD4/CD8 ratio in both groups (CD4/CD8 ratio 1.81 vs 1.57; p=0.738). Memory T cells showed a remarkable decrease in the severe disease group (CD4+ memory T cells 304.99 ± 204.75 vs 682.38 ± 269.26 µL; p <0.001, CD8+ memory T cells 87.61 vs 288.33 µL; p <0.001). The naive, EM, and CM CD4+ T cells were notably reduced in the severe disease group (Naive T cells 175.06 vs 502.68 µL; p <0.001, EM 0 vs 14.61 µL; p=0.015, CM 475.74 vs 1051.68 µL; p <0.001). Furthermore, the naive CD4+ T cell/CD4+ effector memory T cells ratio was significantly impaired in patients with severe disease (median 0 vs 35.10; p <0.001). The CD3+CD4+CD25+ T cell subset was significantly lower in the severe disease group (374.42 vs 965.96 µL; p <0.001). As a new finding, CD4+CD8+ double-positive (DP) T lymphocytes in peripheral blood decreased significantly in the severe disease group (DP T cells 1.95 vs 11.12 µL; p < 0.001, respectively). The comparison of lymphocyte subsets is presented schematically in Figure 1.

DISCUSSION

Many studies have shown that advanced age and concomitant diseases are risk factors for severe COVID-19. In severe COVID-19 cases, adaptive immunity cannot overcome the disease and the virus spreads through the blood and damages tissues. In addition to virus-induced direct cytopathic damage, the development of organ injuries secondary to the uncontrolled release of cytokines (called cytokine storm) is believed to be responsible for the pathogenesis of the disease in severe cases[5,14,15].

Previous publications have reported that the neutralizing Ig-G antibodies last over 1-2 years in SARS-CoV and MERS-CoV infections[16–19]. However, recent studies have reported that neutralizing antibodies begin to decrease after 2-3 months in convalescent COVID-19 patients and many of them become negative in a short time[19–21]. The short-term reduction and disappearance of neutralizing antibodies in convalescent COVID-19 patients may suggest that the virus causes deeper and permanent impairments to the immune system's memory functions.

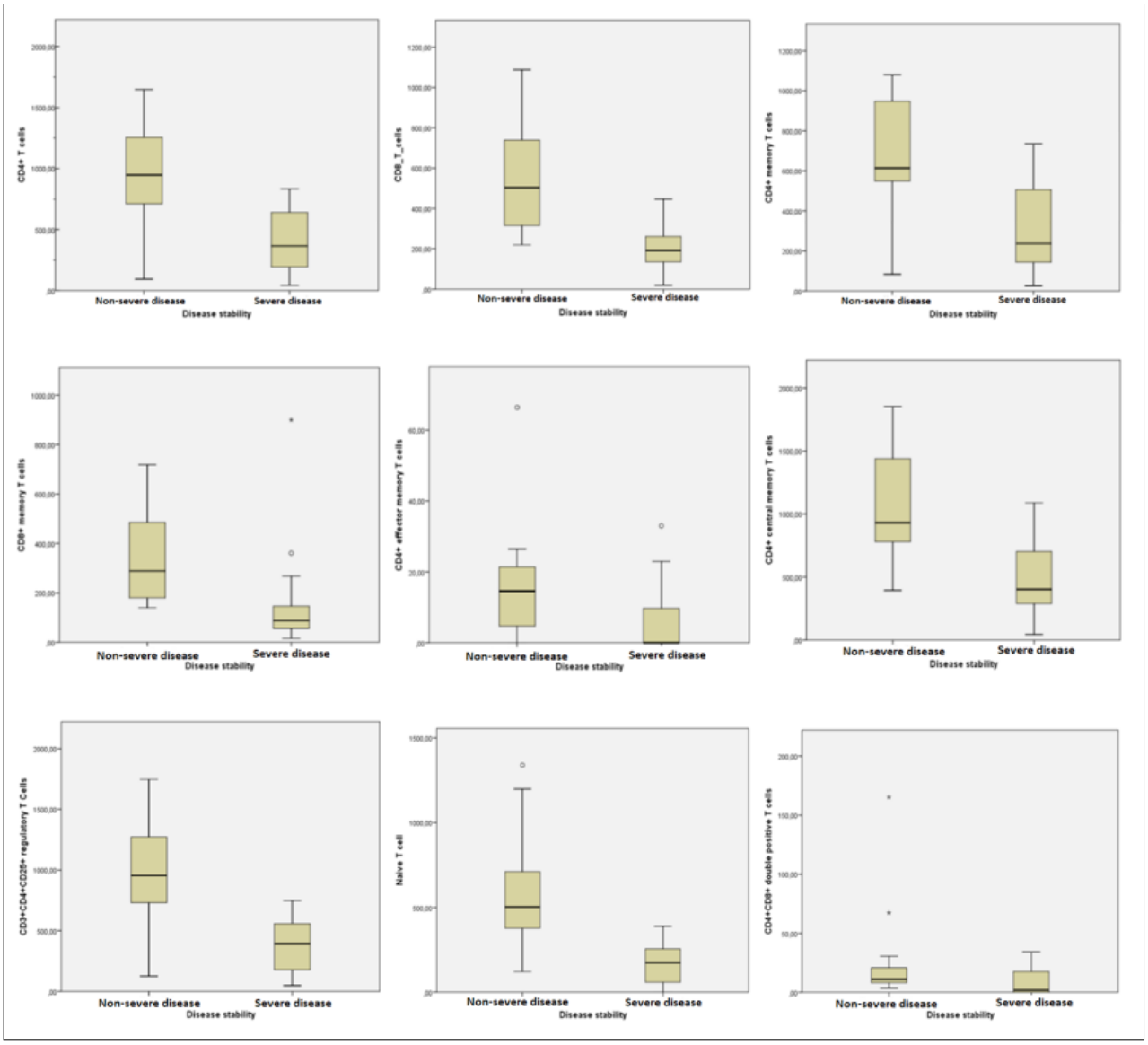
In this study, we aimed to contribute to the explanation of the pathogenesis of the disease by comparing T lymphocyte subsets in severe and nonsevere COVID-19 patients and revealing disorders of the T-cell-mediated immune response. Thus, we analyzed CD4+ T helper cells, CD8+ cytotoxic T cells, memory T cells (CD4 and CD8 positive), CD3+CD4+CD25+ T cells, CD4+CD8+ double-positive (DP) T cells, which are important components of T-cell-mediated immunity.

The severe cases of the disease were significantly older (Table 1). The production of naive T cells and memory T cells decreases with aging[22]. The weakened adaptive immune response in the elderly can explain the increased severity of the disease with age. We found that CRP, ESR, procalcitonin level, which show

TABLE 2. COMPARISON OF LYMPHOCYTES SUBSETS BY CLINICAL SEVERITY OF THE DISEASE.

	Nonsevere patients (n=20)	Severe patients (n=20)	P-value
CD4+ T cells, µL	958.83 ± 416.24	395.45 ± 237.59	<0.001
CD8+ T cells, µL	504.15 (313.61 – 786.22)	192.00 (135.52 – 261.80)	<0.001
CD4+ memory T cells, µL	682.38 ± 269.26	304.99 ± 204.75	<0.001
CD8+ memory T cells, µL	288.33 (178.94 – 492.92)	87.61 (52.15 – 148.52)	<0.001
CD4+ effector memory T cells, µL	14.61 (4.34 – 21.47)	0 (0 – 12.15)	0.015
CD4+ central memory T cells, µL	1051.68 ± 427.16	475.74 ± 298.57	<0.001
CD3+CD4+CD25+ T Cells, µL	965.96 ± 416.58	374.42 ± 224.00	<0.001
Naive CD4+ T cell, µL	502.68 (358.26 – 746.01)	175.06 (58.20 – 259.61)	<0.001
CD4+CD8+ double positive T cells µL	11.12 (7.61 – 21.71)	1.95 (0 -17.66)	<0.001
CD4/CD8 lymphocyte ratio	1.57 (1.38-2.85)	1.81 (1.16-3.13)	0.738
Naive CD4+ T cell/ CD4+ memory T cells ratio	0.92 ± 0.40	0.80 ± 0.82	0.583
Naive CD4+ T cell/ CD4+ central memory T cells ratio	0.59 ± 0.31	0.59 ± 0.96	0.998
Naive CD4+ T cell/ CD4+ effector memory T cells ratio	35.10 (15.83-102.20)	0 (0-14.14)	<0.001

FIGURE 1. LYMPHOCYTE SUBTYPES ACCORDING TO THE CLINICAL SEVERITY OF THE DISEASE DEMONSTRATED SCHEMATICALLY.



Note. The data defined on the Y axis shows the absolute cell count per 1 microliter.

the severity of the infection and reflect the inflammatory response, were significantly high in patients with severe disease (Table 1). LDH, fibrinogen, d-dimer, ferritin, which are biochemical indicators of inflammation, were higher in patients with severe disease, and albumin was significantly lower in severe disease cases (Table 1). Lymphopenia is the first indicator of impairment in T-cell-mediated immunity. The prognostic ratios such as NLR and PLR increased notably in patients with severe disease as a result of decreased ALC (Table 1).

CD4+ T helper cells activate other immune cells and help B cells in the production of antibodies, while CD8+ cytotoxic T cells kill virus-infected cells directly with their granules. Persistent stimulation of viral infection and increased inflammatory cytokines lead to a depletion of T cells[23]. In addition, the suggestion that SARS-CoV-2 infects T cells through spike-protein and increases apoptosis of T cells is still under investigation[24]. Our study demonstrated that helper and cytotoxic T cells were significantly reduced in patients with severe disease. CD4/CD8 ratio has a crucial role in the management of some diseases, especially in HIV infection. However, we did not find a remarkable difference in the CD4/CD8 ratio between the two groups, therefore this is not a useful marker in COVID-19 management.

Normally, some of the naive T cells differentiate into long-lived memory cells after contact with a pathogen. Thus, they guarantee a much faster and

stronger response when the same pathogen is encountered again. We have demonstrated that CD4+ memory T cells, CD8+ memory T cells, naive T cells, CD4+ EM T cells, and CD4+ CM T cells are critically reduced in severe disease cases (Table 2). We would like to point out that the naive T cell/CD4+ EM T cell ratio was impaired. As far as we know, a similar finding has been previously reported only by Chuan Qin et al., who found that the naive-to-memory CD4+ T cell ratio was impaired in patients with severe disease[5]. These results suggest that the differentiation from naive T cells to EM T cells is also impaired.

We encountered a new finding to predict disease severity that has not been reported in previous studies. CD4+CD8+ double-positive (DP) T lymphocytes were remarkably lower in severe disease. DP T cells are present in peripheral blood in small numbers. Their roles in the pathogenesis of autoimmune diseases, viral infections, and cancers are under ongoing debate[25–27]. Some studies have suggested that DP T cells are developing T cells released from the thymus, while others suggest that they are differentiated effector memory cells and have anti-viral effects[27,28]. Michelina Nascimbeni et al. have offered that peripheral DP T cells are involved in the adaptive immune response to viral pathogens[27]. The prominent reduction of peripheral DP T cells suggests that the adaptive immune response is seriously impaired in severe disease. DP T cells can be an important marker to predict severity if supported by larger studies.

Tregs regulates the immune response by suppressing the activation, proliferation, and cytokine production of CD4+ T cells and CD8+ T cells and are thought to suppress B cells and dendritic cells[29,30]. Otherwise, excessive immune response and inflammation damage all tissues. As disease severity increases in COVID-19 infection, inflammation caused by uncontrolled cytokines becomes more and more severe, leading to organ failure. This well-known entity is called a cytokine storm. In our study, we compared the subset of CD3+ CD4+CD25+ T cells and found it to be significantly lower in patients with severe disease. Doubtless, CD25+ T cells do not fully represent Tregs, but they also contain them. Possibly the impairment in the regulatory functions is one of the leading pathologies that cause cytokine storms in patients with severe disease.

Our study has several limitations. First, this is a cross-sectional and single-center study with 40 patients. A prospective study with more participants and successive flow cytometric analysis would undoubtedly provide more valuable information. However, the high statistical significance in our results makes our study precious. Second, we could not compare all lymphocyte subsets due to the limited antibody supply and we couldn't identify Tregs exactly since CD127/FOX-P3 was not available.

Despite the limitations, we obtained precious data regarding the adaptive immune response. In particular, we have demonstrated the importance of memory T cells and DP T cells in severe disease.

CONCLUSION

We have demonstrated that CD4+ helper T cells, CD8+ cytotoxic T cells, and memory T cells are significantly reduced in severe disease. The relative impairment in naive T cell/CD4+ effector memory T cell refers to deeper disorders in adaptive immune functions of patients with severe disease. The decrease in DP T cells is a new and useful marker for predicting disease severity.

Conflicts of interest

All authors declare that there is no potential conflict of interest relevant to this article.

Author's Contribution

Writing - Review and Editing: Yasin Kalpakci; Validation: Tuba Hacibekiroglu; Supervision: Gulay Trak; Resources: Cengiz Karacaer; Methods: Taner Demirci; Data curation: Havva Kocayigit; Conceptualization: Cenk Sunu; Visualization: Ceyhun Varim; Investigation: Mesude Falay.

RESUMO

OBJETIVO: *A pandemia de COVID-19 tem afetado o mundo todo, constituindo uma ameaça grave para a saúde humana. As células T desempenham um papel crítico na imunidade celular contra infecções virais. Procuramos desvendar a relação entre sub*

GRUPOS DE CÉLULAS T E A SEVERIDADE DA DOENÇA.

MÉTODOS: *Um total de 40 pacientes com COVID-19 foram aleatoriamente recrutados para o presente estudo transversal. Todos os casos foram confirmados por RT-PCR quantitativo. Os pacientes foram divididos em dois grupos equivalentes, um grave e um não-grave. Os dados da avaliação clínica, laboratorial e da citometria de fluxo foram obtidos para ambos os grupos e comparados.*

RESULTADOS: *Os subconjuntos de linfócitos, células T CD4+ e CD8+, células T de memória CD4+, células T de memória CD8+, células T CD4+ virgens, células T efetoras CD4+, células T de memória central CD4+ e células T CD3+ CD4+ CD25+ estavam significativamente mais baixas nos pacientes graves. A razão células T virgens/células T efetoras TCD4+, que é um indicador da diferenciação entre células T virgens e células de memória, estava relativamente reduzida em casos graves da doença. As células T duplo-positivas CD4+CD8+ periféricas estavam notavelmente mais baixas em casos graves da doença (mediana das células T DP: 11,12 µL vs. 1,95 µL; p< 0,001).*

CONCLUSÃO: *Conforme aumenta a gravidade da doença nos casos de COVID-19, o número de subconjuntos de células T diminui significativamente. A supressão da diferenciação de células T virgens para células T efetoras é o resultado do comprometimento grave das funções imunológicas adaptativas. As células T duplo-positivas CD4+CD8+ periféricas estavam notavelmente mais baixas em casos graves da doença e podem ser um marcador útil para predizer a severidade da doença.*

PALAVRAS-CHAVE: *COVID-19, Subconjuntos de linfócitos, Imunidade adaptativa.*

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ORIGINAL ARTICLE

Frontal plane QRS-T angle may be a predictor for post-coronary artery bypass graft surgery atrial fibrillation

Mevlüt Serdar Kuyumcu[1]
Dinçer Uysal[2]
Mustafa Bilal Özbay[3]
Oğuz Aydın[1]
Erdoğan İbrişim[2]

1. Süleyman Demirel University, Faculty of Medicine, Department of Cardiology, Isparta, Turkey.
2. Süleyman Demirel University, Faculty of Medicine, Department of Cardiovascular Surgery, Isparta, Turkey.
3. Ankara State Hospital, Department of Cardiology, Ankara, Turkey.

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SUMMARY

BACKGROUND: *New-onset postoperative atrial fibrillation (POAF) is the most common arrhythmia following coronary artery bypass graft surgery (CABG) and is associated with prolonged hospitalization, stroke, and mortality. The frontal plane QRS-T [f(QRS-T)] angle, which is defined as the angle between the directions of ventricular depolarization (QRS-axis) and repolarization (T-axis), is a novel marker of ventricular repolarization heterogeneity. The f(QRS-T) angle is associated with adverse cardiac outcomes. In light of these findings, in this study, we aimed to investigate the potential relationship between the f(QRS-T) angle and POAF.*

METHODS: *180 patients who underwent CABG between August 2017 and September 2018 were included in the study retrospectively. Two groups were established as patients who preserved postoperative sinus rhythm (n=130) and those who developed POAF (n=50). The f(QRS-T) angle and all other data were compared between groups.*

RESULTS: *The fF(QRS-T) angle (p<0.001), SYNTAX score (p=0.039), serum high-sensitivity CRP levels (p=0.026), mean age (p<0.001), electrocardiographic left ventricular hypertrophy rate (LVH) (p=0.019), and hypertension rate (p=0.007) were higher, and the mean left ventricular ejection fraction (LVEF) (p<0.001) was lower in the POAF group. Multivariable logistic regression analyses demonstrated that lower LVEF (p=0.004), LVH (p=0.041), and higher age (p=0.008) and f(QRS-T) angle (p<0.001) were independently associated with POAF.*

CONCLUSIONS: *High f(QRS-T) angle level is closely associated with the development of POAF. The f(QRS-T) angle can be a potential indicator of POAF.*

KEYWORDS: *Atrial fibrillation. Coronary artery bypass. Myocardial revascularization. Postoperative complications. Electrocardiography.*

INTRODUCTION

New-onset POAF is the most common arrhythmia following CABG, affecting about one-third of patients in the post-operative period[1]. It typically peaks on the postoperative day 2[2]. POAF is associated with increased risk of stroke, heart failure (HF), prolonged hospitalization, short-term and long-term mortality[3]. Thus, determining significant pre-operative risk factors for the development of POAF will improve risk

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CORRESPONDING AUTHOR: Mevlüt Serdar Kuyumcu
Süleyman Demirel University, Faculty of Medicine, Department of Cardiology, Isparta, Turkey – 32000
Tel: +90 505 221-1458
E-mail: kuyumcuserdar@hotmail.com

stratification and help clinicians execute perioperative prophylactic strategies.

A twelve-lead electrocardiogram (ECG) is a low-cost routine cardiac examination used in daily practice

that is noninvasive, rapidly deployable, and available in every hospital. The frontal plane QRS-T [f(QRS-T)] angle, which is defined as the angle between the directions of ventricular depolarization (QRS axis) and repolarization (T axis), is a novel marker of ventricular repolarization heterogeneity[4]. Abnormal f(QRS-T) angle has been shown to have a prognostic value for mortality in the general population, as well as in patients with congestive HF and acute coronary syndrome[5]. Increased f(QRS-T) angle has also been found to be associated with an increased risk of AF in the elderly[6]. But, to our knowledge, there are no studies investigating the risk of new-onset POAF after CABG surgery in patients with increased f(QRS-T) angle.

In this study, we aimed to investigate the predictive value of abnormal ventricular repolarization for new-onset POAF by using the f(QRS-T) angle.

METHODS

A total of 208 patients who underwent isolated on-pump coronary artery bypass grafting at the Suleyman Demirel University Faculty of Medicine Education and Research Hospital were evaluated retrospectively. Patients with risk factors associated with the development of atrial fibrillation (AF) such as chronic obstructive pulmonary disease (n=2) and valvular heart diseases (n=5), as well as patients with existing preoperative AF or flutter (n=8), renal insufficiency (n=3), and patients which required additional surgical intervention (n=4), preoperative inotropic or mechanical support (n=2), redo surgery or emergency coronary surgery (n=4) were excluded from the study.

The diagnosis of hypertension was made when the systolic blood pressure was 140 mmHg or higher, or if the diastolic blood pressure was 90 mmHg or higher in at least three different measurements, or when there was use of anti-hypertensive medication. The diagnosis of diabetes mellitus was established when the fasting blood glucose was 126 mg/dL or higher, or when there was use of anti-diabetic medication. Hyperlipidemia was defined as total cholesterol levels of 200 mg/dL or higher, or a history of statin use except in the previous three months. Patients who were smoking before hospitalization were considered smokers.

The study protocol was approved by the local Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and International Conference on Harmonisation guidelines.

Electrocardiography

The 12-lead ECG was recorded at a paper speed of 50 mm/s in the supine position (Nihon Kohden, Tokyo, Japan). ECGs taken at the first hospitalization of the patients were used. ECG intervals were calculated according to the guidelines[7]. Frontal QRS and T-wave axes were present in the automatic reports of the ECG machine. The calculation of the f(QRS-T) angle was made from these axes as the absolute difference between the frontal plane QRS axis and the frontal plane T axis. In case the angle exceeded 180°, it was calculated by subtracting it from 360°[4]. The subjective component of the individual measurements was ruled out by calculating the f(QRS-T) angle based on an automatic report of the ECG machine.

Echocardiography and surgical procedure

Echocardiography was performed in all patients before surgery. LVEF was calculated by using the modified Simpson method. Standard cardiopulmonary bypass (CPB) was performed with median sternotomy and mild hypothermia (32°C). The CPB was performed with two-stage aortovenous cannulation. An X-clamp was placed to the ascending aorta and cardiac arrest was provided with cold antegrade cardioplegia (10 to 15 mL/kg) with high potassium. Cardiac arrest was maintained with blood cardioplegia, which was given every 15 to 20 min. The CPB was established with a roller pump with a membrane oxygenator and an arterial line filter at pump flow rates of 2 to 2.4 L/min/m2. The left internal thoracic artery and the saphenous vein graft were prepared. The distal anastomoses were constructed during a single period of total X-clamp, and proximal anastomoses were established with partial clamping of the aorta. Hot blood shot cardioplegia was given immediately before the X-clamp was removed. Extubation was performed at the earliest stage possible following the provision of hemodynamic stability.

POAF assessment

The presence of AF documented by ECG for at least 5 minutes was recorded and analyzed as POAF[1]. The development of AF was assessed by continuously monitoring the patients on the first 4 postoperative

days and on the following days until discharge by regularly performing 12-lead ECG, 3 times a day. AF was also evaluated using 12-lead ECG when patients complained of palpitations.

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 21.0 (SPSS, Chicago, IL, USA). The minimum number of individuals that should be sampled with 90% power and 0.05 Type I error was at least 46 (R 3.0.1. open-source program). The primary effect variable was determined as the QRS angle. A 1% change in the total f(QRS-T) angle [3.6 degrees on the f(QRS-T) plane] was accepted as clinically relevant. The standard deviation of the primary effect variable was calculated as ± 0.36. For the descriptive statistics of the data, mean, standard deviation, rate, and frequency values were used. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. For the analysis of parametric data, the Student's t-test was used. For the analysis of non-parametric data, the Mann-Whitney U-test was used. The chi-square test was used to compare the categorical variables between groups. Logistic regression analysis was used to determine the impact of variables. The standardized beta coefficients and 95% confidence intervals were calculated. Statistical significance was defined as p-values < 0.05.

RESULTS

The baseline clinical and demographic characteristics of the study population are shown in Table 1. There was no difference between the groups except that the mean age (p<0.001) and hypertension rate (p=0.007) were higher in the POAF group. The echocardiographic measurements and surgery features of the groups are shown in Table 2. The mean SYNTAX score was higher in the POAF group (p=0.039). The mean LVEF was lower in the POAF group (p<0.001). The electrocardiographic features of the groups are shown in Table 3. LVH was higher in the POAF group (p=0.019). The mean f(QRS-T) angle was higher in the POAF group (p<0.001). The laboratory parameters of the study groups are shown in Table 4. There were no differences between the groups, except for the high-sensitivity C-reactive protein (hs-CRP) (p=0.026).

We performed univariate and multiple linear regression analyses for the predictors of POAF, as depicted in Tables 1, 2, 3, and 4 (Table 5). In univariate regression analysis, older age (p<0.001), Hypertension (p=0.010), lower LVEF (p<0.001), higher hs-CRP levels (p=0.032), higher f(QRS-T) angle (p<0.001), and LVH (p=0.029) were associated with POAF. Older age (p=0.008), LVEF (p=0.004), higher f(QRS-T) angle (p<0.001), and LVH (p=0.041) were detected as independent predictors for POAF after multiple linear regression analysis.

DISCUSSION

In our study, we found that an increased f(QRS-T) angle in CABG patients may be predictive, as well as age and other factors, which are the classic risk factors for POAF.

There are many potential pathophysiological mechanisms that lead to POAF. The most common and consistent variable for developing POAF across the studies is aging[8]. Other preoperative risk factors include a previous history of AF, obesity, diabetes mellitus, increased left atrial size, chronic obstructive pulmonary disease, valvular disease, withdrawal from beta-blocker treatment, and electrolyte imbalances like hypokalemia and hypomagnesemia[8]. In our study, increased age, low LVEF, LVH, and larger f(QRS-T) angle were independent predictors for POAF.

Aberrant ventricular repolarization is one of the known mechanisms of arrhythmiogenesis[9]. The development of aberrancy in ventricular repolarization has also been found to be associated with AF. Long QT syndrome patients have increased atrial action potential durations which can result in polymorphic atrial tachyarrhythmias[10]. These polymorphic atrial arrhythmias are also likely to degenerate into AF[11]. Increased activity of late sodium channels currents leading to initiation of atrial ectopic activity may be another explanation for the occurrence of AF[12]. The association of abnormal ventricular repolarization with AF may be due to common comorbid conditions such as diabetes and hypertension. An increased f(QRS-T) is closely related to abnormal ventricular repolarization[5].

Another pathophysiological mechanism involved in the pathophysiology of atrial fibrillation is increased adrenergic hyperactivity[13]. Variations in cardiac autonomic neural tone and elevated sympathetic activity on the ventricular myocardium are related to the total dispersion of repolarization and an increased risk of AF. The total dispersion of repolarization reflects the heterogeneity like the f(QRS-T) angle[4].

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY GROUPS.

Variables	Postoperative sinus rhythm (n = 130)	Postoperative Atrial fibrillation (n = 50)	p-value
Age, years	59.87 ± 12.45	68.10 ± 8.83	< 0.001
Female, n(%)	41 (31.5%)	10 (20.0%)	0.124
BMI, kg/m^2	29.01 ± 5.35	27.97 ± 4.60	0.227
Diabetes Mellitus, n(%)	54 (41.5%)	26 (52.0%)	0.206
Hypertension, n(%)	92 (70.8%)	45 (90.0%)	0.007
Smoking, n(%)	43 (33.1%)	23 (46.0%)	0.107
Cerebrovascular event history, n(%)	18 (13.8%)	7 (14.0%)	0.979
Peripheral vascular disease, n(%)	13 (10.0%)	5 (10.0%)	0.998

Data are given as mean ± SD, n, or median (interquartile range). HDL: high-density lipoprotein; Hs-CRP: high-sensitivity C-reactive protein; LDL: low-density lipoprotein; WBC: white blood cells; BMI: body mass index.

TABLE 2. ECHOCARDIOGRAPHIC AND SURGICAL CHARACTERISTICS OF THE GROUPS.

Variables	Postoperative sinus rhythm (n = 130)	Postoperative Atrial fibrillation (n = 50)	p-value
In-hospital mortality, n(%)	4 (3.1%)	2 (4.0%)	0.757
SYNTAX score	29.98 ± 8.45	34.74± 5.27	0.039
Graft number	2.56 ± 0.81	2.74± 0.72	0.174
Cardiopulmonary bypass time, minutes	78.84 ± 27.41	83.76 ± 34.71	0.321
Aortic cross clamp time, minutes	47.61 ± 19.09	46.31 ± 16.86	0.674
Left ventricular ejection fraction, %	56.54 ± 9.46	49.27 ± 11.94	<0.001
Left ventricular diastolic diameter, mm	45.80 ± 5.47	47.29 ± 8.46	0.168
Left ventricular systolic diameter, mm	29.67 ± 6.85	31.42 ± 5.86	0.120
Interventricular septum diameter, mm	11.05 ± 3.24	11.30 ± 3.51	0.652
Posterior wall thickness, mm	10.47 ± 2.28	0.97 ± 2.28	0.590
Left atrial diameter, mm	39.09 ± 6.20	41.26± 7.85	0.056
Systolic pulmonary artery pressure, mmHg	26.65 ± 9.08	28.26 ± 6.68	0.257

Data are given as mean ± SD, n, or median (interquartile range).

TABLE 3. ELECTROCARDIOGRAPHIC FEATURES OF THE GROUPS.

Variables	Postoperative sinus rhythm (n = 130)	Postoperative Atrial fibrillation (n = 50)	p-value
Sinus rhythm	84 (64.6%)	50 (18.2%)	0.072
Left bundle branch block	5 (3.8%)	4 (8.0%)	0.252
Left anterior fascicular block	24 (18.2%)	13 (26.0%)	0.265
Left posterior fascicular block	0	0	
Right bundle branch block	12 (9.2%)	2 (4.0%)	0.241
Right bundle branch block + Left anterior fascicular block	1 (0.8%)	1 (2.0%)	0.480
Left ventricular hypertrophy	4 (3.1%)	6 (12.0%)	0.019
QT, ms	361.3 ± 32.3	368.5 ± 29.9	0.174
QTc, ms	400.2 ± 34.4	404.2 ± 23.4	0.452
Tpe, ms	81.6 ± 12.1	84.8 ± 16.0	0.150
Tpe/QTc	0.20 ± 0.04	0.21 ± 0.04	0.481
f(QRS)-T (°)	57.9 ± 17.5	71.1 ± 13.6	<0.001

Data are given as mean ± SD, n, or median (interquartile range). QTc: corrected QT interval; f(QRS)-T: frontal QRS-T angle

TABLE 4. LABORATORY PARAMETERS OF THE STUDY GROUPS.

Variables	Postoperative sinus rhythm (n = 130)	Postoperative Atrial fibrillation (n = 50)	p-value
Glucose, mg/dL	142.54 ± 67.23	161.46 ± 80.35	0.121
Creatinine, mg/dL	1.09 ± 0.33	1.14 ± 0.29	0.318
Sodium, mmol/l	136.1 ± 2.7	138 ± 3.1	0.696
Potassium, mmol/l	3.91 ± 0.36	3.87 ± 0.41	0.731
Magnesium, mg/dL	1.73 ± 0.24	1.65 ± 0.43	0.534
WBC,10^3/mm^3	8.89 ± 4.27	8.46 ± 2.44	0.419
Hemoglobin, g/dL	13.77 ± 1.85	13.74 ± 1.77	0.922
Platelet, 10^3/mm^3	232.34 ± 63.08	227.64 ± 72.84	0.669
Hs-CRP, mg/L	6.24 ± 4.85	11.24 ± 7.54	0.026
Total cholesterol, mg/dL	196.33 ± 48.11	196.8 ± 45.54	0.798
LDL-C, mg/dL	129.58 ± 51.06	126.26 ± 38.45	0.749
HDL-C, mg/dL	42.14± 10.72	41.22 ± 9.45	0.675
Triglyceride, mg/dL	147.30 ± 59.66	150.50 ± 62.10	0.798

Data are given as mean ± SD, n, or median (interquartile range). HDL, high-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; WBC, white blood cells.

TABLE 5. MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR PREDICTING POAF.

	Univariable OR (95% CI)	P value	Multivariable OR (95% CI)	p-value
Age	1.081 (1.040 - 1.124)	< 0.001	1.068 (1.017 - 1.122)	0.008
Hypertension	3.717 (1.370 - 10.087)	0.010	1.156 (0.970 - 1.376)	0.064
SYNTAX score	1.290 (1.001 - 1.579)	0.066		
Left ventricular ejection fraction	0.939 (0.908 - 0.971)	< 0.001	0.939 (0.900 - 0.981)	0.004
Hs-CRP, mg/L	1.025 (1.002 - 1.049)	0.032	1.016 (0.983 - 1.050)	0.341
f(QRS)-T	1.051 (1.028 - 1.076)	< 0.001	1.079 (1.005 - 1.153)	< 0.001
Left ventricular hypertrophy	4.295 (1.158 - 15.934)	0.029	8.368 (1.657 - 42.255)	0.041

CI: confidence interval; OR: odds ratio; Hs-CRP: high-sensitivity C-reactive protein; f(QRS)-T: frontal QRS-T angle.

As determined in our study, common myocardial ischemia associated with a high SYNTAX score is one of the causes of POAF[2]. Zhang et al.[14] detected that an increase in the f(QRS-T) angle was associated with myocardial ischemia and found that the f(QRS-T) angle was normalized after successful revascularization therapy. An increased f(QRS-T) angle may be indicative of total ischemic load in POAF patients.

It is a well-known fact that inflammation is associated with cardiovascular diseases[15]. Indicators of electrocardiographic ventricular repolarization have been found to be correlated with systemic inflammation in a study[16]. Increased inflammatory activity may be responsible for the pathogenesis of arrhythmia either by direct arrhythmogenic effects by locally activating complements or by inducing oxidative stress and apoptosis[17]. POAF has also been linked to increased inflammatory activity and oxidative stress[18]. We have found higher Hs-CRP in patients with POAF compared to patients with sinus rhythm. Thus, an abnormal f(QRS-T) angle caused by systemic inflammation may be an explanation for patients with POAF.

CONCLUSIONS

The measurement of the f(QRS-T) angle, which is a basic and low-cost parameter, may allow physicians to predict POAF after CABG surgery. This novel parameter is more reliable, consistent, and less susceptible to false calculation and definition than other traditional electrocardiographic myocardial repolarization parameters. This may allow physicians to take tighter precautions and modify risk factors in advance by predicting POAF development. However, further studies are needed to determine the relationship between POAF and the f(QRS-T) angle.

Limitations of the study

The present study has a cross-sectional design with a relatively small sample size. We do not have data on major adverse cardiovascular events during follow-up.

Author's Contribution

Mevlüt Serdar Kuyumcu - Methodology, writing of the original draft; Dinçer Uysal - Data curation, investigation; Mustafa Bilal Özbay - Writing of the original draft; Oğuz Aydın, Erdoğan İbrişim - Supervision.

RESUMO

OBJETIVO: *A fibrilação atrial pós-operatória de início recente (Poaf) é a arritmia mais comum após a cirurgia de revascularização do miocárdio (CABG) e associada a hospitalização prolongada, acidente vascular cerebral e mortalidade. O ângulo QRS-T [f(QRS-T)] do plano frontal, que é definido como o ângulo entre as direções da despolarização ventricular (eixo-QRS) e repolarização (eixo-T), é um novo marcador da heterogeneidade da repolarização ventricular. O ângulo f(QRS-T) está associado a desfechos cardíacos adversos. À luz desses achados, neste estudo, objetivamos investigar a relação potencial entre o ângulo f(QRS-T) e a Poaf.*

MÉTODOS: *Cento e oitenta pacientes submetidos a CABG entre agosto de 2017 e setembro de 2018 foram incluídos no estudo retrospectivamente. Dois grupos foram estabelecidos como pacientes com ritmo sinusal pós-operatório (n=130) e com Poaf (n=50). O ângulo f(QRS-T) e todos os dados foram comparados entre os grupos.*

RESULTADOS: *Ângulo f(QRS-T) (p<0,001), escore Syntax (p=0,039), níveis séricos de PCR de alta sensibilidade (p=0,026), idade média (p<0,001), taxa de hipertrofia ventricular esquerda eletrocardiográfica (LVH) (p=0,019) e taxa de hipertensão (p=0,007) foram maiores; a fração de ejeção média do ventrículo esquerdo (LVEF) (p<0,001) foi menor no grupo com Poaf. As análises de regressão logística multivariável demonstraram que menor LVEF (p=0,004), LVH (p=0,041), maior idade (p=0,008) e maior ângulo f(QRS-T) (p<0,001) foram independentemente associados à Poaf.*

CONCLUSÕES: *Níveis de ângulo altos f(QRS-T) estão intimamente associados à Poaf. O ângulo f(QRS-T) pode ser um indicador potencial de Poaf.*

PALAVRAS-CHAVE: *Fibrilação atrial. Ponte de artéria coronária. Revascularização miocárdica. Complicações pós-operatórias. Eletrocardiografia.*

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ORIGINAL ARTICLE

Smoking and comorbidities are associated with COVID-19 severity and mortality in 565 patients treated in Turkey: a retrospective observational study

Tayfun Caliskan[1]
Bengu Saylan[2]

1. Assistant Professor, Department of Pulmonology, Sultan 2.Abdulhamit Han Training and Research Hospital, Health Sciences University, Istanbul, Turkey.
2. Pulmonologist, Department of Pulmonology, Sultan 2.Abdulhamit Han Training and Research Hospital, Health Sciences University, Istanbul, Turkey.

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SUMMARY

OBJECTIVE: *We aimed to explore the prevalence of smoking rates and comorbidities and evaluate the relationship between them and disease severity and mortality in inpatients with COVID-19.*

METHODS: *COVID-19 patients were divided into the following groups: clinic group, intensive care unit (ICU) group, survivors, and non-survivors. Non-COVID-19 patients were included as a control group. The groups were compared.*

RESULTS: *There was no difference between patients with and without COVID-19 in terms of smoking, asthma, diabetes, dementia, coronary artery disease (CAD), hypertension, chronic renal failure and arrhythmia (p>0.05). Older age (Odds ratio (OR), 1.061; 95% confidence interval (CI): 1.041-1.082; p< 0.0001), chronic obstructive pulmonary disease (COPD) (OR, 2.775; 95% CI: 1.128-6.829; p=0.026) and CAD (OR, 2.696; 95% CI: 1.216-5.974; p=0.015) were significantly associated with ICU admission. Current smoking (OR, 5.101; 95% CI: 2.382-10.927; p<0.0001) and former smoking (OR, 3.789; 95% CI: 1.845-7.780; p<0.0001) were risk factors for ICU admission. Older age (OR; 1.082; 95% CI: 1.056-1.109; p<0.0001), COPD (OR, 3.213; 95% CI: 1.224-8.431; p=0.018), CAD (OR, 6.252; 95% CI: 2.171-18.004; p=0.001) and congestive heart failure (CHF) (OR, 5.917; 95% CI 1.069-32.258; p=0.042), were significantly associated with mortality. Current smoking (OR, 13.014; 95% CI: 5.058-33.480; p<0.0001) and former smoking (OR, 6.507; 95% CI 2.731-15.501; p<0.0001) were also risk factors for mortality.*

CONCLUSION: *Smoking, older age, COPD, and CAD were risk factors for ICU admission and mortality in patients with COVID-19. CHF was not a risk factor for ICU admission; however, it was a risk factor for mortality.*

KEYWORDS: *Smoking, Mortality, COVID-19, Comorbidity, Severity*

INTRODUCTION

The COVID-19 pandemic, caused by SARS-CoV-2 is still ongoing and has caused hundreds of thousands of deaths worldwide. The lack of currently approved treatment and vaccination makes it difficult to fight against the disease. Identifying and understanding the risk factors for mortality and poor prognosis of the disease will determine the clinical approach and treatments to be applied to patients.

In 2019, around a fourth (24.9%) of the global population (both sexes combined) aged 15 years and older

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CORRESPONDING AUTHOR: Tayfun Caliskan
Department of Pulmonology, Sultan 2. Abdulhamit Han Training and Research Hospital, Health Sciences University
Tibbiye Avenue, Selimiye Street, Uskudar, Istanbul, Turkey – 34688
Tel: +90 021 6542-2020
E-mail: drtcaliskan@yahoo.com

were current users of some form of tobacco, and more than 8 million people died from tobacco use including both tobacco smoking and smokeless tobacco use in 2017.[1] Active smoking has been reported to increase the risk of viral respiratory diseases.[2] Smokers have an increased risk for community-acquired pneumonia.[3] About 20% of COVID-19 patients develop pulmonary infiltrates and some of these will develop very severe disease, hypoxia, and progression to ARDS.[4] The angiotensin-converting enzyme (ACE-2) protein, which is expressed on the surface of lung type-2 pneumocytes, is an entry receptor for SARS-CoV-2.[4] ACE-2 gene expression and is higher in smokers than in non-smokers, which may explain the increased risk of severe COVID-19 in this population.[5] On the other hand, Lippi et al. reported that smoking was not associated with COVID-19 severity.[6]

Comorbidities were associated with the severity of COVID-19.[7] The fatality rate also increased in patients with comorbidities.[8] Older age, male sex, and comorbidities including chronic cardiac disease, non-asthmatic chronic pulmonary disease, chronic kidney disease, liver disease, and obesity were associated with higher in-hospital mortality.[9] There are conflicting results in studies for the prevalence of comorbidities and the factors affecting COVID-19 severity and mortality. The interpretation of the effects of smoking on COVID-19 is difficult, because one of the factors affecting smoking status is gender, and cardiovascular diseases are more common in smokers. Combined hormonal contraception with other risk factors increases venous thromboembolism risk, especially in patients hospitalized with COVID-19.[10] Menopausal hormone therapy also affects the risk of cardiovascular diseases.[11]

We aimed to explore the prevalence of smoking rates and comorbidities and evaluate the relationship between smoking and comorbidities with disease severity and mortality in inpatients with and without COVID-19 in this study.

METHODS

This retrospective observational study was conducted in Sultan 2, in the Abdulhamit Han Training& Research Hospital, which has also been a COVID-19 reference center during the pandemic, in Istanbul, Turkey, between March 15 and May 10, 2020. The study was approved by the local ethics committee (179/May 12, 2020). Adult patients (≥18 years old) diagnosed with COVID-19 by polymerase chain reaction (PCR) and whose COVID-19 diagnosis was based on clinical, laboratory, and radiological findings, especially with chest computed tomography findings, despite COVID-19 PCR negativity were included. Non-COVID-19 patients who were hospitalized in the department of pulmonology due to diseases other than COVID-19 were included as the control group. The data were extracted from electronic medical records of the hospital. Age, smoking status, comorbidities including hypertension, coronary artery disease (CAD), arrhythmia, congestive heart failure (CHF), diabetes, chronic obstructive pulmonary disease (COPD), asthma, dementia, and chronic renal failure (CRF) were recorded based on patients' self-reporting or information received from patient relatives. The smoking status was classified as current smoker, former smoker, and never smoked. Former smokers were patients who reported having smoked ≥100 cigarettes during their lifetime and were not smoking for at least the past three months. The patients with COVID-19 were divided into two groups: a clinic group and an intensive care unit (ICU) group. In addition, the patients were divided into two groups: survivors and non-survivors. The groups were compared in terms of smoking, age, and comorbidities.

Patient data collected in the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 21.0 package program (Statistical Package for the Social Sciences, Chicago, IL, USA). Discrete data is given as frequency and percentage. The mean ± standard deviation for continuous data is given as descriptive value. The Mann Whitney U-test was used to compare the two groups. The Pearson Chi-Square Test and Fisher's Exact Test were used to compare the two categorical groups. Logistic Regression Analysis was used to examine the risk factors for mortality and intensive care unit admission. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

The study included 565 patients hospitalized with COVID-19 and 248 patients hospitalized with non-COVID-19 diseases. The mean ages of the patients with and without COVID-19 were 48.0 ± 19.7 and 52.0 ± 21.1 (Table 1). There was no statistically significant difference between patients with and without COVID-19 in terms of smoking, asthma, diabetes, dementia,

CAD, hypertension, CRF, and arrhythmia (p>0.05). Non-COVID-19 patients were older than COVID-19 patients (p = 0.014). COPD and CHF were more common in non-COVID-19 patients than in patients with COVID-19 (18.9%, 9.6% vs. 6.5%, 2.5%, p <0.001). Hypertension (22.7%), diabetes (12.7%), CAD (7.4%) and COPD (6.5%), were the most common comorbidities in patients with COVID-19. The ICU group had a significantly higher proportion of patients with current and former smoking than the clinic group (24.2%, 37.4%, vs. 20.3%, 9.5%, p <0.0001) (Table 2). The patients in the ICU group were significantly older than those in the clinic group (p <0.0001). COPD, diabetes, dementia, CAD, hypertension, CRF, and arrhythmia were found to be significantly more common in the ICU group than in the clinic group (p<0.05). There was no difference between the two groups for asthma and CHF (p>0.05). The results of the logistic regression analysis demonstrated that older age [Odds ratio (OR), 1.061; 95% confidence interval (CI): 1.041-1.082; p < 0.0001], COPD (OR, 2.775; 95% CI: 1.128-6.829; p = 0.026), and CAD (OR, 2.696; 95% CI: 1.216-5.974; p = 0.015) were significantly associated with ICU admission (Table 3). Current smoking (OR, 5.101; 95% CI: 2.382-10.927; p < 0.0001) and former smoking (OR, 3.789; 95% CI: 1.845-7.780; p < 0.0001) were risk factors for ICU admission. Diabetes, dementia, hypertension, CRF, and arrhythmia were not significantly associated with ICU admission.

Non-survivors were older than survivors (p<0.0001) (Table 2). Current smokers and former smokers were more common in non-survivors (p<0.0001). COPD,

TABLE 1. COMPARISON BETWEEN PATIENTS WITH AND WITHOUT COVID-19

	COVID-19 (N= 565) N (%)	Non-COVID-19 (N=248) N (%)	p
Age, median (IQR)	48 ± 19.664	52 ± 21.105	0.014
Current smoker	118 (20.9)	47 (18.9)	0.738
Former smoker	79 (14.0)	33 (13.3)	
Never smoked	368 (65.1)	169 (67.9)	
COPD	37 (6.5)	47 (18.9)	<0.0001
Asthma	21 (3.7)	8 (3.2)	0.839
Diabetes	72 (12.7)	41 (16.5)	0.186
Dementia	12 (2.1)	3 (1.2)	0.572
CAD	42 (7.4)	11 (4.4)	0.124
Hypertension	128 (22.7)	53 (21.3)	0.715
Chronic renal failure	12 (2.1)	9 (3.6)	0.234
CHF	14 (2.5)	24 (9.6)	<0.0001
Arrhythmia	14 (2.5)	8 (3.2)	0.639

IQR: interquartile range; COPD: Chronic pulmonary obstructive disease; CAD: Coronary artery disease; CHF: Congestive heart failure.

TABLE 2. SMOKING STATUS AND COMORBIDITIES OF THE PATIENTS WITH COVID-19

	Clinic (N= 474) N (%)	ICU (N=91) N (%)	p	Survivors (N= 490) N (%)	Non-survivors (N=75) N (%)	p
Current smoker	96 (20.3)	22 (24.2)	<0.0001	97 (19.8)	21 (28.0)	<0.0001
Former smoker	45 (9.5)	34 (37.4)		48 (9.8)	31 (41.3)	
Never smoked	333 (70.3)	35 (38.5)		345 (70.4)	23 (30.7)	
Age, median (IQR)	44 (26-57)	69 (58-79)	<0.0001	44 (27-57)	70 (62-80)	<0.0001
COPD	12 (2.5)	25 (27.5)	<0.0001	12 (2.4)	25 (33.3)	<0.0001
Asthma	17 (3.6)	4 (4.4)	0.761	17 (3.5)	4 (5.3)	0.506
Diabetes	49 (10.3)	23 (25.3)	<0.0001	54 (11.0)	18 (24.0)	0.004
Dementia	7 (1.5)	5 (5.5)	0.030	7 (1.4)	5 (6.7)	0.013
CAD	22 (4.6)	20 (22.0)	<0.0001	24 (4.9)	18 (24.0)	<0.0001
Hypertension	81 (17.1)	47 (51.6)	<0.0001	88 (18.0)	40 (53.3)	<0.0001
Chronic renal failure	6 (1.3)	6 (6.6)	0.006	7 (1.4)	5 (6.7)	0.013
CHF	9 (1.9)	5 (5.5)	0.059	9 (1.8)	5 (6.7)	0.027
Arrhythmia	8 (1.7)	6 (6.6)	0.015	8 (1.6)	6 (8.0)	0.006

ICU: Intensive care unit; IQR: interquartile range; COPD: Chronic pulmonary obstructive disease; CAD: Coronary artery disease; CHF: Congestive heart failure.

diabetes, dementia, CAD, hypertension, CRF, CHF, and arrhythmia were significantly more common among non-survivors than in survivors (p<0.05). There was no difference between non-survivors and survivors in terms of asthma (p>0.05). The logistic regression analysis indicated that older age (OR; 1.082; 95% CI: 1.056-1.109; p<0.0001), COPD (OR, 3.213; 95% CI: 1.224-8.431; p = 0.018), CAD (OR, 6.252; 95% CI: 2.171-18.004; p = 0.001), and CHF (OR, 5.917; 95% CI 1.069-32.258; p = 0.042), were significantly associated with mortality (Table 3). Current smoking (OR, 13.014; 95% CI: 5.058-33.480; p< 0.0001) and former smoking (OR, 6.507; 95% CI 2.731-15.501; p<0.0001) were risk factors for mortality. Diabetes, dementia, hypertension, CHF, and arrhythmia were not significantly associated with mortality (p>0.05).

DISCUSSION

In this study, the prevalence of comorbidities and the factors affecting COVID-19 prognosis were examined. Revealing these risk factors can be decisive and guide clinicians towards the effective treatment and proper management of patients with COVID-19.

There was no difference in smoking between COVID-19 and non-COVID-19 patients. The rate of active smokers and former smokers were significantly higher among patients who were admitted to the ICU. Current smoking was associated with 5-fold and 13-fold increased risks respectively for ICU admission and mortality in the present study. Former smokers were 3.7 times more likely to be admitted to ICU and 6.5 times more likely to die than non-smokers. Lippi et al. reported that smoking did not affect the severity of COVID-19.[6] However, active cigarette smoking was associated with the risk of severe COVID-19 in the re-analysis of the data.[12] In studies conducted in COVID-19 patients in China, smoking rates were lower than among the general population.[13] This may be related to the inability to receive patient data, record data, and health system deficiencies during the pandemic. In the study by Zhou F et al., there was no difference between patients who recovered and died in terms of active smoking (4% vs. 9% p = 0.21).[14] Conversely, Guan W et al. indicated that the smoking rate was higher in patients with severe COVID-19 patients.[15]

COPD was more common in non-COVID-19 patients than in COVID-19 patients, but this was due to the fact that patients were hospitalized in the department of pulmonology. In a meta-analysis, patients with COPD had a 5-fold increased risk of severe COVID-19.[16] Patients with COPD have also a higher risk for in-hospital mortality due to COVID-19.[17] The results of the present study were compatible with previous reports, and COPD was a risk factor for ICU admission and mortality. The increased risk for severe COVID-19 in patients with asthma has not been proven in studies yet. The prevalence of asthma in COVID-19 patients in China was lower than in the general population.[18] Asthma was not a risk factor for ICU admission and mortality in the present study.

Hypertension was reported to be associated with a 2.5-fold increased risk for COVID-19 severity and mortality, especially in the elderly.[19] The mortality risk of hypertensive COVID-19 patients was increased, with

TABLE 3. MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF VARIABLES RELATED TO ICU ADMISSION AND MORTALITY

Risk factor	For ICU admission		For mortality	
	OR (%95 CI)	P-value	OR (%95 CI)	P-value
Former smoker	3.789 (1.845-7.780)	<0.0001	6.507 (2.731-15.501)	<0.0001
Current smoker	5.101 (2.382-10.927)	<0.0001	13.014 (5.058-33.480)	<0.0001
Age	1.061 (1.041-1.082)	<0.0001	1.082 (1.056-1.109)	<0.0001
COPD	2.775 (1.128-6.829)	0.026	3.213 (1.224-8.431)	0.018
Diabetes	1.152 (0.553-2.396)	0.706	1.037 (0.445-2.421)	0.932
Dementia	1.249 (0.315-4.952)	0.751	1.762 (0.408-7.607)	0.448
CAD	2.696 (1.216-5.974)	0.015	6.252 (2.171-18.004)	0.001
Hypertension	1.571 (0.818-3.016)	0.175	1.458 (0.690-3.079)	0.323
Chronic renal failure	3.685 (0.983-13.811)	0.053	3.982 (0.939-16.891)	0.061
CHF	NA	NA	5.917 (1.069-32.258)	0.042
Arrhythmia	1.649 (0.446-6.097)	0.453	2.804 (0.689-11.412)	0.150

ICU: Intensive care unit; OR: Odds ratio; CI: Confidence interval; COPD: Chronic pulmonary obstructive disease; CAD: Coronary artery disease; CHF: Congestive heart failure; NA: Not applicable.

an OR of 3.36.[20] Although hypertension was the most common comorbidity in the present study, it was not a risk factor for ICU admission and mortality. According to this study, arrhythmia was not a risk factor for ICU admission and mortality. CAD was a risk factor for both ICU admission and mortality, and CHF was not a risk factor for ICU admission but was a risk factor for mortality in the present study. Non-COVID-19 patients were older patients who needed inpatient treatment; therefore CHF was more common in non-COVID-19 patients than in COVID-19 patients. Cardiac injury was a common comorbidity in COVID-19 patients treated in a hospital in Wuhan and increased mortality.[21] ACE-2 levels were higher in patients with cardiovascular disease.[22] On the other hand, SARS-CoV-2 can reduce ACE-2, causing excessive accumulation of angiotensin II, which induces myocarditis and acute respiratory distress syndrome.[22]

In a previous study, diabetes was associated with COVID-19 severity and mortality, and this was particularly evident in younger and less hypertensive patients.[23] Diabetes was the second most common comorbidity in the present study but it was not a risk factor for ICU admission and mortality. CRF has been associated with an increased risk of severe COVID-19.[24] Another study concluded that renal disease did not increase the risk of COVID-19.[25] CRF was not a risk factor for ICU admission and mortality in this study. Patients with Alzheimer's disease and related dementias have an increased risk of COVID-19.[26] Therefore, concerns have arisen regarding these patients during the COVID-19 pandemic. Dementia was not a risk factor for ICU admission and mortality in the present study. It has been reported that in-hospital mortality was higher in patients over 65 years old with COVID-19.[17] Older age was also a risk factor for ICU admission and mortality in the present study.

The most important limitation of this study was its retrospective design. Therefore, data about patients' medical records in the hospital system were used. Comorbidities were also checked from the e-pulse system to prevent a lack of data. Missing data about smoking status was completed by contacting the patient or relatives by phone.

CONCLUSION

Smoking, older age, COPD, and CAD were risk factors for ICU admission and mortality in inpatients treated for COVID-19. Asthma, diabetes, dementia, hypertension, CRF, and arrhythmia were not associated with ICU admission and mortality. CHF was not a risk factor for ICU admission; however, it was a risk factor for mortality.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethics

Informed consent was waived because of the retrospective nature of the study. This study was approved by the Ethics Committee of the Umraniye Training and Research Hospital (No: 179, Date: May 12, 2020).

Author's Contribution

TC, BS designed the study; BS collected the data; TC performed the literature review; BS performed the statistical analysis; TC wrote the paper; TC, BS performed the critical review of the manuscript.

RESUMO

OBJETIVO: *Buscamos explorar as taxas de prevalência de tabagismo e de comorbidades e avaliar a relação entre elas e a severidade e mortalidade da doença em pacientes hospitalizados com COVID-19.*

MÉTODOS: *Pacientes com COVID-19 foram divididos nos seguintes grupos: grupo clínico, grupo da unidade de terapia intensiva (UTI), grupo de sobreviventes e não-sobreviventes. Pacientes sem COVID-19 foram incluídos em um grupo de controle. Os grupos foram comparados.*

RESULTADOS: *Não houve diferença entre os pacientes com e sem COVID-19 em termos de tabagismo, asma, diabetes, demência, doença arterial coronariana (DAC), hipertensão arterial, insuficiência renal crônica e arritmia (p>0,05). Idade mais avançada (odds ratio (OR), 1,061; 95% de intervalo de confiança (IC): 1,041-1,082; p< 0,0001), doença pulmonar obstrutiva crônica (DPOC) (OR, 2,775; 95% IC: 1,128-6,829; p=0,026) e DAC (OR, 2,696; 95% IC: 1,216-5,974; p=0,015) estavam significativamente associados com a admissão na UTI. O tabagismo atual (OR, 5,101; 95% IC: 2,382-10,927; p < 0,0001) e tabagismo prévio (OR, 3,789; 95% IC: 1,845-7,780; p< 0,0001)*

foram fatores de risco para admissão na UTI. Idade mais avançada (OR; 1,082; 95% IC: 1,056-1,109;< 0,0001), DPOC (OR, 3,213; 95% IC: 1,224-8,431; p=0,018), DAC (OR, 6,252; 95% IC: 2,171-18,004; p=0,001) e insuficiência cardíaca congestiva (ICC) (OR, 5,917; 95% IC 1,069-32,258; p=0,042) estavam significativamente associados com mortalidade. O tabagismo atual (OR, 13,014; 95% IC: 5,058-33,480; p<0,0001) e o tabagismo prévio (OR, 6,507; 95% IC 2,731-15,501; p<0,0001) também foram fatores de risco para mortalidade.

CONCLUSÃO: *O tabagismo, a idade avançada, DPOC e DAC foram fatores de risco para admissão na UTI e mortalidade em pacientes com COVID-19. ICC não foi um fator de risco para admissão na UTI; no entanto, foi um fator de risco para mortalidade.*

PALAVRAS-CHAVE: *Tabagismo, Mortalidade, COVID-19, Comorbidade, Gravidade*

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ORIGINAL ARTICLE

Is ionizing radiation a risk factor for anxiety in employees?

Aslı Kurtar Mansiroglu[1]
Murat Erer[2]
Mehmet Coşgun[1]
Isa Sincer[1]
Yılmaz Güneş[1]

1. Abant Izzet Baysal University, Faculty of Medicine, Department of Cardiology, Bolu, Turkey
2. Kırıkkale University, Faculty of Medicine, Department of Cardiology, Kırıkkale, Turkey

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SUMMARY

OBJECTIVE: *Workers describe many physical and mental symptoms when working in radiation areas. This study aimed to assess these symptoms in radiation workers using the Beck Anxiety Inventory (BAI).*

METHODS: *A total of 42 radiation workers (22 males and 20 females, mean age 34±7 years) and 47 control subjects (22 males and 27 females, mean age 31± 8 years) who work in non-radiation areas in the hospital were included in the study. All participants anonymously filled out the Beck Anxiety Inventory (BAI) questionnaire.*

RESULTS: *The demographic data of workers were not significantly different between groups. In the BAI, the dizzy or lightheaded (p =0.01), terrified (p= 0.01), unsteady (p=0.02), heart-pounding and racing (p=0.02) items were significantly higher in the radiation-exposed group compared to the control group. |The BAI score was also significantly higher in the radiation-exposed group (11.1±6.8 vs. 8.7±3.8, p =0.04)*

CONCLUSION: *These results suggest the possibility that radiation may play a role in the psychometric properties of workers. The effects of radiation on the health of employees need to be further investigated and understood.*

KEYWORDS: *Radiation, Ionizing. Anxiety. Psychological Tests. Stress, psychological/etiology. Occupational diseases.*

INTRODUCTION

Radiation is an important part of both the diagnosis and treatment management of many diseases in medicine.

The types of radiation are alpha, beta, gamma, and x-rays, and one can be exposed to exposed radiation externally and internally. Ionizing radiation has sufficient energy to affect the atoms in cells and thereby damage their genetic material (DNA)[1,2]. If this damage is not repaired correctly, a cell may die or eventually become cancerous[1]. Exposure to radiation can have acute health effects, such as skin burns, and long-term health effects, such as cancer and cardiovascular disease. Radiation effects have been shown by numerous studies in the literature, particularly in radiotherapy patients, atomic bomb survivors, and radiation industry workers[3].

Anxiety is a very common health problem in every part of society. Anxiety is seen in all humans, but

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CORRESPONDING AUTHOR: Asli Kurtar Mansiroglu
Abant Izzet Baysal University Hospital, Department of Cardiology, Golkoy, 14280, Bolu, Turkey; Phone: +903742534656; Fax: +903742534615
E-mail: dr.asli.kurtar@gmail.com

the level of anxiety may change with some triggers. Although many scales are used in the literature to determine the level of anxiety, the Beck Anxiety Inventory (BAI) is one of the most commonly used. Since the test construction by Beck and colleagues in 1988, it has been employed in numerous empirical studies[4]. BAI is used to evaluate patients' psychological levels of anxiety. Patients describe how frequently the symptoms have been true for them during the past 2 weeks, including the test day.

The level of job-related stress is determined by many factors including age, sex, working area, workload, working hours, and individual aspects. Anxiety and stress levels among hospital workers have been studied in the literature[5-7]. However, there is limited data on depression and anxiety in radiation workers.

The effects of radiation on depression and anxiety in radiotherapy patients and seasonal global radiation differ in the literature[8-10].

Radiotherapy patients have common complaints such as drowsiness, headache, hand tremor, palpitation, dormancy, general weakness, discomfort, and flushing on therapy days[11-14]. Although there is no study available in the literature, in our daily practice we observe similar symptoms among invasive cardiologists and radiologists. This observation was the basis of our study.

This study aimed to compare the anxiety levels among radiation workers and non-radiation workers using the BAI and to attract attention to these complaints.

METHODS

This is a two-centered cross-sectional study conducted between June and December 2019 at the Bolu University Hospital and Kırıkkale University Hospital.

A total of 42 radiation workers (38 of them were interventional cardiologists and catheterization lab staff, 4 of them were interventional radiologists) and 47 control subjects matched for age and sex working in non-radiation areas were included in the study.

We received informed consent from each participant after approval of the study protocol by the Local Ethics Committees.

All participants filled out a self-reporting questionnaire; the Beck Anxiety Inventory. There were 21 questions in the test. In these questions, patients chose one of a total of 4 frequency scales: none, mild, moderate, severe. Each marked option had a score: the 'none' option was worth 0 points; 'mild', 1 point, 'moderate', 2 points; 'severe', 3 points. After marking 21 questions in this way, these points were added up and the total score was in the range of 0 to 63. Scores over 25 indicated severe anxiety, those from 16 to 25 indicated moderate anxiety, those from 8 to 15 indicated mild anxiety; those from 0 to 7 indicated no/minimal anxiety[15].

The presence of hypertension (HT) was considered when blood pressure was ≥140/90 mmHg or when there was the use of antihypertensive drugs. Smoking was understood as active smoking or a history of >10 years of smoking.

Exclusion criteria were as follows: absent consent, younger than 18 years old, having cognitive deficits, neurologic impairment and /or communicative disabilities, history of psychiatric diagnoses, or psychiatric drug use, and pregnancy.

Radiation workers undergo regular examinations in their institutions including blood tests, eye examination, dermatological examination, and thyroid ultrasound. No employee had a history of radiation accidents.

STATISTICAL ANALYSIS

The quantitative variables were presented as mean± standard deviation (SD), and qualitative variables were expressed in numbers and percentages. The differences between independent groups were analyzed by the Student *t*-test in the case of normal distribution. The Chi-Square test was used for the qualitative variables. Spearman's correlation analyses were used to assess the correlations of feeling heart-pounding or racing and unsteady with the radiation exposed group. A p-value < 0.05 was considered significant. Statistical analysis was carried out using the SPSS 18.0 Statistical Package Program for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

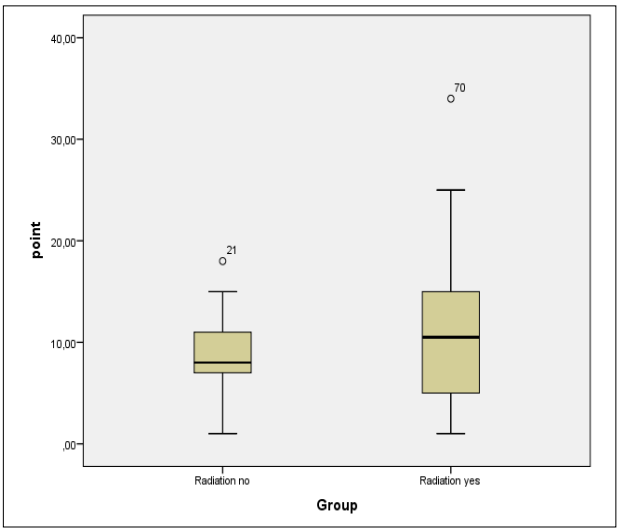
The final analyses in the present study included 89 workers; there were no significant differences between the radiation and control groups regarding demographic characteristics such as age, gender, presence of HT, and smoking (Table 1). There were 12 (28.6%) doctors, 16 (38.1%) technicians, and 14 (33.3%) nurses in the radiation worker group; in the non-radiation group, there were 7 (14.9%) doctors, 22 (46.8%) nurses, and 18 (38.3%)

TABLE 1. DEMOGRAPHIC CHARACTERISTICS AND BAI SCORE

Baseline characteristics	Radiation exposed (N=42)	Control (N=47)	p
Age (years) (Mean ±SD)	34±7	31± 8	0.12
Male/female	22/20	22/25	0.60
Hypertension (%)	4 (9%)	2 (4%)	0.32
Smoking (%)	16 (38%)	12 (25%)	0.20
Nightshifts (%) BAI Score (Mean ±SD)	38 (90%) 11.1±6.8	34 (72%) 8.7±3.8	0.03 0.04
BAI Items			
Numbness or tingling	21 (%50)	23 (49%)	0.58
Feeling hot	23 (55%)	19 (40%)	0.29
Wobbliness in legs	23 (55%)	20 (47%)	0.56
Unable to relax	18 (43%)	22 (47%)	0.32
Fear of the worst happening	13 (31%)	18 (43%)	0.39
Dizzy or lightheaded	31 (66%)	20 (48%)	0.01
Heart-pounding or racing	28 (66%)	15 (32%)	0.02
Unsteady	33 (%78)	25 (53%)	0.02
Terrified or afraid	38 (90%)	35 (74%)	0.01
Nervous	30 (71%)	39 (83%)	0.17
Feeling of choking	9 (21%)	14 (30%)	0.65
Hands trembling	17 (41%)	20 (43%)	0.88
Shaky or unsteady	8 (19%)	6 (13%)	0.48
Difficulty in breathing	9 (21%)	10 (21%)	0.56
Fear of dying	5 (12%)	8 (17%)	0.55
Scared	13 (28%)	12 (29%)	0.36
Indigestion or discomfort in the abdomen	20 (48%)	21 (66%)	0.26
Faint	8 (17%)	3 (7%)	0.11
Face flushed	20 (48%)	17 (%36)	0.50
Sweating	20 (48%)	13 (28%)	0.08
Fear of losing control	12 (25%)	8 (19%)	0.46

Significant at alpha p < 0.05. SD standard deviation

FIGURE 1. IN SPEARMAN'S CORRELATION TEST, THE HEART-POUNDING OR RACING (R=0.44, P=0.003) AND UNSTEADY (R=0.34, P=0.022) ITEMS WERE SIGNIFICANTLY CORRELATED WITH THE RADIATION-EXPOSED GROUP.



office staff. In the radiation group, 38 (90%) employees had nightshifts, while 4 (10%) employees did not. In the control group, 34 (72%) employees had nightshifts, while 13 (18%) employees did not.

Scores on the BAI for radiation and control groups are also summarized in Table 1. The total BAI score of the radiation-worker group (11.1±6.8) differed significantly from that of the non-radiation-worker group (8.7±3.8; *p*=0.04); also, there were significant group differences for several BAI subscales.

The BAI subscale scores for feeling dizzy or lightheaded (p=0.01), feeling heart-pounding or racing (p=0.02), feeling unsteady (p=0.02), and feeling terrified or afraid (p=0.01) were significantly higher in radiation workers than in those without radiation.

There were no significant differences on items relating to numbness or tingling, feeling hot, wobbliness in legs, being unable to relax, fearing the worst happening, feeling nervous, feeling of choking, hands trembling, shaky, difficulty in breathing, fear of dying, scared, indigestion or discomfort in the abdomen, faint, face flushed, or sweating.

DISCUSSION

In this study, we examined differences of anxiety levels using the BAI in working areas with or without radiation exposure at two university hospitals. We found that the frequencies of the dizzy or lightheaded, terrified, unsteady, heart-pounding, and racing items and the BAI score were significantly higher among health employees working in radiation areas.

Depression and anxiety are common health problems in society. The BAI is among the most used self-rating scales worldwide for measuring anxiety[4].

While many studies about job stress or anxiety have attracted attention, emotional or psychological events in employees who are exposed to ionizing radiation at work have not been well defined.

Studies show that radiation causes oxidative stress, sperm/testicular damage, apoptosis, cellular DNA damage, endocrine changes[2]. Radiation has effects on the cardiovascular, hematopoietic, reproductive, endocrine, nervous, respiratory, and gastrointestinal sysytems[2,16].

It is known that all forms of ionizing radiation have the potential to produce toxicity in the central

nervous system, and the mechanisms of neurotoxicity and neurodegeneration following ionizing radiation are poorly understood[11].

Ionizing radiation disrupts serotonin, norepinephrine, and dopamine neurotransmission, synthesis, and exchange of other neurotransmitters, which are responsible for the neurochemical effects of radiation[12,13].

Following radiation accidents, the incidence of stress-related disorders, anxiety, and depression have been reported to be significantly increased[12]. Also, recent studies showed that after an atomic bombing, the survivors got easily tired, lost interest in life, had a depressed mood, and were introverted and even autistic[14].

It is known that conventional radiotherapy that is applied to the temporal bone causes vestibular dysfunction in approximately 30% of patients, and the patients perceived dizziness[17]. The mechanism underlying vestibular dysfunction is cell death in the vestibular nerve following radiation exposure[18]. Also, studies show that the amount of radiation that interventional cardiologists or radiologists are exposed to the head area annually is approximately 10 times higher than the whole-body exposure, and the left side of the head is exposed two times more than the right side[19].

The conduction system may also be affected by radiation[20]. Radiation stimulates the sympathetic nervous system by increasing beta-adrenergic receptors[21]. Also, radiation-induced fibrosis can damage the conduction system[2]. These may be the mechanisms underlying the feeling of heart-pounding or racing in workers.

Feeling terrified and unsteady are some of the depressive symptoms. It has been shown that in radiotherapy patients and atomic bomb survivors depression and depressive symptoms are increased[8-10,12]. In our current study, these complaints were significantly higher among healthy employees working in radiation areas too.

Radiation causes different symptoms in workers. Since the dose is much less than the dose received in nuclear accidents, atomic bombing, or radiotherapy patients, it is more difficult to explain these symptoms in this group. However, these complaints by health employees working in radiation areas should be taken into consideration and should be searched in larger sized and longer follow-up protocols.

Limitations

There are some limitations to this study. We tried to choose employees from similar work environments, though there were some differences between the working conditions of the two groups. While all employees in the radiation group worked face to face and had voice to voice interactions with patients, some workers in the control group were working with patients inwards, while others were working in the office environment. In the control group, doctors, nurses, and the office staff were selected from the cardiology unit. Thus, we tried to match a similar working area, stress, and workload. While the daytime hours were similar between the two groups, there was a difference in nightshifts, and this is one of the limitations of our study.

CONCLUSION

This study carefully suggests that radiation is likely to be related to some complaints by radiation workers. In this study, we tried to document the neuropsychiatric effects of radiation among occupational workers using the BAI. It is recommended to organize the system of long-term neuropsychiatric care and control programs for radiation workers.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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None

Author's Contribution

Concept: Y.G., M.C.; Design: I.S.; Supervision: A.K.M.; Materials: M.C.; Data collection &/or processing: A.K.M., M.E.; Analysis &/or interpretation: I.S.; Literature search: A.K.M.

Writing: A.K.M.; Critical review – I.S., Y.G.

RESUMO

OBJETIVO: *Ao trabalhar em áreas de radiação, trabalhadores descrevem muitos sintomas físicos e mentais. Este estudo teve como objetivo avaliar esses sintomas em trabalhadores expostos à radiação utilizando a Escala de Ansiedade de Beck (BAI - Beck Anxiety Inventory).*

MÉTODOS: *Um total de 42 trabalhadores expostos à radiação (22 homens e 20 mulheres, com idade média de 34±7 anos) e 47 controles (22 do sexo masculino e 27 do sexo feminino, com idade média de 31±8 anos) que trabalham em áreas do hospital sem radiação foram incluídos no estudo. Todos os participantes responderam anonimamente ao questionário da BAI.*

RESULTADOS: *Os dados demográficos dos trabalhadores dos dois grupos não apresentaram diferenças significativas. Na BAI, os itens de tonturas ou vertigens (p =0,01), medo (p= 0,01), instabilidade (p=0,02) e batimento cardíaco mais forte e acelerado (p=0,02) foram significativamente mais elevados no grupo exposto à radiação em comparação ao grupo de controle. A pontuação da BAI também foi significativamente maior no grupo exposto à radiação (11,1 ± 6,8 versus 8,7 ± 3,8, p = 0,04).*

CONCLUSÃO: *Esses resultados sugerem a possibilidade de que a radiação pode desempenhar um papel importante nas propriedades psicométricas dos trabalhadores. Os efeitos da radiação na saúde dos funcionários precisam ser mais bem investigados e compreendidos.*

PALAVRAS-CHAVE: *Radiação Ionizante. Ansiedade. Testes Psicológicos. Estresse psicológico/etiologia. Doenças profissionais.*

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ORIGINAL ARTICLE

Evaluation of re-hospitalized COVID-19 patients in a hospital

Ensar Durmus[1]
Fatih Guneysu[1]

1. Sakarya University Training and Research Hospital Emergency Department, Sakarya, Turkey

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SUMMARY

PURPOSE: *This study intends to investigate the reasons for re-hospitalization, complaints, and prognoses of COVID-19 patients after being discharged.*

METHODS: *COVID-19 patients who were re-hospitalized at the Sakarya University Training and Research Hospital were examined. Reverse transcriptase-polymerase chain reaction (RT-PCR), tomography and laboratory results, demographic characteristics, and prognostic results were recorded retrospectively.*

RESULTS: *A total of 60 patients, including 26 males (43.3%) and 34 females (56.7%), with repeated admissions to the hospital for COVID-19 symptoms, were included in the study with a mean age of 56.9 (± 22.5) (median value = 61, age range = 3–88). The number of days of the second hospitalization was statistically significantly higher ($p < 0.05$). Patient age and number of days of hospitalization were strongly positively correlated ($p < 0.01$). A total of 11 patients (18%) had negative results in their first RT-PCR and subsequently tested positive in their second hospitalization. In addition, 10 (17.5%) of the patients who underwent thoracic tomography had unilateral involvement, 34 (59.6%) had bilateral involvement, and 13 (22.8%) had no significant results. Note that 4 (6.6%) of the patients re-hospitalized died in the hospital, while 56 (93.4%) were discharged once more. All of the four patients that died were female with a mean age of 81.5 years.*

CONCLUSION: *Particularly patients with advanced age and comorbidities should be examined more carefully when discharged; if their complaints are repeated, they should be advised to quickly contact the emergency service.*

KEYWORDS: *COVID-19, re-hospitalization, coronavirus*

INTRODUCTION

The disease caused by the SARS-CoV-2 virus, which is part of the family of Coronaviruses, has been named COVID-19 [1]. After being infected by the SARS-CoV-2 virus, the average incubation period is reported to be of 6.4 days (2.1–11.1 days) [2]. COVID-19 has a wide range of clinical manifestations, from symptoms such as asymptomatic disease and mild upper respiratory tract infection to severe pneumonia accompanied by respiratory failure, which might subsequently result in death [3]2019, Wuhan, China,

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CORRESPONDING AUTHOR: Ensar Durmuş
Sakarya Universitesi Egitim ve Arastırma hastanesi – Adapazarı –Sakarya – Turkey – 54100
E-mail: ensar.durmus@saglik.gov.tr

has experienced an outbreak of coronavirus disease 2019 (COVID-19. Some studies report hospitalizations of 19 days, while the average length of hospitalizations varies depending on the country [4]. There are healthcare centers that suggest patient discharge after ensuring that the patient meets criteria such as improvement in symptoms, improvement in check-up thoracic tomography, testing negative in at least two RT-PCR tests with an interval of 24h, and having no fever for at least three days[3]2019, Wuhan, China, has experienced an outbreak of coronavirus disease 2019 (COVID-19. Patients who are discharged from the hospital might then be admitted asymptomatically or symptomatically and require re-hospitalization [5]. This study intends to help clinical physicians estimate which COVID-19 patients might have a higher potential of re-hospitalization.

METHODS

The Sakarya University Training and Research Hospital (SUEAH) is a 1000-bed, fully equipped tertiary public hospital located in the Sakarya Province of Turkey and has served as a pandemic hospital since March 03, 2020.

After obtaining written permission from the chief physician's office, the patient data were collected through retrospective reviewing of patient files and the hospital information system covering between March 16 and May 8, 2020.

Thoracic tomography was then performed using Toshiba Alexion 16 tomography scanner as a low-dose non-contrast tomography.

Inclusion criteria: Re-hospitalized RT-PCR-positive COVID-19 patients and the hospital records of all age groups were examined and included in the study.

Exclusion criteria: Those re-hospitalized for reasons other than COVID-19, despite testing positive in the RT-PCR test or negative in the RT-PCR test while their tomography findings supported COVID-19 disease were excluded from the study.

Statistical analysis: The data obtained were statistically analyzed using SPSS 21. The Chi-square test was used to compare categorical data, and $p < 0.05$ was considered to be statistically significant. The Kolmogorov–Smirnov test was used to assess the normality of the data in terms of distribution. For the statistical assessment of non-normally distributed data, nonparametric tests were used. The Mann–Whitney U test was then used to compare two independent variables, while the Kruskal–Wallis H test was used to compare more than two independent variables; $p < 0.05$ was accepted to be statistically significant.

RESULTS

The number of patients re-hospitalized was 60; 26 of them (43.3%) were male and 34 (56.7%) were female. The mean age of the patients was 56.9 (± 22.5) and the age range was 3–88 years. Moreover, the mean length of the initial hospitalization was 4.6 days (±2.87 days). The mean time until re-hospitalization after discharge was 6.8 days (±4.75); however, no correlation was found between the time until re-hospitalization after discharge and the second hospitalization ($p > 0.05$). The patients had a mean length of the second hospitalization of 7.4 days (±6.8) and the duration of the stay was between 1 and 36 days. There was a moderate, positive, statistically significant correlation between the number of days of the first hospitalization and the number of days of the second hospitalization ($p < 0.05$). Accordingly, compared to the first hospitalization, the second one was longer in 34 patients (56.7%), shorter in 17 patients (28.3%), and the same in 9 patients (15%). The patients were then divided into three age groups, i.e., 0–17 years, 18–64 years, and >65 years. After analyzing the correlation between the age groups and their hospitalizations, there was a significant difference between the first hospitalization, the second hospitalization, and the total length of hospitalization ($p < 0.05$). According to the post-hoc analysis, there was a significant difference between the group of 0–17 years and the groups of 18–64 years and of >65 years in terms of second hospitalization ($p < 0.05$). Moreover, there was no significant difference in the age group of 18–64 years and the group of >65 years in terms of second hospitalization and the length of hospitalization ($p > 0.05$). Nevertheless, there was no significant correlation between patient age and the time until re-hospitalization after discharge ($p > 0.05$).

Note that 49 (81.7%) of these patients tested positive, and 11 (18.3%) tested negative in the RT-PCR tests during their first hospitalization. Of the 49 patients who tested positive in the RT-PCR tests, 22 were not tested again, 17 tested negative, and 10 tested positive again during their second hospitalization. The first RT-PCR result was negative in 11 patients (18%), who subsequently tested positive in

their second hospitalization. Of the total number of patients, 57 (95%) had CT. Note that for 17 patients (28.3%), CT was performed only during their first admission; for 39 patients (65%), it was during both first and second hospitalizations; and for one patient (1.7%) it was performed during their second admission. Of the patients who underwent thorax CT, 10 patients (17.5%) had unilateral involvement, 34 (59.6%) had bilateral involvement, and 13 (22.8%) had no significant findings. There was a significant difference between those without significant CT findings and those with bilateral involvement in terms of initial hospitalization ($p < 0.05$).

Note that 4 (6.6%) of the re-hospitalized patients died in the hospital, and all of them were female. They had an age range of 73–86 years and a mean age of 81.5 years and bilateral involvement in their thoracic CT. They had an initial hospitalization range of 3–10 days and their mean initial hospitalization length was 6 days. Moreover, three of them were re-hospitalized for impaired consciousness and syncope and 1 for cough and fever 1-7 days after their first discharge. All of these patients had at least two (2–6) concomitant chronic diseases. Three of the patients died on the seventh day of their second hospitalization and one died on the ninth day of the second hospitalization.

Of the re-hospitalized patients, 23 (38.3%) had cough, 20 (33.3%) dyspnea, 13 (21.6%) fever, while 10 (20%) had no symptoms at all. The symptoms of the patients are listed in Table 1.

When examining the comorbidity status of the patients re-hospitalized, 19 (31.7%) did not have any chronic disease, 14 (23.3%) had three comorbidities, and 11 (18.3%) had two comorbidities. Table 2 lists the number of comorbidities. There was a statistically significant correlation between the presence of comorbidity and the number of days of the first hospitalization and the total number of hospitalization days ($p < 0.05$). The initial hospitalization of patients with comorbidities was longer than those without comorbidities. There was a positive correlation of statistically moderate significance between the number of concomitant chronic diseases and the number of days of the first and second hospitalization ($p < 0.05$). As for comorbidities, 32 patients (53.3%) had hypertension, 19 (31.7%) had cardiovascular diseases (CVH), and 16 (26.7%) had diabetes. The comorbid diseases of the patients are shown in Table 3.

After comparing the values of lactate, ferritin, d-Dimer, white blood cell (WBC), platelets (PLT), lymphocyte (LYM), lactate dehydrogenase (LDH), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and C-reactive protein (CRP) during the first and second hospitalization, there was a statistically significant difference in lactate, ferritin, d-Dimer, platelet, CRP, and LDH ($p < 0.05$). Table 4 lists the evaluation of the laboratory results.

TABLE 1. SYMPTOMS OF RE-HOSPITALIZED PATIENTS

Symptom	Patient Count	Percent %
Cough	23	38.3
Dyspnea	20	33.3
Fever	13	21.7
Weakness	10	16.7
Asymptomatic	10	16.7
Syncope	5	8.3
Chest pain	5	8.3
Back pain	4	6.7
Diarrhea	4	6.7
Sore throat	3	5
Stomachache	3	5
Anosmia	2	3.3
Nausea	2	3.3
Headache	1	1.7

TABLE 2. NUMBER OF COMORBIDITIES OF RE-HOSPITALIZED COVID-19 PATIENTS

Number of Comorbidities	Number of Patients	Percent %
0	19	31.7
1	7	11.7
2	11	18.3
3	14	23.3
4	1	1.7
5	6	10
6	2	3.3

TABLE 3. TYPES OF COMORBIDITIES OF RE-HOSPITALIZED COVID-19 PATIENTS

Comorbidity	Number of Patients	Percent %
Hypertension	32	53.3
Cardiovascular diseases	19	31.7
Diabetes	16	26.7
Cerebrovascular diseases	11	18.3
Psychiatric diseases	10	16.7
Chronic lung diseases	9	15
Cancer	4	6.7
Chronic renal failure	3	5
Immunosuppressive states	1	1.7

DISCUSSION

There is only a limited volume of information about COVID-19 patients who are re-hospitalized after discharge. Chen et al. reported that 11 patients were re-hospitalized, and Peng et al. reported that 7 patients were re-hospitalized after discharge [5,6]. In a prospective study conducted by Wang et al., 5 (3.82%) of 13 COVID-19 patients discharged were re-hospitalized [7]distribution of quarantine locations, and the infection status of the contacts of COVID-19 patients after discharge.\n \n \n Design\n A prospective cohort study\n \n \n Methods\n Demographics, baseline characteristics of 131 COVID-19 patients discharged from February 3 to 21, 2020 in Wuhan, China were collected and analyzed by reviewing the medical records retrospectively. Post-hospitalization data related to clinical outcomes, quarantine locations and close contact history were obtained by following up the patients every week up to 4 weeks.\n \n \n Results\n 53 (40.05%. Moreover, Wang et al. reported that two patients discharged after recovering from pneumonia were re-hospitalized because of intestinal infections caused by Sars-Cov-2 [8]. Cao et al. reported that 8 out of 108 patients who were discharged between February 10 and April 13, 2020, were re-hospitalized [9]. Richardson et al. examined 5700 patients who were hospitalized for COVID-19 in New York City and reported that 45 patients (2.2%) were re-hospitalized [10]. In our study, the number of patients who were admitted to the hospital for different reasons after discharge is 60.

In the study of Safiya et al., [22] (48.8%) of the re-hospitalized COVID-19 patients were aged >65 years, and 22 (48.8%) were aged between 18 and 64 years[10]. The mean age of patients was 48.4 years in the study by Chen et al., [54].3 years in the study by Cao et al., and 48.7 years in the study by XingyuWang et al. [6,9,11]. The mean age of the patients included in our study is 56.9. Based on these results, the mean age of the patients in our study is older than that of the patients in other studies. Chen et al. reported that re-admitted patients were re-hospitalized 16 ± 7.14 days after discharge, while Wang et al. reported that five patients were re-hospitalized within 1–2 weeks and 3 patients within 3–4 weeks [6,11]. Safiya et al. reported that 45 discharged patients were re-hospitalized on an average of 3 days after discharge, while Wang et al. reported that two patients were re-hospitalized one day after discharge [8,10]. In our study, the mean time until re-hospitalization after discharge is 6.82 ± 4.75 days, which is shorter than that reported by Chen et al. and longer than that reported by Safiya et al. and Wang et al. Although Chen et al. and Peng et al. reported that there was a shortening in the lengths of second hospitalizations [5,6], 56.7% of the patients had longer durations in the second hospitalization, and these results did not coincide with the results of Chen et al. To better understand the cause of this noncoincidence, it may be useful to examine the reasons for patient hospitalizations. In most of the abovementioned studies that cover information about re-hospitalization, the RT-PCR tests were negative when patients were discharged. In this case, it is observed that the eight patients that Cao et al. included in their study had no symptoms but were re-hospitalized only because their RT-PCR test came back positive [9]. In support of this study, Peng et al. reported seven re-hospitalized patients, and five of these patients were re-hospitalized despite having no symptoms and only because their RT-PCR test results came back positive or they had abnormalities in their tomography images [5]. However, all of the 11 patients that Chen et al. included in their study had at least one of the complaints of cough (54.5%), fever (27.3%), or malaise (27.3%) [6]. In our study, 10 patients (16.6%) were re-hospitalized based on tomography findings and RT-PCR test results, although they were asymptomatic during the second admission, while 50 patients (83.4%) had one or several complaints. The top three symptoms include cough in 23 patients (38.3%), dyspnea in 20 patients (33.3%), and fever in 13 patients (21.7%). Moreover, severe symptoms such as syncope (8.3%) and chest pain (8.3%) were observed in some patients. Complaints during the second admission include more serious symptoms such as dyspnea, syncope, and chest pain, which may be considered to affect the higher number of days of hospitalization in the second admission. The mean hospitalization of 10 asymptomatic patients in the first hospitalization was 4.8 days; however, on average, this period was reduced to 3.3 days in the second admission. Nevertheless, the mean duration of the first hospitalization in patients with one or more symptoms was 4.5 days, while the mean number of days in the second hospitalization increased to 8.26 days. However, our study does not coincide with the study by Chen et al. since the hospitalization of the symptomatic patients included in our study increased in their second admission. Moreover, as mentioned above, the mean age of our patients is higher than that of patients in other studies, which may affect the longer duration of their second hospitalization. In other studies, limited information was

given about the mortality of re-hospitalized patients. Of the patients included in our study, four (6.6%) died in the hospital during their second hospitalization.

Note that there is no sufficient information in the literature concerning the comorbidities of re-hospitalized patients. In 19 (31.6%) of the patients included in our study, there were no additional diseases, while 41 patients (68.4%) had at least one concomitant disease. According to a meta-analysis investigating comorbidities of COVID-19 patients, the four most common comorbidities are as follows: hypertension (14%–22%), diabetes (6%–11%), cardiovascular diseases (4%–7%), and chronic lung diseases (1%–3%), respectively [12]. The most common comorbidities of the patients in our study include hypertension, cardiovascular diseases, diabetes, and cerebrovascular events. The concomitant diseases of the patients in our study are similar to the comorbidities of overall COVID-19 patients.

CONCLUSION

Re-hospitalized patients may have at least one additional disease, there might not be very specific symptoms during the second admission, and comorbidities may increase mortality especially in elderly women when they are re-admitted with serious symptoms such as syncope. When being discharged, such patients can be advised to be more careful and immediately contact the hospital on any negative development. For asymptomatic patients, a more detailed assessment of their second hospitalization may help reduce unnecessary hospitalization.

Permission

Obtained from Sakarya University Training and Research Hospital Administration on 01/04/2020.

Conflict

There is no conflict of interest regarding the article. No financial aid has been received from any institution or organization.

Author's Contribution

Fatih GUNEYSU -visualization, resources, editing.

Ensar DURMUS - data curation, formal analysis, methodology, writing.

RESUMO

OBJETIVO: *Este estudo pretende investigar as causas para re-hospitalizações, as reclamações e os prognósticos de pacientes com COVID-19 após a alta hospitalar.*

MÉTODOS: *Pacientes com COVID-19 internados que foram re-hospitalizados no Sakarya University Training and Research Hospital foram examinados. Os resultados da reação em cadeia de polimerase precedida de transcrição reversa (RT-PCR), tomografia e dos exames laboratoriais, as características demográficas e os resultados prognósticos foram registrados retrospectivamente.*

RESULTADOS: *Um total de 60 pacientes, 26 do sexo masculino (43,3%) e 34 do sexo feminino (56,7%), com internações repetidas devido a sintomas de COVID-19 foram incluídos no estudo, com uma idade média de 56,9 (± 22,5) (mediana = 61, faixa etária = 3-88). O número de dias da segunda internação foi estatisticamente significativamente maior (p < 0,05). A idade do paciente e o número de dias de internação apresentaram uma forte correlação positiva (p < 0,01). Um total de 11 pacientes (18%) apresentaram resultados negativos no primeiro RT-PCR e posteriormente tiveram resultados positivos na segunda internação. Além disso, 10 (17,5%) dos pacientes submetidos a tomografia de tórax apresentaram envolvimento unilateral, 34 (59,6%) bilateral, e 13 (22,8%) não apresentaram resultados significativos. Nota-se que 4 (6,6%) dos pacientes re-hospitalizados morreram no hospital, enquanto 56 (93,4%) receberam alta mais uma vez. Todos os quatro pacientes que morreram eram do sexo feminino, com idade média de 81,5 anos.*

CONCLUSÃO: *Principalmente pacientes com idade avançada e comorbidades devem ser examinados com mais cuidado no momento da alta hospitalar; caso suas queixas se repitam, eles devem ser aconselhados a contatar o serviço de emergência o quanto antes.*

PALAVRAS-CHAVE: *COVID-19, re-hospitalização, coronavírus*

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ORIGINAL ARTICLE

Bilateral simultaneous percutaneous nephrolithotomy versus staged approach: a critical analysis of complications and renal function

Fabio C. M. Torricelli[1]
Regina S. Carvalho[2]
Giovanni S. Marchini[1]
Alexandre Danilovic[1]
Fabio C. Vicentini[1]
Carlos A. Batagello[1]
Miguel Srougi[1]
William C. Nahas[1]
Eduardo Mazzucchi[1]

1. Divisão de Urologia, Departamento de Cirurgia, Faculdade Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.
2. Faculdades das Américas, São Paulo, SP, Brasil.

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SUMMARY

INTRODUCTION: *Patients with bilateral kidney stones and burdened by large stones are challenging cases for endourologists. Simultaneous bilateral percutaneous nephrolithotomy (sbPCNL) is an option; however, it may be accompanied by important morbidity. An alternative is a staged PCNL, operating one side each time. Herein, we compare the impact of sbPCNL and staged PCNL on complication rates and renal function.*

METHODS: *Patients who underwent sbPCNL or staged bilateral PCNL with a frame time of 6 months were searched in our prospectively collected kidney stone database. Groups were compared for age, gender, body mass index (BMI), comorbidities (classification by the American Society of Anesthesiology - ASA), stone size, Guy's score, stone-free status, renal function, blood loss, blood transfusion rate, complication rate, and length of hospital stay.*

RESULTS: *Twenty-six patients and 52 kidney units were enrolled. The mean operative time was 134.7 min. Only 11.3% of cases had complications, all of them minor (Clavien ≤ 2). Overall, the stone-free rate was 61.50%. Comparing the groups, there was a significantly longer operative time in the sbPCNL group (172.5 vs. 126.3 min; p=0.016), as well as a higher transfusion rate (12.5% vs. 5.6%; p=0.036). There was no statistically significant difference in creatinine levels between the groups. Regarding the stone-free rate, there was a significantly higher proportion of patients in the staged PCNL group (64.9% vs. 43.8%; p=0.012).*

CONCLUSION: *sbPCNL is a safe procedure; however, when compared to staged procedures it has a higher transfusion and lower stone-free rate.*

KEYWORDS: *Complications; Kidney; Lithotripsy; Urinary calculi.*

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CORRESPONDING AUTHOR: Fábio César Miranda Torricelli
Av. Vereador Jose Diniz, 3300, conj. 208, São Paulo, SP, Brasil – 04604-006
Tel: +55 11 5533-4900
E-mail: fctorricelli@yahoo.com.br

INTRODUCTION

Urolithiasis is a common disease in urological practice with a high socio-economic impact. It is estimated that approximately 10% of the worldwide population has kidney stones. In the United States, the disease affects about 1 in 11 people.[1] In addition, the risk of recurrence is also high. Once diagnosed, 50% of adult patients relapse in 5 to 10 years and 75% in 20 years.[2] Recent studies have shown that the prevalence of urolithiasis has been increasing in the last decades in developed and developing countries.[1,3] This trend is believed to be associated with changes in lifestyle, such as lack of physical activity, changes in eating habits, and global warming.[4,5] Stones in the urinary tract can be unilateral or bilateral. The real incidence of bilateral nephrolithiasis in kidney stone formers is unknown and estimated to be from 12% to 26%.[6] Thus patients with bilateral kidney stones may not be so infrequent and represent a challenge for endourologists.

Percutaneous nephrolithotomy (PCNL) replaced open renal surgery and became the treatment of choice for large (> 2.0 cm) or complex kidney stones.[7,8] As a strategy to reduce the morbidity of bilateral surgery as much as possible, traditionally, patients with large or complex bilateral kidney stones are treated in procedures performed in 2 stages, operating one side at a time. PCNL has been changed and improved, minimizing its morbidity and invasiveness, with a notable improvement in efficacy and operative time. With the implementation of new devices and technology, simultaneous bilateral PCNL (sbPCNL) has emerged as a safe and effective option.[9] Recently, different authors have demonstrated favorable results with sbPCNL.[6,10-12] Advantages include less sedative agents, faster return to daily activities, less need for reoperation, and shorter surgical time, decreasing the overall hospital stay and the cost of surgery. However, the lack of studies evaluating its safety compared to staged procedures makes its applicability still restricted to a few specialized centers. The concerns are increased blood loss, prolonged surgical time, and acute renal failure.

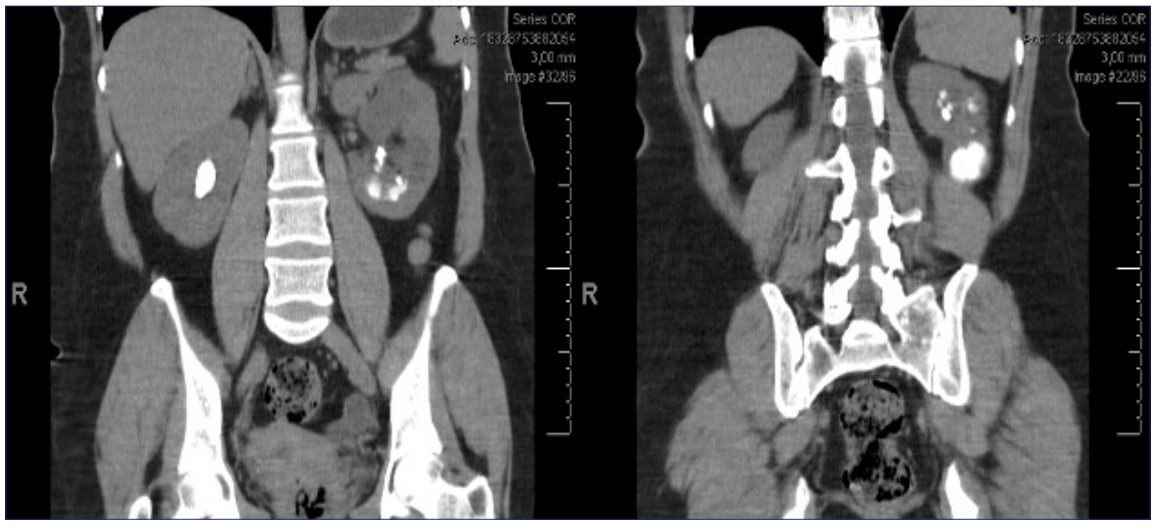
The main objective of this study is to assess renal function alteration and complication rates in patients undergoing sbPCNL in comparison to staged bilateral procedures. The secondary objective is to analyze the stone-free rate.

METHODS

Study design

After Institutional review board approval (IRB approval number 3.227.010), we searched our prospectively collected kidney stone database for patients who underwent bilateral PCNL from January 2011 through December 2018. Electronic charts were reviewed, searching for patients with bilateral stones who underwent sbPCNL or staged bilateral PCNL with a time frame of 6 months. Only patients older than 18 years were enrolled in the study. Patients with anatomical abnormalities such as pelvic kidney or ureteral duplicity were excluded, as were patients with abnormal renal function before surgery. Figure 1 shows a computed tomography scan of a patient with bilateral kidney stones eligible for bilateral PCNL.

FIGURE 1. COMPUTED TOMOGRAPHY SCAN SHOWING A LARGE BILATERAL KIDNEY STONE SUBMITTED TO BILATERAL PERCUTANEOUS NEPHROLITHOTOMY



The groups were compared for age, gender, body mass index (BMI), comorbidities (classification by the American Society of Anesthesiology - ASA), stone size, Guy's stone score,[13] post-operative stone-free status, renal function, blood loss assessed by the postoperative variation of hemoglobin level, blood transfusion rate, complication rate (according to Clavien-Dindo score modified for PCNL)[14] and length of hospital stay. Stone-free status was evaluated by a non-contrast computed tomography performed on the 1st postoperative day as routinely performed in our Institution. Renal function was evaluated by the variation of creatinine level between the day before surgery, the first day after surgery, and between 30 to 60 days after the procedure.

Surgical technique

Patients with preoperative positive urine culture received appropriate antibiotic therapy for one week according to germ susceptibility, whereas patients with negative urine culture received prophylactic antibiotics (third-generation cephalosporin) starting 24 hours before surgery or at anesthesia induction.

All procedures were performed under general anesthesia. Patients were positioned in the prone or supine position based on the surgeon's preference. A 6-Fr ureteral catheter was placed through cystoscopy. After retrograde pyelography, the selected calyx was punctured under fluoroscopy guidance. A hydrophilic guidewire was inserted and passed into the ureter. If this guidewire did not reach the ureter, a PTFE guidewire was used to replace it. The tract was dilated with fascial dilators and a 30-Fr Amplatz sheath was placed. A 26-Fr rigid nephroscope was used for nephroscopy and a ultrasonic lithotripter was used for stone fragmentation and suction (Swiss LithoClast® Master, EMD, Dallas, Texas, USA). Irrigation was performed with saline solution at 25°C and pressure of 30-40 cmH_2O. Flexible nephroscopy to assess residual fragments was routinely performed at the end of the procedure when conditions allowed it. An 18-Fr nephrostomy tube was placed at the end of the procedure in cases of bleeding, residual stones, renal pelvis perforation, or multiple accesses. The ureteral catheter was maintained for 12 hours in the postoperative time, or a double-J stent for one to two weeks was left in place at surgeon discretion.

Operative time was considered from the beginning of the cystoscopy for ureteral catheter placement until the end of nephrostomy tube placement. Patients with residual stones were submitted to a second look PCNL, flexible ureteroscopy, or shock wave lithotripsy based on residual stone burden and location.

Statistical analysis

Categorical data were described in frequencies, and continuous parameters were described as mean and standard deviation. Categorical variables were compared using the chi-square and Fisher exact tests, whereas continuous variables were compared using the Student t-test for independent groups. All statistical analyses were performed using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA). The significance level was set at $p < 0.05$.

RESULTS

Twenty-six patients and 52 kidney units were enrolled in the study. The patient's mean age was 42.8 years, mean BMI was 30.1 Kg/m², and 73.07% of the patients were female. Most patients were healthy – ASA 1 (53.8%), while 46.2% had at least one comorbidity.

Overall, the mean operative time was 134.7 min. Complications occurred in 5 cases (11.3%; 5 of 44 surgeries), all of them minor (Clavien ≤ 2). The most common complication was bleeding that required transfusion (4 cases, 7.7%). The mean drop in hemoglobin level was 1.9 mg/dL, while creatinine showed a slight increase on the 1st postoperative day, but returned to the baseline level between the first and the second postoperative month. The transfusion rate was 7.7% and the mean hospital stay was 2.7 days. Overall, the stone-free rate was 61.50%. Table 1 shows all the descriptive data.

TABLE 1. INTRA AND POSTOPERATIVE DATA.

	Overall
Operative time (min)	134.7 ± 49.8
Complications (%)	11.3%
Major Complications (Clavien ≥3)	0
Preop hemoglobin (mg/dL); mean ± SD	13.3 ± 1.5
Postop hemoglobin (mg/dL); mean ± SD	11.4 ± 1.9
Drop of hemoglobin (mg/dL); mean ± SD	1.9 ± 1.4
Blood Transfusion (%)	7.7%
Length of hospital stay (days); mean ± SD	2.7 ± 1.2
Preop creatinine (mg/dL); mean ± SD	0.9 ± 0.3
1 POD creatinine (mg/dL); mean ± SD	1.2 ± 0.4
30-60 POD creatinine (mg/dL); mean ± SD	0.9 ± 0.3
Stone-free rate – 1st POD (%)	61.5%

Of the 26 patients, eight underwent sbPCNL and 18 underwent staged NLPC. Patients with staged NLPC were older (45.9 vs. 33.3 years; p = 0.028); however, there were no statistically significant differences regarding gender, BMI, comorbidities (ASA score), and Guys score between the groups. Table 2 shows the demographic data of both groups. Staged PCNL was performed with a mean interval time of 34.4 days.

Comparing both groups, there was a significantly longer operative time in the sbPCNL group (172.5 vs. 126.3 min; p=0.016). There was a trend for more complications in the sbPCNL (25% vs. 8.3%; p=0.070); however, there was no major complication in the sbPCNL or staged PCNL groups. There was also no significant difference in the drop of hemoglobin levels (2.5 vs. 1.7 mg/dL; p=0.347); however, the transfusion rate was statistically significantly higher in the sbPCNL group (12.5% vs. 5.6%; p=0.036). Regarding the length of hospital stay, there was a trend for longer hospitalization in patients with sbPCNL (3.5 vs. 2.5 days; p=0.071). There was no statistically significant difference in creatinine levels between the groups. Both presented with a slight increase on the 1st postoperative day but returned to the baseline level after one month. Regarding the stone-free rate, there was a significantly higher proportion of patients in the staged PCNL group (64.9% vs. 43.8%; p=0.012). Table 3 summarizes the comparison between the groups. Figure 2 shows the main postoperative outcomes.

DISCUSSION

According to our results, sbPCNL is a safe procedure that is not accompanied by major complications or a significant increase in the creatinine level.

FIGURE 2. MAIN POSTOPERATIVE OUTCOMES OF SIMULTANEOUS AND STAGED BILATERAL PERCUTANEOUS NEPHROLITHOTOMY

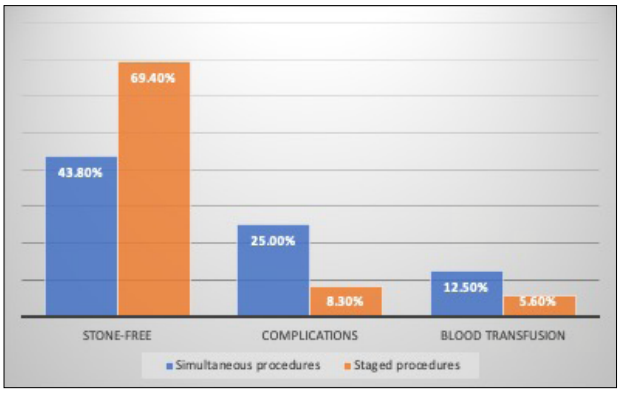


TABLE 2. DEMOGRAPHIC DATA OF SIMULTANEOUS AND STAGED-PERCUTANEOUS NEPHROLITHOTOMY.

	Simultaneous procedures	Staged procedures	p-value
Age (years); mean ± SD	33.3 ± 12.3	45.9 ± 10.6	0.028
Gender (female)	75.00%	72.2%	0.639
Body Mass Index (kg/m2); mean ± SD	31.6 ± 5.1	29.4 ± 8.3	0.424
ASA score	62.5% ASA 1 37.5% ASA 2	50% ASA 1 50% ASA 2	0.437
Guys score (all renal units)	Guys 1 50% Guys 2 30% Guys 3 20% Guys 4	25% Guys 1 25% Guys 2 30.6% Guys 3 19.4% Guys 4	0.250

TABLE 3. INTRA E POSTOPERATIVE DATA OF SIMULTANEOUS AND STAGED-PERCUTANEOUS NEPHROLITHOTOMY.

	Simultaneous procedures	Staged procedures	p-value
Operative time (min)	172.5 ± 59.7	126.3 ± 44.0	0.016
Complications	(n = 2) 25.0%	(n = 3) 8.3%	0.070
Major Complications (Clavien ≥3)	0,00%	0,00%	1,000
Preop hemoglobin (mg/dL); mean ± SD	13.1 ± 1.4	13.4 ± 1.5	0.663
Postop hemoglobin (mg/dL); mean ± SD	10.6 ± 1.9	11.6 ± 1.9	0.221
Drop of hemoglobin (mg/dL); mean ± SD	2.5 ± 1.9	1.7 ± 1.2	0.347
Blood Transfusion	12.5%	5.6%	0.036
Length of hospital stay (days); mean ± SD	3.5 ± 1.1	2.5 ± 1.2	0.071
Preop creatinine (mg/dL); mean ± SD	0.9 ± 0.3	0.9 ± 0.3	0.967
1 POD creatinine (mg/dL); mean ± SD	1.0 ± 0.5	1.2 ± 0.4	0.5
30-60 POD creatinine (mg/dL); mean ± SD	0.8 ± 0.2	0.9 ± 0.3	0.45
Stone-free rate	43.8%	69.4%	0.012

However, when compared to staged procedures, we observed a trend for more minor complications, a higher transfusion rate and a lower stone-free rate in the sbPCNL group. To our knowledge, we present one of the few comparative studies available regarding this subject.

Other authors have already shown favorable outcomes with sbPCNL.[9-12,15-17] Adhikari et al published a descriptive study with 52 patients submitted to sbPCNL and reported a success rate of 94% when considering residual fragments up to 4mm. In this study, almost 85% of cases were Guys 1 or 2, which means cases of low complexity. In our study, almost 50% were complex cases, which lead us to a success rate close to 65%. In accordance with our data, those authors also reported a relatively low hemoglobin drop and no significant change in serum creatinine levels. Differently of our findings, those authors had two major complications; one patient had hydrothorax that required thoracic drainage and another required surgical evacuation of clots from the bladder.[12] Sofer et al, in a comparative study of bilateral versus unilateral PCNL, showed that sbPCNL is associated with a significantly increased postoperative creatinine level, a decreased postoperative hemoglobin level, a higher blood transfusion rate (9% versus 2%), and a longer hospital stay.[15] In our study, there was no significant difference in postoperative serum creatinine between simultaneous and staged procedures; however, we also found a higher transfusion rate and a tendency for longer hospital stays in sbPCNL. Rivera et al, in another study comparing bilateral versus unilateral PCNL, reported that patients submitted to sbPCNL had longer procedures, were more likely to undergo a secondary procedure, and had a longer hospital stay. Notably, there were no differences in the number or the severity of complications between the groups.[18] These findings are very similar to ours, which leads us to conclude that staged procedures have outcomes that are very close to those found in unilateral procedures.

In a systematic review of studies reporting sbPCNL outcomes, including descriptive reports, the authors found a mean initial stone-free rate of 72.6% with a mean operative time of 171.1 minutes and mean hospital stay of 3.9 days. The mean complication rate per study was 23.4% and most were Clavien grade 1.[16] Once again, these findings are close to those presented in our study. However, when compared to staged procedures, we could note that transfusion rate and minor complications are higher in sbPCNL. That may be the key for an optimal outcome, recognizing when bilateral kidney stones can be treated at the same time and when procedures should be planned in two stages. This decision is not always easy and may require surgeon experience.

The literature reports one study comparing the outcomes of sbMini-PCNL versus staged bilateral Mini-PCNL.[17] Simultaneous procedures had a significantly shorter cumulative operative time, shorter cumulative hospital stay, and higher hemoglobin loss than staged surgeries. There were no differences between the groups for blood transfusion and complication rates.[17] In our comparative study, there was a higher transfusion rate and a tendency for a higher minor complication rate in sbPCNL, this could be explained by the fact that we performed the conventional 30 Fr approach to both kidneys and not a small tract as in Mini-PCNL.

Our study has limitations, such as its retrospective design and small sample size, which makes our comparison between the two groups limited. However, simultaneous bilateral PCNL is not a daily approach. In our Institution, we opt for simultaneous bilateral procedures in selected cases, such as those of patients with no co-morbidities and stones classified as Guys I or II bilaterally.

CONCLUSION

sbPCNL is a safe procedure; however, when compared to staged procedures it has a higher transfusion and lower stone-free rates. sbPCNL should be reserved for selected cases.

Acknowledgments

None

Author disclosure statement

No competing financial interests exist.

Author's Contribution

Fabio C. M. Torricelli – drafting of the manuscript and study design; Regina S. Carvalho – drafting of the manuscript; Giovanni S. Marchini, Alexandre Danilovic, Fabio C. Vicentini, Carlos A. Batagello, – data acquision; Miguel Srougi, William C. Nahas, – supervision; Eduardo Mazzucchi – critical review.

RESUMO

INTRODUÇÃO: *Paciente com cálculos renais bilaterais e de grande volume são casos desafiadores para os endourologistas. A nefrolitotripsia percutânea bilateral simultânea (NLPbs) é um opção, entretanto esse procedimento pode ser acompanhado de morbidade importante. Uma alternativa é a NLP estagiada, operando um lado de cada vez. Aqui, nós comparamos o impacto da NLPbs e da NLP estagiada nas taxas de complicações e função renal.*

MÉTODOS: *Pacientes que foram submetidos a NLPsb ou NLP stagiada com intervalo de até 6 meses foram pesquisados em nossa base de dados de cálculos renais prospectivamente coletada. Os grupos foram comparados em idade, gênero, índice de massa corpórea (IMC), comorbidades (classificação da Sociedade Americana de Anestesiologia – ASA), tamanho do cálculo, Classificação de Guys, taxa de pacientes livres de cálculos, função renal, perda sanguínea, taxa de transfusão, taxa de complicações e tempo de internação hospitalar.*

RESULTADOS: *Vinte e seis paciente e 52 unidades renais foram incluídas. O tempo operatório médio foi de 134,7 min. Apenas 11.3% dos casos tiveram complicações, sendo todas menores (Clavien ≤ 2). No geral, a taxa de pacientes livres de cálculos foi de 61,5%. Comparando os grupos houve um tempo operatório significativamente maior no grupo NLPbs(172,5 vs. 126,3 min; p=0,016), assim como uma maior taxa de transfusão (12,5% vs. 5,6%; p=0.036). Não houve diferença significante nos níveis de creatinina entre os grupos. Em relação a taxa de doentes livre de cálculos houve uma proporção significativamente maior de pacientes livres de cálculos na NLP estagiada (64,9% vs. 43,8%; p=0,012).*

CONCLUSÃO: *A NLPsb é um procedimento seguro, entretando quando comparada ao procedimento estagiado apresenta uma maior taxa de transfusão e uma menor taxa de pacientes livres de cálculos.*

PALAVRAS CHAVES: *Complicações; Rim; Litotripsia, Cálculo urinário.*

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ORIGINAL ARTICLE

Femoral fractures in the elderly in Brasil - incidence, lethality, and costs (2008-2018)

Paula Antas Barbosa de Vasconcelos[1]
Anderson de Jesus Rocha[1]
Rodrigo Jorge de Souza Fonseca[1]
Thiago Rhangel Gomes Teixeira[2]
Enilton de Santana Ribeiro Mattos[3]
Alex Guedes[4]

1. Residente, Programa de Residência Médica em Ortopedia e Traumatologia do Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, BA, Brasil.
2. Mestrando, Programa de Mestrado Profissional em Saúde Coletiva, Universidade Federal da Bahia, Salvador, BA, Brasil.
3. Preceptor, PRM em Ortopedia e Traumatologia do Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, BA, Brasil.
4. Professor Associado-Doutor, Chefe da Disciplina Cirurgia do Aparelho Locomotor, Departamento de Cirurgia Experimental e Especialidades Cirúrgicas, Faculdade de Medicina da Bahia, Universidade Federal da Bahia, Salvador, BA, Brasil

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SUMMARY

OBJECTIVES: *To describe the incidence by gender and region, lethality, and costs associated with the treatment of femoral fractures in the elderly (≥ 60 years) hospitalized in the Unified Health System (SUS) of Brasil between 2008 and 2018.*

METHODS: *This is a cross-sectional, descriptive, retrospective study of hospitalizations of elderly people due to femoral fractures by analyzing secondary data obtained from the SUS Hospital Information System (SIH/SUS) between 2008 and 2018; for calculation of epidemiological coefficients, we used information from demographic censuses (2000 and 2010) of the Brazilian Geography and Statistics Institute (IBGE).*

RESULTS: *A total of 478,274 hospitalizations were recorded in the period; the incidence was 1.7 times higher in females (overall average of 274.91/100,000 for women and 161/100,000 for men). The Southeast region had the highest absolute number of hospitalizations and the South region presented the highest annual overall average incidence (224.02/100,000). The average annual cost for SUS for the treatment of femoral fractures in the elderly was R$ 99,718,574.30.*

CONCLUSIONS: *In the evaluated period (2008-2018), femoral fractures in the elderly had a high incidence (478,274 hospitalizations; 224.02 cases/100,000 elderly), a predominance of females (1.7F/1.0M), a higher absolute number of hospitalizations in the Southeast region and a higher incidence in the South region; the lethality was high (an increase of 17.46%; overall mean coefficient of 4.99%/year); and the costs for the SUS were huge (an increase of 126.24%; average annual expenditure of R$ 99,718,574.30).*

KEYWORDS: *Femoral fractures. Health services for the aged. Hospitalization. Health policy. Health systems agencies.*

INTRODUCTION

Femoral fractures have a great social impact and financial burden on the health system. This impact is associated with extensive surgical procedures, long periods of hospitalization and patient recovery, and the potential for complications, sequels, and deaths inherent to this condition.

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CORRESPONDING AUTHOR: Alex Guedes
Endereço: Rua Padre Feijó 139, Ap. 203, Canela, Salvador-BA, Brasil, CEP 40.110-170.
E-mail: alexguedes2003@yahoo.com.br

The process of human aging is directly related to femoral fractures because the progressive physiological decrease of structural and functional reserves can create the conditions for a loss of bone continuity during moments of overload of the musculoskeletal system.

Femoral fractures in the elderly are associated with a greater length of hospitalization and rehabilitation and generate a high economic and social burden to their family members and the health system. In this age group, such fractures have a high incidence and are related to unfavorable outcomes, including the loss or decline of autonomy and quality of life: many of these patients do not resume their previous daily life activities (DLAs), suffer from psychological impact imposed by this condition, in addition to fearing new falls[1,2]. There are direct effects on the overall health of these individuals, particularly on account of immobilism, leading to increased rates of hospitalization and mortality[2,3].

Studies highlight poor prognoses in elderly patients during the first postoperative year after the surgical treatment of fractures of the proximal segment of the femur[2]. Regarding hospital readmission, the study by Paula et al.[2] showed a rate of 17.8% in the first year of follow-up postoperatively, especially secondary to surgical complications.

Campos et al.[4] presented mortality rates at 3, 6, 9, and 12 months of the postoperative follow-up of an elderly population who suffered a fracture of the proximal segment of the femur, identifying values of 21.2%, 25%, 28.8%, and 34.6% for men and 7.8%, 13.5%, 19.2%, and 21.4% for women.

Given these findings, it appears that femoral fractures represent an actual public health problem, considering their significant negative impact in terms of overall incidence, lethality, and costs and their consequences for the public sector. In this sense, it is vital to identify the main epidemiological data related to this disease in the Brazilian population to support health policies and actions that can contribute to their prevention, the reduction of morbidity and lethality, and the reduction of costs by defining and standardizing guidelines to care for and follow-up on these patients, based on the best scientific evidence available.

The purpose of this study is to describe the incidence by gender and region, lethality, and costs associated with the treatment of femoral fractures in patients aged 60 years or older, hospitalized in the Single Health System (SUS) in Brasil between 2008 and 2018.

METHODS

A descriptive, retrospective, cross-sectional study was conducted on the hospitalizations of elderly patients with femoral fractures in the Single Health System (SUS) in Brasil between 2008 and 2018. We analyzed secondary data obtained from the Hospital Information System of SUS (SIH/SUS), of the Ministry of Health, and included cases of femoral fractures of the femur, based on the tenth revision of the International Classification of Diseases (ICD-10), in people aged 60 years or older.

The number of hospitalizations was evaluated in all regions of Brasil. The demographic data for calculation of epidemiological coefficients were obtained from the population censuses of 2000 and 2010, conducted by the Brazilian Institute of Geography and Statistics (IBGE). The data relating to the costs involved in elderly hospitalizations, i.e., the victims of femoral fractures, were obtained by tabulating the information available in the SIH/SUS. Microsoft Excel® version 2010 spreadsheets were used to tabulate the data and make the statistical calculations.

No approval by the Human Research Ethics Committee was necessary because we used secondary information from a public domain database, per Resolution of the National Council of Health (CNS) No 466/2012.

RESULTS

This study recorded 478,274 hospitalizations due to femoral fractures in people aged age 60 years or older in the SUS hospitals between January 2008 and December 2018, generating treatment costs that exceeded a billion reais (Table 1).

The overall incidence of femoral fractures in the Brazilian elderly had, in the analyzed period, an overall average of 224.02 cases per one 100,000 elderly individuals. The coefficient of lethality, i.e., hospitalized patients who progressed to death, showed an increase of 17.46%, and the overall average of the coefficient, by 4.99% per year, while the costs increased 126.24% and had an average annual expenditure of R$ 99,718.574.30. The costs decreased only in the last year (Figure 1).

The incidence of femoral fractures by sex was 1.7 times higher in females when compared to males; in percentage values, there was a variation of 68.03% in 2008 and 68.22% in 2018, with an overall average of

274.91 per 100,000 for elderly women and 161 per 100,000 for elderly men.

The Southeast region had the greatest number of hospitalizations for all the years analyzed. However, the incidence of femoral fractures in the elderly in the South region, between 2008 and 2018, surpassed those in the Southeast region. From the year 2017, the Northeastern region had the lowest incidence of these fractures, but the figures were very close to those of the North region.

Regarding the lethality associated with hospitalizations due to femoral fractures, we observed a descending oscillation in the years 2009, 2013, and 2017 (Figure 2). On the other years, the coefficient of lethality remained on the rise, peaking in 2017.

DISCUSSION

In this study, we analyzed descriptively information relating to the distribution of hospitalizations of the elderly (60 years or older) due to femoral fractures in accredited hospitals of the public health system in Brasil, between January 2008 and December 2018, in addition to the coefficients of lethality and of the treatment costs relating to this disease. The results analyzed showed alarming numbers, considering the

FIGURE 2. OVERALL LETHALITY COEFFICIENT OF FEMORAL FRACTURES IN THE ELDERLY IN BRASIL, 2008 TO 2018. SOURCE: MINISTRY OF HEALTH- HOSPITAL INFORMATION SYSTEM OF SUS (SIH/SUS).

Year	Overall elderly population	No. of hospitalizations / year	No. of deaths	Mortality	Mort. Specific per 100,000
2008	14.536.029	34.052	1.501	4,41	10,33
2009	14.536.029	35.847	1.711	4,77	11,77
2010	20.590.599	35.903	1.689	4,70	8,20
2011	20.590.599	38.297	1.837	4,80	8,92
2012	20.590.599	39.298	1.940	4,94	9,42
2013	20.590.599	41.839	2.118	5,06	10,29
2014	20.590.599	44.613	2.245	5,03	10,90
2015	20.590.599	46.974	2.415	5,14	11,73
2016	20.590.599	52.359	2.721	5,20	13,21
2017	20.590.599	55.654	2.943	5,29	14,29
2018	20.590.599	53.438	2.769	5,18	13,45
Total	-	478.274	23.889	4,99	-

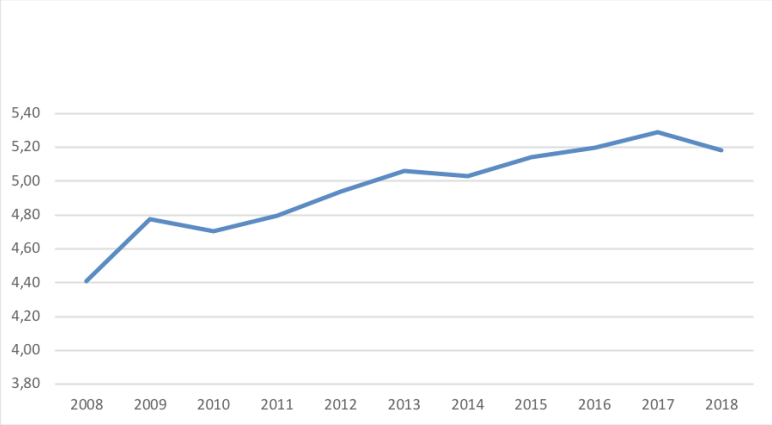

TABLE 1. HOSPITALIZATIONS DUE TO FEMORAL FRACTURES IN THE ELDERLY IN BRASIL, 2008 TO 2018.

Year of Treatment	Hospitalizations	Deaths	Elderly Population	Incidence per 100,000	Lethality	Costs in Brazilian Reais/ SUS (Public and Private)
2008	34,052	1,501	14,536,029	234.26	4.41	61,244,495.61
2009	35,847	1,711	14,536,029	246.61	4.77	68,731,712.18
2010	35,903	1,689	20,590,599	174.37	4.70	73,622,107.30
2011	38,297	1,837	20,590,599	185.99	4.80	79,272,817.63
2012	39,298	1,940	20,590,599	190.85	4.94	83,720,115.34
2013	41,839	2,118	20,590,599	203.19	5.06	98,328,317.70
2014	44,613	2,245	20,590,599	216.67	5.03	108,382,558.85
2015	46,974	2,415	20,590,599	228.13	5.14	114,525,068.42
2016	52,359	2,721	20,590,599	254.29	5.20	130,097,650.56
2017	55,654	2,943	20,590,599	270.29	5.29	140,416,282.49
2018	53,438	2,769	20,590,599	259.53	5.18	138,563,191.21
TOTAL	478,274	23,889	–	–	4.99	1,096,904,317.29
Average/year	43,479	2,172	19,489,768	–	–	99,718,574.30

Source: Ministry of Health - Hospital Information System of SUS (SIH/SUS).

high rates of incidence and the important impact on the public budget.

Between 2008 and 2018, more than 470,000 hospitalizations were recorded due to femoral fractures in the elderly in the context of the SUS, with an average of approximately 43,479.45 cases per year. These data are corroborated by the study by Soares et al.[5], which found, from a five-year analysis considering the same profile of fractures and population, an expansion in the number of hospitalizations and an annual average of 32,600 cases.

Many of these elderly individuals have, in addition to a femoral fracture, multiple comorbidities, which further increase the risk for complications, hospital readmissions, and deaths[2].

In addition to the high incidence and lethality observed, the total expenses with these fractures have exceeded 1 billion reais in the analyzed period, keeping the average annual costs with immediate and late treatments close to 99 million reais per year. The high costs imposed by this type of fracture were also was observed in other countries, such as Israel[1], United States[5], Switzerland[6] and Canada[7]; in the USA, the average cost of hospitalizations due to femoral fractures in 26,000 dollars[5].

We found that most episodes of hospitalization due to femoral fractures in the elderly happened in females, with a percentage of 68.05% vs. 31.95% in males. These data are consistent with other studies carried out in Brasil and in other countries, which have shown a predominance of the incidence of this disease among the elderly[5,7,8]. Studies suggest that this happens because women begin the process of loss of bone mineral density before man[3,7,8].

A scientific bibliographic survey indicates that biological aging has structural and functional repercussions that accumulate progressively with the passing of the years, thus decreasing the motor capacity of the elderly[9]. Along with other comorbidities, this physiological decline means a greater chance of bone fragility and, consequently, fractures[3,7,8].

As a result of the analysis of these data, it is clear that we are facing an important issue of public health, one that is complex and challenging due to five main factors: the high incidence of these fractures in recent years; the high cost of immediate and late treatments that burden the health budget; the psychological problems generated on patients due to the fear of new fractures, causing restrictions to DLAs[3.10]; the high lethality linked to this profile of patients and pathology; and finally, world aging is considered an irreversible process that must be dealt with in different ways in different countries but always with the intention of optimizing this process and reducing major diseases[7,8,10].

The data in this research allude only to hospitalizations due to femoral fractures in the population aged 60 years or older who used public health services in Brasil. Thus, if we take into account that a large portion of the population also makes use of the private health system, surely it is possible to infer that the situation may be even worse.

A limitation of this study is the underreporting of events and possible coding errors regarding femoral fracture; the Hospital Information System (SIH) is considered a limited and flawed database, with imperfections regarding the overall reliability of its data. This is due to the absence of records regarding some services in partnership with the private system and complementary health system, managed by private health insurance companies and health cooperatives[11].

It is clear that this is a disease with significant implications for the population in this age group, among them the decline in autonomy, functional capacity, and quality of life, constituting a complex public health problem.

Although the findings of this study are the results of a descriptive approach, they can be useful to better understand the occurrence of these fractures in the analyzed period and support the planning of public policies targeted at the elderly, thus aiming to inform and educate the population on the occurrence of these fractures, its complications, and health policies in favor of mechanisms to prevent new episodes, particularly in the more vulnerable age groups.

This way, we will be able to intervene effectively in factors that influence the events that lead to femoral fractures, thus reducing expenses and implementing better and lasting actions within the health and socioeconomic sphere.

CONCLUSIONS

In the assessed period (2008-2018), we identified that femoral fractures in the elderly in Brasil had a high incidence (478,274 hospitalizations, 224.02 cases/ 100,000 elderly), and females were more affected than males (1.7F/1.0M). The Southeast

region had the highest absolute number of hospitalizations; however, it was surpassed in incidence by the South region.

There was a high lethality (an increase of 17.46%, and an overall average of 4.99% per year for the coefficient).

There were substantial costs to the public health system (an increase of 126.24% and an average annual expenditure of R$ 99,718,574.30).

Conflicts of interest

The authors declare there are no conflicts of interest.

Author's contributions

Paula Antas Barbosa de Vasconcelos - Anderson de Jesus Rocha - concept and design, acquisition of data, or analysis and interpretation of data; drafting of the article or critical review of important intellectual content; Rodrigo Jorge de Souza da Fonseca - Thiago Rhangel Gomes Teixeira -concept and design, acquisition of data collection, or analysis, and interpretation; Enilton de Santana Ribeiro Mattos - (ICMJE) final approval of the version to be published; Alex Guedes - (ICMJE) final approval of the version to be published; accepted as responsible for all aspects of the work, ensuring that issues related to the accuracy or completeness of any part of the work are duly investigated and resolved.

RESUMO

OBJETIVOS: *Descrever a incidência por gênero e região, a letalidade e os custos associados ao tratamento de fraturas do fêmur em idosos (≥ 60 anos) internados no Sistema Único de Saúde (SUS) do Brasil entre 2008 e 2018.*

MÉTODOS: *Estudo transversal, descritivo e retrospectivo das internações de idosos por fraturas do fêmur mediante análise dos dados secundários obtidos do Sistema de Informações Hospitalares do SUS (SIH/SUS) entre 2008 e 2018; para cálculo dos coeficientes epidemiológicos, utilizamos informações dos censos demográficos (2000 e 2010) do Instituto Brasileiro de Geografia e Estatística (IBGE).*

RESULTADOS: *Foram registradas 478.274 mil internações no período. A incidência foi 1,7 vezes maior no gênero feminino (média geral de 274,91/100.000 para mulheres e 161/100.000 para homens). A região sudeste obteve maior número absoluto de internações e na região sul apresentou a maior incidência média geral anual (224,02/100.000). O custo médio anual do SUS para o tratamento das fraturas de fêmur em idosos foi de R$ 99.718.574,30.*

CONCLUSÕES: *No período avaliado (2008-2018), as fraturas do fêmur em idosos apresentaram alta incidência (478.274 mil internações; 224,02 casos/100.000 idosos), predomínio do gênero feminino (1,7F/1,0M), maior número absoluto de internações na região sudeste e maior incidência na região sul; a letalidade foi elevada (aumento de 17,46%; média geral do coeficiente de 4,99%/ano); e, os custos para o SUS foram vultuosos (aumento de 126,24%, média anual de gastos de R$ 99.718.574,30).*

PALAVRAS-CHAVE: *Fraturas do fêmur. Serviços de saúde para idosos. Hospitalização. Política de saúde. Órgãos dos sistemas de saúde.*

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ORIGINAL ARTICLE

Intraocular pressure predicts premature coronary atherosclerosis

Mehmet Kaplan[1]
Ozge Ozcan Abacıoglu[2]
Fethi Yavuz[2]
Gizem Ilgın Kaplan[3]
Betül Düzen[4]
Nurbanu Bursa[5]
Ferhat Zorlu[6]

1. Gaziantep University School of Medicine, Department of Cardiology. 27100, Gaziantep, Turkey.
2. Adana City Training & Research Hospital, Department of Cardiology, Adana, Turkey
3. Ersin Arslan Training & Research Hospital, Department of Internal Medicine, Gaziantep, Turkey.
4. Ersin Arslan Training & Research Hospital, Department of Ophthalmology,, Gaziantep, Turkey.
5. Hacettepe University, Department of Statistics, Ankara, Turkey
6. Adana City Training & Research Hospital, Department of Ophthalmology, Adana, Turkey

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SUMMARY

OBJECTIVE: *The aim of this study was to investigate the association between intraocular pressure (IOP) and premature atherosclerotic coronary artery disease (PACAD) by comparing central corneal thicknesses (CCTs) measurements.*

METHODS: *One hundred-eighty-six subjects were enrolled in this cross-sectional study, 100 in the PACAD group and 86 in the control group. All participants underwent a physical examination and routine biochemical tests. Ophthalmological examinations, including IOP and CCTs measurements, were performed for each subject. Additionally, pulse wave velocity measurements were obtained and recorded.*

RESULTS: *Participants with PACAD showed significantly higher IOP values than those without atherosclerosis ($p = 0.001$), and there was no statistically significant difference between the groups in terms of CCT ($p = 0.343$). Also, pulse wave velocity (PWV) values were statistically significantly higher in the PACAD group ($p = 0.001$). High IOP was not significantly associated with metabolic syndrome parameters ($p > 0.05$).*

CONCLUSIONS: *A relationship was found between PACAD and IOP, but CCTs were not associated with PACAD. The IOP measurement is affected by CCT; therefore, CCT is used to correct IOP values. To our knowledge, this is the first study to report a positive relationship between PACAD and IOP based on CCTs measurements.*

KEYWORDS: *Intraocular pressure. Atherosclerosis. Glaucoma.*

INTRODUCTION

Glaucoma is a significant cause of irreversible blindness worldwide, and intraocular pressure (IOP) is a modifiable risk factor for glaucoma. IOP is the pressure exerted on the cornea and sclera by the aqueous humor that fills the anterior and posterior chambers, and the mean IOP is 16±3 mm Hg. In glaucoma, inflammation has been recognized as a common mechanism[1].

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CORRESPONDING AUTHOR: Mehmet Kaplan
Gaziantep University School of Medicine, Department of Cardiology. Tel/Fax: + 90 532 463 12 85 / + 90 342 3606060.
E-mail: kardiomehmet27@hotmail.com.

Coronary artery disease (CAD) has become increasingly important due to its prevalence, which has recently risen among younger people[2]. Premature atherosclerotic coronary artery disease (PACAD) has been defined in some studies as CAD occurring in individuals 40 years old or younger, but other studies used a lower age limit of 30 or 50 years[3]. For this study, we used 40 years of age and younger to define PACAD.

Studies have demonstrated that single-vessel involvement is more common in patients with premature CAD than in older patients[4]. In addition, patients with PACAD are more likely to have a family history of hypercholesterolemia, smoking, and CAD[5]. More careful management of risk factors has been recommended in premature CAD due to its poor prognosis[6]. In the present study, we aimed to determine changes in IOP measures and PWV in patients diagnosed with PACAD.

METHODS

Study sample

In this cross-sectional study, we analyzed 186 subjects in two groups: one group of 100 patients with PACAD and a control group of 86 healthy subjects. Approval for the study protocol was granted by the Adana City institutional review board and the study was conducted in accordance with the principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. The study exclusion criteria included a history of glaucoma, surgery, use of steroid drugs or diabetic retinopathy, known cardiac disease, renal dysfunction (glomerular filtration rate<15 L/min), chronic liver disease, rheumatic and hematological diseases, malignancies, active infection, known medication use or chronic conditions that require transfusions.

Data collection

The study was conducted with two groups, i.e., a PACAD group and a control group. Patients 40 years old or younger with a positive exercise test or ischemia detected by myocardial perfusion scintigraphy and >50% lesions in at least one vessel were defined as having PACAD. Obstructive CAD was defined as ≥50% stenosis in any epicardial coronary artery[7]. Individuals with normal coronary arteries as demonstrated by coronary angiography were included in the control group. Coronary lesions were evaluated by two independent cardiologists. All participants underwent a physical examination and biochemical tests. Blood samples were obtained after at least 10 hours of fasting. Hypertension (HT) was defined as systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg, or current use of antihypertensive medication[8]. Diabetes mellitus (DM) was defined as a fasting serum glucose ≥126 mg/dL, hemoglobin-A1C ≥6.5%, or the use of blood glucose lowering agents[9]. Hypercholesterolemia was defined as low-density lipoprotein (LDL) cholesterol level of ≥130 mg/dL[10]. The components of metabolic syndrome (MS) included a waist circumference of >102 cm in men or > 88 cm in women, triglycerides ≥ 150 mg per 100 ml, HDL < 40 mg per 100 ml in men or < 50 mg per 100 ml in women (Low-HDL definition), blood pressure ≥ 130/85 mmHg, and fasting glucose ≥ 100 mg per 100 ml. Metabolic syndrome was defined as having at least three of the five components[10].

Ophthalmological examinations were performed and included the best-corrected visual acuity, refraction, IOP measurement by non-contact and dilated fundus examination. A trained nurse took three consecutive measurements for each eye, and the average IOP of the right eye was used for analysis. Ocular hypertension was defined as an IOP ≥ 21 mm Hg in the right eye[1]. Five CCT measurements were obtained from each eye with a Sonomed ultrasound pachymeter (California, USA) and the median reading was taken.

Pulse wave measurement

After resting, the device was connected to the patient using the Mobil-o-Graph on the right brachial artery traction. PWV was measured with an oscillometric measuring device for at least ten cycles. Three measurements were taken for each patient at 5-minute intervals as recommended. Due to varying heart rates among participants and repeated individual measurements, a correction at a heart rate of 75 beats per minute was automatically calculated and recorded by software for the Augmentation index(AIx@75).

Statistical Analysis

Statistical analyses were performed using SPSS 23 for Windows (SPSS, USA). The distribution of data was investigated by using the Kolmogorov-Smirnov test. Continuous variables, according to their normal distribution or not, were presented as mean ± standard deviation or median ± standard deviation, respectively. Categorical variables were presented as percentages. In the comparison between PACAD and control groups,

the independent t-test or Mann-Whitney U-test was used for demographic characteristics, biochemical variables, and examinations. In addition, the Spearman correlation coefficient was used in order to analyze the degree of association between parameters. Binomial logistic regression analysis also was performed to evaluate the impact of IOP and PWV measurements on the odds ratio of the event of PACAD. In all analyses, a two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

A total of 186 participants 40 years old or younger were enrolled in the study, including 100 patients presenting clinical symptoms of stable angina pectoris and 86 age-matched healthy subjects with normal coronary arteries as detected by coronary angiography. The baseline demographic characteristics and laboratory findings of the groups are shown in Table 1. There was no significant difference in CCTs between the study and control groups but the IOP values were significantly greater in the PACAD group (p=0.001). Since no meaningful difference was observed between patients and the significance value of the analysis was very close to 0.05, we considered that the left ventricular ejection fractions did not differ significantly between the groups (p=0.043). However, significantly higher PWV measurements were found in the PACAD group (p=0.001) (Table 2). A high IOP was not significantly associated with MS parameters ($p > 0.05$). In table 2, because the IOP and PWV measurements were significant variables, the effects of these measurements on the odds ratio of the event of PACAD were examined with binomial logistic regression analysis. In the analysis, participants with PACAD were used as a reference, and the IOP and PWV measurements were statistically different from the reference. When IOP measurement rises 1 unit, a participant is 2.194 times more likely to belong to the PACAD group than to the control group. Similarly, when the PWV measurement rises 1 unit, a participant is 1.969 times more likely to belong to the PACAD group than to the control group (Table 3). These odds ratios indicate that the IOP and PWV measurements correlate positively with having PACAD.

DISCUSSION

The relationship between IOP and cardiovascular risk factors suggests that an increased IOP may be associated with cardiovascular disease. To our best knowledge, however, no study in the literature

TABLE 1. BASELINE DEMOGRAPHIC CHARACTERISTICS AND BIOCHEMICAL VARIABLES OF THE GROUPS

Demographic properties	PACAD (n=100)	Control (n=86)	p value
Age (years, mean)	34.7 ±4.5	34.3 ±7.5	0.860
Sex (female, %)	48 (48 %)	48 (56 %)	0.290
Hypertension (n, %)	20 (20 %)	24 (28 %)	0.206
Diabetes Mellitus (n, %)	19 (19 %)	19 (22 %)	0.602
Smoking (n, %)	35 (35 %)	16 (19 %)	0.012*
Hypercholesterolemia (n, %)	48 (48%)	22 (25 %)	0.042*
Family history for CAD (n, %)	36 (36 %)	24 (28 %)	0.386
Metabolic syndrome (n, %)	17 (17 %)	14 (16 %)	0.752
Biochemical variables			
Hemoglobin (g/dL)	12.9±1.7	12.8±0.9	0.438
Leukocyte ($\times 10^3$ /μL)	8.7 ±1.7	10.4±3.3	0.086
Platelet ($\times 10^3$ /μL)	309±90	346±315	0.600
Plasma fasting glucose (mg/dL)	84±11	87±11	0.403
Creatinine (mg/dL)	0.63±0.18	0.64±0.14	0.466
LDL-C (mg/dL)	109±30	110±28	0.727
HDL-C (mg/dL)	44±11	44±8	0.887
BNP (pg/mL)	43±16	37±19	0.853
High sensitivity CRP (mg/L)	1.3±0.6	1.0±0.7	0.063

Abbreviations: CA, coronary artery disease; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; BNP, brain natriuretic peptide. *p<0.05 is significant.

TABLE 2. OPHTHALMOLOGICAL AND CARDIOLOGIC EXAMINATION FINDINGS OF THE GROUPS

Ophthalmological examination	PACAD (n=100)	Control (n=86)	p-value
Intraocular pressure (mm Hg)	14.4 ±4.5	13.0±1.0	0.000*
Central corneal thickness (μm)	537±14	535±19	0.800
Ocular hypertension (n, %)	3 (%3)	1 (%1.5)	0.242
Cardiologic examination			
Ejection Fraction (%)	62.6±3	61.4±4	0.043*
Pulse wave velocity (m/s)	6 ±1.1	5.5±0.7	0.001*

*p<0.05 is significant.

TABLE 3. RELATIONSHIPS OF INTRAOCULAR PRESSURE AND PULSE WAVE VELOCITY WITH PACAD

Term	estimates with standard errors	OR	p-value
Constant)	-14.439±2.392	-	<0.001*
Intraocular pressure (mm Hg)	0.786±0.140	2.194	<0.001*
Pulse wave velocity (m/s)	0.677±0.224	1.969	0.002*

*p<0.05 is significant.

examined the relationship between IOP and the risk of cardiovascular disease. Longitudinal studies have shown that the IOP is influenced by diabetes, body mass index (BMI), and BP levels[11]. In the present study, we found elevated IOP in patients with PACAD.

It is well known that the aqueous humor production and outflow facility alters with age[12],[13]. Given the consistent association of IOP with systemic blood pressure (BP) levels and hypertension, it is clear that the influence of age and systemic BP on IOP is complex and cannot be examined in isolation[12]. In one study, individuals who had diabetes were hypertensive with higher BMI and higher SBP and DBP levels and exhibited higher IOP levels[14]. Several cohort studies and population-based cross-sectional studies consistently suggest that blood pressure (BP) and history of DM are positively correlated with IOP[15]. Furthermore, several studies have demonstrated the positive association between IOP and cardiometabolic risk factors or metabolic syndrome[16].

The mechanism underlying the relationship between BP and IOP is unclear based on the data currently available. However, it may be related to an increase in ocular perfusion pressure which is often associated with increased aqueous humor production[17]. In our study, there was no significant difference between HT and non-HT patients in terms of IOP. Obesity and other metabolic conditions could be considered for the possible shared pathophysiology between CAD and higher IOP. The result of one study showed increased LDL-C, decreased HDL-C, increased triglycerides, and obesity features based on BMI and waist circumference were associated with higher IOP[18]. Sahinoglu-Keskek et al.[19] investigated the relative importance of each component of MS and found that elevated IOP was affected by distinct metabolic variables in individual patient groups. However, the subgroups were also examined in detail with respect to the components of metabolic syndrome in our study, and no significant difference was found between the subgroups in terms of IOP and CCT.

Former studies have found that diabetic patients have greater CCTs compared to those without the disease, which may artefactually increase IOP readings depending on the IOP measurements[20]. Among diabetic patients, the CCT was significantly correlated with diabetic duration[21]. However, our study sample included 38 diabetic patients, and the CCT and IOP were not significantly different between DM and non-DM patients. Further studies are warranted to understand the effect of diabetes on the development of glaucoma.

Limited data are available on the link between IOP and CAD. In one study, higher levels of IOP were shown to be significantly associated with the presence of coronary artery calcium (CAC), independent of conventional cardiovascular risk factors. Interestingly, the association between IOP quartiles and the prevalence of detectable coronary calcium artery score was stronger in diabetic subjects, even though the interaction of IOP quartiles and diabetes for calcium artery score was not statistically significant. That study provided more insight into understanding the process of subclinical atherosclerosis in CVD and the relationship with higher IOP as a common pathophysiology[18]. The results of the aforementioned study are interesting and support our findings.

Considerable evidence suggests that systemic and ocular biometric parameters are affected by genetic and environmental factors. Our sample consisted of patients 40 years old or younger presenting with stable angina pectoris diagnosed with severe coronary artery disease, most of them without comorbidities. Higher IOP values found in these patients compared to the control subjects might be related to genetic and environmental factors or individual inflammatory response. Thus, PACAD patients under 40 years of age might be explained by pathophysiology that predominantly involves inflammation rather than a manifest risk factor.

In major studies, pulse pressure and PWV predicted cardiovascular events[22]. Since PWV is the most widely used and validated technique for estimating arterial stiffness, high PWV represents an early sign of arteriosclerosis/atherosclerosis[23]. Consistently, both IOP and PWV were elevated in patients with PACAD in our study. The higher value of IOP and PWV seems to support the inflammatory process.

CONCLUSION

Although there is a limited number of studies on IOP, we believe that, along with a clinical evaluation, IOP measurement can predict premature atherosclerosis.

Limitations: The cross-sectional design of the current study limits its ability to establish a causal relationship between PACAD and IOP. Further longitudinal studies are needed to clarify the relationship between PACAD and IOP.

Author's Contribution

Mehmet Kaplan: all parts. Ozge Ozcan Abacıoglu: conceptualization, formal analysis, methodology, supervision. Fethi Yavuz: data curation, formal analysis, investigation, visualization, writing -original draft. Gizem Ilgın Kaplan: methodology, software, visualization, writing - original draft. Betül Düzen: data curation, visualization. Nurbanu Bursa: formal analysis, methodology, software, validation. Ferhat Zorlu: data curation.

RESUMO

OBJETIVO: *O objetivo deste estudo é investigar a associação entre a pressão intra-ocular (PIO) e a doença aterosclerótica arterial coronariana prematura (DAACP) compar ando as medidas das espessuras corneanas centrais (ECCs).*

MÉTODOS: *Cento e oitenta e seis indivíduos foram incluídos no presente estudo transversal, 100 no grupo DAACP e 86 no grupo de controle. Todos os participantes foram submetidos a um exame físico e exames bioquímicos de rotina. Exames oftalmológicos, incluindo PIO e medições das ECCs, foram realizados em cada participante. Além disso, medições de velocidade da onda de pulso foram obtidas e registradas.*

RESULTADOS: *Os participantes com DAACP apresentaram valores de PIO significativamente maiores do que os daqueles sem aterosclerose (p = 0,001) e não houve diferença estatisticamente significativa entre os grupos em relação ECC (p = 0,343). Além disso, os valores das velocidades da onda de pulso (VOP) foram estatisticamente significativamente maiores no grupo DAACP (p = 0,001). Um valor elevado de PIO não estava significativamente associado com os parâmetros de síndrome metabólica (p > 0,05).*

CONCLUSÃO: *Encontramos uma relação entre DAACP e PIO, mas as ECCs não estavam associadas com DAACP. A medição da PIO é afetada pela ECC; portanto, a ECC é utilizada para corrigir os valores da PIO. Até onde sabemos, este é o primeiro estudo a relatar uma relação positiva entre DAACP e a PIO com base em medições da ECC.*

PALAVRAS-CHAVE: *Pressão intraocular. Aterosclerose. Glaucoma.*

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ORIGINAL ARTICLE

Vitamin D status influences cytokine production and MALAT1 expression from the PBMCs of patients with coronary artery disease and healthy controls

Peyman Nowrouzi-Sohrabi[1,2]
Mehdi Kalani[3]
Peyman Izadpanah[4]
Hassan Ahmadvand[5,6]
Masoumeh Fakhour[7]
Reza Fadaei[8]
Meghdad Khorshidifar[4]
Atefeh Seghatoleslam[1,9]

1. Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Cardiology Department, Shiraz University of Medical Sciences, Shiraz, Iran.
5. Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.
6. Razi Herbal Researches Center, Lorestan University of Medical Sciences, Khorramabad, Iran.
7. Faculty of Allied Medical Sciences, Zabol University of Medical Sciences, Zabol, Iran.
8. Sleep Disorders Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
9. Research Center for Traditional Medicine and History of Medicine, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.

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SUMMARY

OBJECTIVE: *This study aimed to investigate the long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (lncRNA MALAT1) expression and its role in cytokine production from peripheral blood mononuclear cells (PBMCs) in patients with coronary artery disease (CAD) and non-CAD participants (NCAD).*

METHODS: *Blood samples were taken from 15 patients with CAD and 15 NCAD individuals. The plasma was used for biochemical analyses. MALAT1 and CD36 expressions were evaluated in the isolated peripheral blood mononuclear cells (PBMCs) by real-time PCR. Furthermore, the levels of inflammatory cytokines e.g. interleukin (IL)-6, IL-10, and IL-22 were measured in the supernatants of the cultured PBMCs by flow cytometry.*

RESULTS: *The levels of MALAT1 and CD36 were not significantly different between the CAD and NCAD groups. However, a lower level of MALAT1 and CD36 was observed in PBMCs of vitamin D deficient (<15 ng/ml) CAD and NCAD participants. Furthermore, the vitamin D deficient (<15 ng/ml) group showed a significantly higher plasma level of IL-6, IL-10, and IL-22 compared to the non-deficient (≥15 ng/ml) group. In addition, significant positive correlations were found between CD36, IL-22, and fasting blood sugar (FBS) with MALAT1.*

CONCLUSION: *Given that in vitamin D deficient individuals a decreased level of MALAT1 was associated with CD36 expression and increased IL-22 production, vitamin D supplementation may play a role in reducing MALAT1/CD36/IL-22 mediated complications such as T2DM and CAD, especially in vitamin D deficiency.*

KEYWORDS: *Vitamin D. CD36 antigens. Interleukins. Coronary artery disease. RNA, long noncoding.*

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CORRESPONDING AUTHOR: Atefeh Seghatoleslam
Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran – P. O. Box: 71348-45794
Tel: +98 917 319-7091 / Fax: +98 71 3230-3029
E-mail: seghatolea@sums.ac.ir

INTRODUCTION

Vitamin D deficiency is associated with both calcium and bone metabolism disorders, as well as vascular and low-grade inflammatory disease[1,2]. Some long non-coding RNAs (lncRNAs), such as lncRNAs HOTAIR and H19, have been found to be regulated by vitamin D through vitamin D receptors in multiple cancers. LncRNAs are transcripts, longer than 200 nucleotides without protein-coding ability, which are involved in both transcriptional and post-transcriptional regulation, and therefore, in the pathophysiology of diseases such as coronary artery disease (CAD)[3]. Amongst the reports, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as NEAT2, might protect against the occurrence of CAD and alleviate inflammation in atherosclerosis. The role of the scavenger receptors B1 or CD63 as receptors for oxidized low-density lipoproteins (ox-LDL) is vital in foam cell formation, which is a critical step in the development of atherosclerosis. Moreover, a recent in-vitro study reported the influence of MALAT1 on the expression of CD36[4].

Previously, the role of inflammatory cytokines has been well evaluated. Interleukin (IL)-22, a member of the IL-10 related cytokine superfamily, has a dual role in inflammation. Gong et al.[5] suggested IL-22 functions as a double-edged sword in type 2 diabetes mellitus (T2DM) and CAD.

Consequently, given the inflammatory nature of atherosclerosis and the role of MALAT1 and CD36 in atherosclerosis, the present study aimed to assess MALAT1/CD36 levels as well as cytokines, such as IL-6, IL-10, and IL-22 in CAD patients compared to healthy controls. However, little is known about the regulation and functions of lncRNAs in the treatment and prevention of cardiovascular diseases with vitamin D.

METHODS

Participants

A cross-sectional study included 15 CAD patients and 15 non-CAD (NCAD) or healthy individuals who were admitted to the Al-Zahra Heart Hospital, Shiraz, Iran, from October 2018 until January 2019, requiring coronary angiography (CA) or CT angiography (CTA). Based on the CA and CTA results, the study population was divided into CAD and NCAD groups. CAD was diagnosed by a cardiologist if there was more than 50% stenosis in at least one coronary artery.

The serum level of 25-hydroxy vitamin D was measured in these two groups and classified as deficient (<15 ng/mL) and non-deficient (≥15 ng/mL)[6,7]. Patients with diabetes mellitus, malignancies, infections, blood diseases, and chronic renal or liver failure, history of inflammatory diseases, and those using immunosuppressive drugs were excluded from the study. Demographic and anthropometric data including age, body mass index (BMI), as well as data regarding medication usage by patients were collected via a questionnaire, and using the hospital patient records.

Laboratory assessments

The biochemical characteristics including fasting blood sugar (FBS), lipid profile, and liver enzyme activities were measured using commercially enzymatic kits by BS 200 autoanalyzer (Mindray, China). Vitamin D was measured using the HPLC method by Tosoh G8 instrument (Tosoh, USA).

PBMCs isolation and cell culture

PBMCs were isolated from 10 ml peripheral blood samples by centrifugation over Ficoll-Hypaque gradients (Lymphodex, InnoTrain, Germany). The cells were washed, using RPMI 1640 medium and resuspended in RPMI-1640 medium supplemented with 50 U/mL penicillin, 50 µg/mL streptomycin, and 10% fetal bovine serum. In order to evaluate the MALAT1 expression level, 2 x 10^6 cells were incubated for 5h at 5% CO_2, 95% humidity, and 37°C. Simultaneously, additional 10^5 cells were incubated for 48h to assess the cytokines level; hence, supernatants were collected and stored at -80 °C until further analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from 2 x 10^6 cells, using the Trizol reagent (Bio Basic Inc., Canada) and then converted into cDNA in reverse transcription reactions using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) based on the manufacturer's instructions.

Quantitative Real-Time PCR

Equal amounts of cDNA for each sample were amplified using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) and specific primers for MALT1, CD36, and beta-actin as a reference gene, based on the manufacturer's protocol. The relative expressions were calculated using $2^{-\Delta CT}$ method.

Cytokine assay

The supernatants of the cultured PBMCs were used to assess the IL-6, IL-10, and IL-22 levels. Cytokines were measured by LEGENDplex™ Human Th22 Panel (Biolegend, USA) using a flow cytometer (FACS Calibur, BD, USA), according to the manufacture's instruction.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 (IBM Inc., USA) and GraphPad Prism version 8.2 (San Diego, CA, USA). The categorical data were tested by Fisher exact test and presented by frequency and percentile. Continuous variables are presented as mean ± standard error of the mean (SEM) and tested by the Mann-Whitney U test and one-way ANOVA. The Spearman correlation test was used to determine the relationship between the variables. A P value < 0.05 was considered statistically significant.

RESULTS

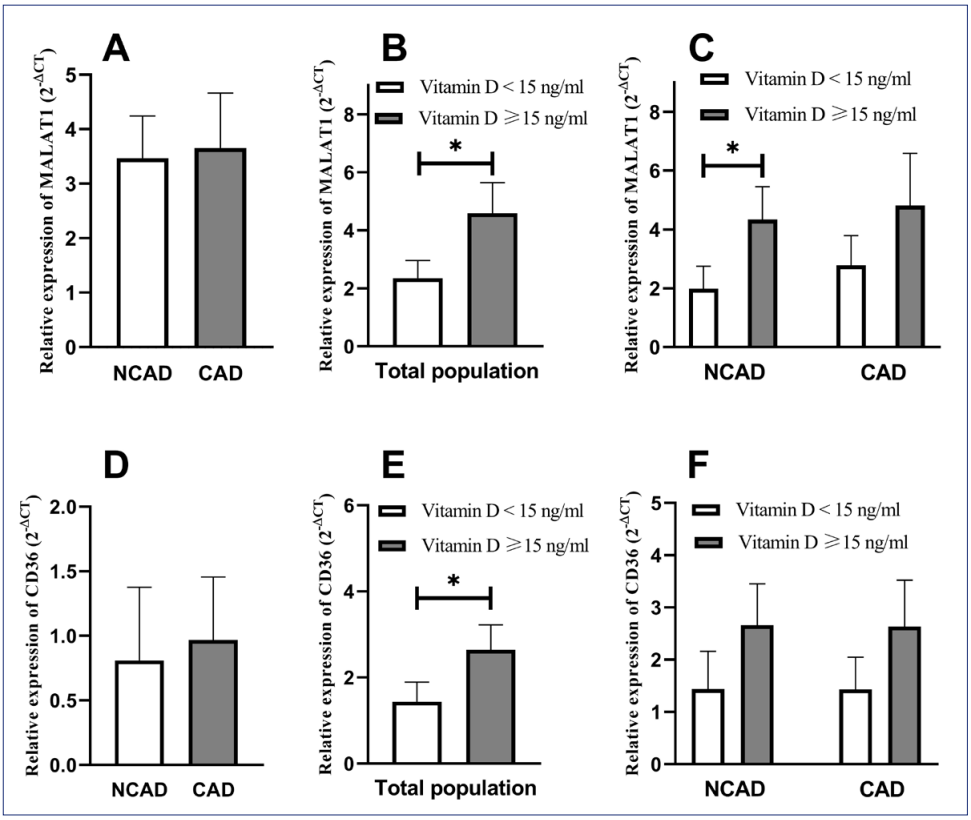
Clinical and laboratory findings of the participants

The demographic and clinical characteristics of participants are shown in Table 1. Hip circumference, echocardiogram ejection fraction, and diastolic blood pressure were significantly lower in patients with CAD compared to NCAD. Furthermore, body mass index, hip circumference, and AST activity were significantly higher in the vitamin D deficient group compared to the non-deficient. For a detailed description, see Table S1 in Supplementary Materials.

The comparison of MALAT1 and CD36 levels between the investigated groups

We first analyzed MALAT1 and CD36 in the PBMCs of CAD and NCAD individuals. We found that there was no significant difference between the CAD and NCAD groups (p=0.238 and p=0.945) (Fig 1A and D). Interestingly, after classification into non-deficient and deficient groups, we noticed that MALAT1 and CD36 expressions were significantly lower when there was vitamin D deficiency (Fig 1B and 1E) (p=0.015 and p=0.022). In addition, the comparison of the MALAT1 levels between the CAD and NCAD groups based on the vitamin D status showed that MALAT1 expression was significantly higher in the NCAD non-deficient group compared to the NCAD deficient group (p=0.023). However, the difference between CAD non-deficient and deficient groups was not statistically significant (Fig 1C).

FIGURE 1



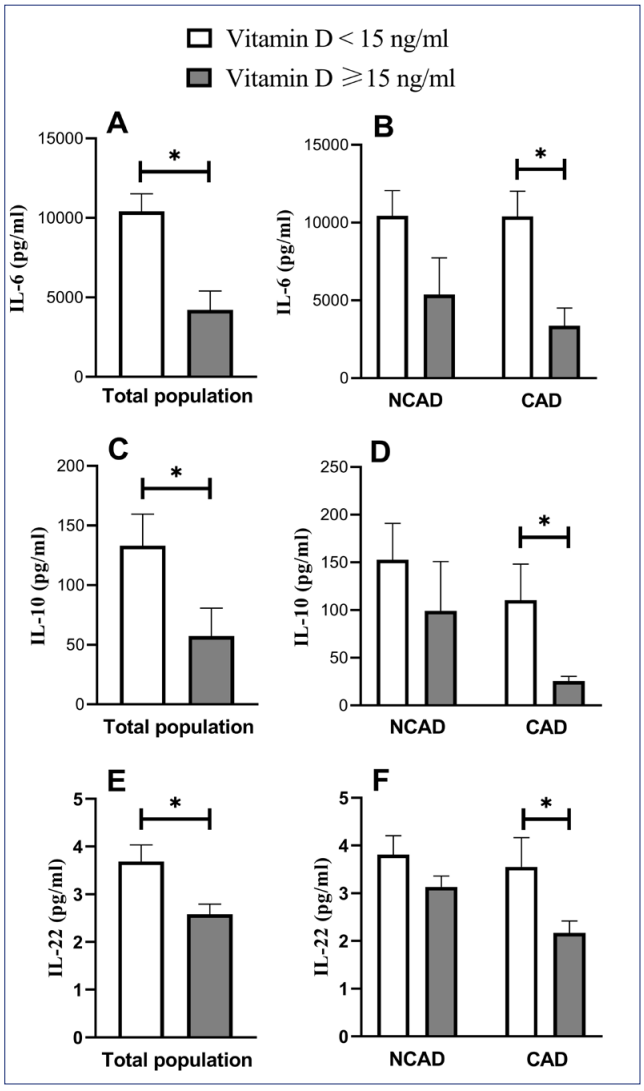
Comparing the cytokine production levels between the study groups

The *ex-vivo* cytokine profiles produced by PBMCs are depicted in Fig 2. The results showed that the production of IL-6, IL-10, and IL-22 were significantly higher in the vitamin D deficient group compared to the non-deficient group (p=0.006, p=0.014, and p=0.018, respectively) (Fig 2A, C and E). When CAD and NCAD individuals were divided based on the vitamin D status, the cytokines levels were significantly higher in the CAD deficient group compared to that in the CAD non-deficient group (p=0.021, p=0.009, and p=0.040, respectively) (Fig 2B, D, and F).

Association between clinical and biochemical characteristics with MALAT1

As presented in Table 2, the spearman correlation test revealed a significant correlation between the MALAT1 and CD36 expressions in the PBMCs of the studied groups. In order to evaluate the association of MALAT1 expression with cytokine production from PBMCs, we analyzed the correlation between MALAT1 expression and cytokine level. MALAT1 showed a significant negative correlation with IL-22 in the total population of the CAD and NCAD groups (r=-0.406, p=0.036). There were no significant correlations between MALAT1 and other cytokines. In addition, MALAT1 expression in PBMCs of the NCAD group correlated directly and indirectly with FBS (r=0.606, p=0.022) and vitamin D (r=-0.693, p=0.006). For a detailed description, see Table S2 in the Supplementary Materials.

FIGURE 2



DISCUSSION

In this study, we observed that the relative expression of MALAT1 and CD36 were comparable between CAD patients and healthy controls. However, dysregulated expression of MALAT1 and CD36 has been reported in different pathological conditions. The unregulated expression of MALAT1 was reported from the PBMCs of type 2 diabetics and patients with MI[8]. However, the function of MALAT1 in inflammation related to vitamin D status and CAD remains unknown. Accordingly, this study investigated the effects of vitamin D status on the expression of MALAT1 as well as cytokines IL-6, IL-10, and IL-22 from the PBMCs of the patients with CAD as well as NCAD individuals.

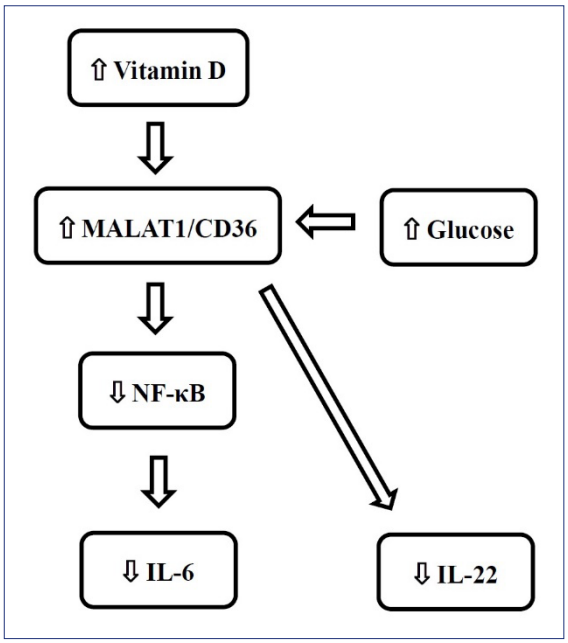
The results revealed that although MALAT1/CD36 expression was higher in the CAD group, upregulated levels of MALAT1/CD36 were not significantly associated with CAD. A similar finding to our study was reported by Toraih et al.[9], who reported that MALAT1 levels, the median MALAT1 expression in the PBMCs of patients with previous cardiac events was comparable with those of control groups. However, MALAT1 is identified and characterized as a regulator of inflammatory response *via* TLR4/ TLR4/NF-κB[8].

Some controversy still exists regarding CD36 expression levels in CAD[10,11]. In the present study, CD36 was highly correlated with MALAT1. According to previous studies, it is suggested that MALAT1 might be a contributing factor to CD36 upregulation in the PBMCs of CAD patients. In this line, Huangfu et al.[4] reported that MALAT1 through the accumulation of beta-catenin on the CD36 promoter raises CD36 transcription.

Interestingly, we observed that the level of MALAT1 was significantly lower in the PBMCs of vitamin D deficient individuals compared to that in non-deficient ones. To the best of our knowledge, this is the first study to investigate the association between vitamin D status and MALAT1 expression. Based on these results, regardless of the CAD status, MALAT1 expression is increased in the PBMCs of vitamin D deficient individuals. The molecular mechanisms underlying increased expression of MALAT1 in the non-deficient group are not well understood; however, the anti-inflammatory effect of vitamin D can be a determining factor. In several studies, the association between MALAT1 and inflammation has been reported[8]. However, the protective effects of vitamin D on colorectal cancer through the modulation of some lncRNAs have been previously suggested[12].

Accordingly, the upregulation of MALAT1 could be proposed as a contributing factor to the anti-inflammatory effects of vitamin D. As a detailed description, regarding the results, vitamin D deficiency was significantly associated with a higher production level of cytokines including IL-6 (2.5 fold), IL-10 (2.3 fold), IL-22 (1.4 fold) compared to the non-deficient group (Fig 2). The effects of vitamin D on the level of inflammatory cytokines have already been investigated. Vitamin D might inhibit the production of pro-inflammatory cytokines (IL-6 and TNF-α) via directly inhibiting the NF-*k*B signaling pathway (Fig 3)[13]. Importantly, in this study, a significant negative correlation was also found between MALAT1 and IL-22.

FIGURE 3



Furthermore, based on the present and previous studies, we found that FBS increases the expression of MALAT1 in the PBMCs of CAD patients[14]. In addition, MALAT1 expressions were negatively correlated with the production of IL-22. To the best of our knowledge, this is the first study to investigate the association between MALAT1 and IL-22. The molecular mechanisms underlying this correlation are not well understood; however, different factors might be involved, such as hyperglycemia, especially because a significant direct correlation has also been observed between IL-22 and FBS (Fig 3). Furthermore, the increased level of IL-22 in the vitamin D deficient group might be partly through MALAT1 (Fig 3).

So far, few studies have been performed on the role of IL-22 in CAD. Shen et al.[15] reported that plasma concentrations of IL-22 are decreased in impaired fasting glucose (IFG) and T2DM patients, and decreased plasma concentrations of IL-22 is an independently susceptible factor for IFG and T2DM. Decreased plasma IL-22 levels increase the morbidity of diabetes.

In this line, Puthanveetil et al.[14] incubated human umbilical vein endothelial cells with high glucose levels, which led to an increase in MALAT1 expression after 12h. IL-22 protects endothelial cells from glucose-induced injury. All these findings suggest that the MALAT1 correlation with IL-22 might be a potential target for the treatment of chronic inflammatory diseases, such as T2DM and CAD[5].

CONCLUSION

The results of the present study suggest that MALAT1 expression is decreased in the PBMCs of the vitamin D deficient group, and this decreased expression appears to be associated with CD36 expression and increased IL-22 production from PBMCs. The protective effects of IL-22 against hyperglycemia and the observed correlation of MALAT1 with CD36 and FBS in the CAD group suggest that MALAT1/CD36/IL-22 might be also potential targets, using vitamin D supplementation for treating vascular complications mediated by hyperglycemia in T2DM and CAD especially in vitamin D deficiency.

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Compliance With Ethical Standards

This study was approved by the local Ethics Committee of the Shiraz University of Medical Sciences (IR.SUMS.REC.1397.687). After explaining the study objectives, written informed consent was obtained from the participants.

Conflict of Interests

The authors have no conflicts of interest to declare.

Author's Contribution

P.N: Performed all experiments, analyzed the data, and wrote the manuscript. P.I., M.Kh.: Analyzed the CA and CTA reports. P.N., H.A., R.F.: Contributed to concept and design. M.F.: Contributed to sampling and experiments. A.S., M.Ka: Contributed to the concept and design, financial support, supervising practical performances, and final approval of the manuscript. All authors read and approved the final manuscript.

RESUMO

OBJETIVO: *O objetivo deste estudo foi investigar a expressão do RNA longo não codificante lncRNA MALAT1 e o seu papel na produção de citocinas a partir de células mononucleares do sangue periférico (PBMCs) em pacientes com doença arterial coronariana (DAC) e participantes sem DAC (NDAC).*

MÉTODOS: *Amostras de sangue foram coletadas de 15 pacientes com DAC e 15 indivíduos NCAD. O plasma foi usado para análises bioquímicas. As expressões de MALAT1 e CD36 foram avaliadas nas células mononucleares do sangue periférico (PBMCs) isoladas por PCR em tempo real. Além disso, os níveis de citocinas inflamatórias, como a interleucina (IL)-6, IL-10 e IL-22 foram medidas no sobrenadante da cultura de PBMCs por citometria de fluxo.*

RESULTADOS: *Os níveis de MALAT1 e CD36 não foram significativamente diferentes entre os grupos DAC e NDAC. No entanto, um nível inferior de MALAT1 e CD36 foi observado nas PBMCs de participantes com deficiência de vitamina D (< 15 ng/ml) tanto no grupo DAC quanto no NDAC. Além disso, o grupo com deficiência de vitamina D (< 15 ng/ml) apresentou um nível plasmático significativamente maior de IL-6, IL-10 e IL-22 em comparação com o grupo sem a deficiência (≥15 ng/ml). Além disso, foram encontradas correlações positivas significativas entre CD36, IL-22, e glicemia de jejum (GJ) e o MALAT1.*

CONCLUSÃO: *Dado que em indivíduos com deficiência de vitamina D a diminuição do nível de MALAT1 foi associada com a expressão de CD36 e produção aumentada de IL-22, a suplementação de vitamina D pode ter um papel importante na redução de complicações mediadas por MALAT1/CD36/IL-22, tais como DMT2 e DAC, especialmente em casos de deficiência de vitamina D.*

PALAVRAS-CHAVE: *Vitamina D. Antígenos CD36. Interleucinas. Doença da artéria coronariana. RNA longo não codificante.*

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REVIEW ARTICLE

Uses and limits of the clinical laboratory in the COVID-19 pandemic: a didactic review

Fernando Antonio Glasner da Rocha Araujo[1]

1. Professor Associado, Departamento de Medicina Interna e Apoio ao Diagnóstico, Faculdade de Medicina da Bahia, Universidade Federal da Bahia, Salvador, BA, Brasil.

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SUMMARY

The world is currently experiencing an unprecedented pandemic of a new disease, the coronavirus disease (COVID-19), which has unusual clinical and immunological presentations. This is especially true regarding the choice and interpretation of laboratory test results. In this review, we have provided didactic information for physicians on the current concepts and practical guidance regarding COVID-19.

KEYWORDS: *Severe acute respiratory syndrome - coronavirus 2, COVID-19, clinical pathology, clinical diagnosis, laboratory.*

INTRODUCTION

The Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 was first identified after an outbreak of pneumonia of unknown etiology in Wuhan, Hubei Province, China in December 2019.[1]

Laboratory tests can play different and important roles in medical decision making during the current COVID-19 pandemic by providing the following:

- Etiological diagnosis of the disease
- Serological diagnosis of the disease
- Immunological status evaluation
- Severity and/or prognosis indicators

The choice of test depends on the inherent characteristics of the test (sensitivity, specificity, and predictive values), the characteristics of the target population (prevalence, incidence, and pre- and post-test probability), and a combination of both (likelihood ratio).

Since COVID-19 is a novel disease, the current knowledge regarding its characteristics, especially laboratory characteristics, is limited. In this review, we have provided didactic information on the current concepts and practical guidance regarding COVID-19 based on the current knowledge to help physicians choose laboratory tests and interpret their results based on different clinical presentations.

METHODS

A literature review was performed to identify studies that met the objective of the research. The following strategies were used. First, a literature search was performed in PubMed and SciELO using the terms SARS-CoV-2, COVID-19, laboratory, and interpretation. Second, an active Google database search was performed using the specific terms SARS-CoV-2, COVID-19, laboratory, interpretation, polymerase chain reaction, and serology. Third, websites of several national (such as Ministry of Health, Brasil and Scientific Societies of Clinical Pathology and Infectious Diseases) and international (such as

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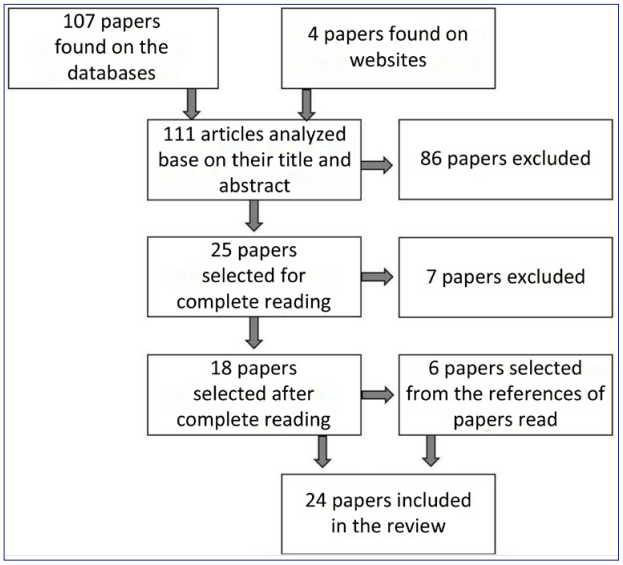
CORRESPONDING AUTHOR: Fernando Antonio Araujo
Faculdade de Medicina da Bahia. Praça XV de novembro, s/n - Largo do Terreiro de Jesus, Salvador, BA, Brasil – 40026-010
Tel: +55 71 3285-5566
E-mail: faraujo.br@hotmail.com

CDC-USA) scientific and government entities were searched for recommendations or practice guidelines. Lastly, articles cited in the previously selected articles and strategies were explored. We searched for articles published in 2020 in English, Portuguese, or Spanish.

RESULTS AND DISCUSSION

Using the aforementioned search strategy, 107 articles were initially found in the databases and 6 on the websites. After excluding the articles that did not meet the study criteria and including 4 articles referenced in previously selected articles, 24 articles were finally analyzed (Figure 1).

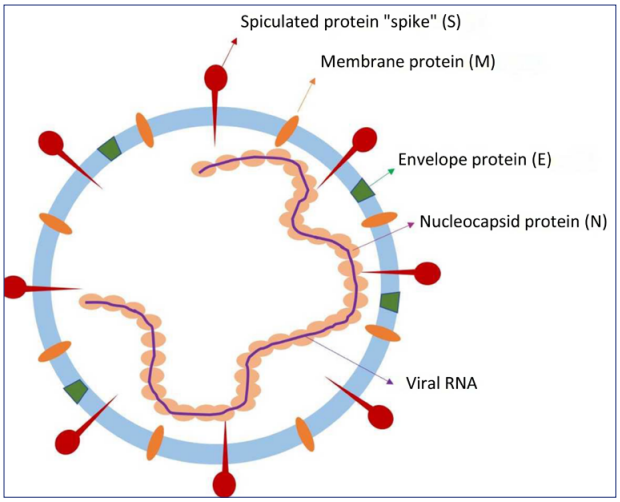
FIGURE 1



Etiological diagnosis (molecular methods)

The structure of SARS-CoV-2 (Figure 2) consists of a single RNA ribbon in the center surrounded by a nucleocapsid (N). This structure is wrapped in a lipid-membranous layer (M) that has different proteins, such as envelope (E) and spiculated or spike (S) proteins, which gives the virus the appearance of thorns or tips of a crown (origin of the term *corona*).[2] Molecular tests are based on the identification of the genes that encode these proteins using reverse transcription-polymerase chain reaction (RT-PCR). Currently, the genes used for identification are those coding for E, N, S, and RNA-dependent RNA polymerase. The tests usually identify more than one genetic marker, and most of them search for genes coding for N and E.

FIGURE 2



Reverse transcriptase-polymerase chain reaction

RT-PCR is the most widely used method for genetic identification of the virus and is considered the gold standard.

The ideal use of this test depends on factors such as the time and site of sample collection.

Kucirka et al.[3] reported that the best time to collect samples for SARS-CoV-2 RT-PCR is between the third and fourth days after symptom onset (Table 1). Further, they reported that all patients tested in their pre-symptomatic phase were negative; therefore, testing asymptomatic patients would not be justified. However, Sakurai et al.[4] observed that during the COVID-19 outbreak on the Diamond Princess ship, 712 of the 3,711 people aboard tested positive for COVID-19. Moreover, 410 of 712 (58%) people were asymptomatic at the time of sample collection. These two reports, although containing contradictory findings, represent two distinct pre-test conditions. The first includes patients who, with no history of suspicious contact, wish to undergo the RT-PCR test to know if they were infected. The second includes an important precedent of prior contact. Therefore, only the latter scenario would justify the testing of asymptomatic individuals, considering that about half of them would test negative. The duration from the initial test to symptom onset was a mean of 4 days (range, 3–7 days).[4]

Furthermore, only 8 of 32 (25%) people on the Diamond Press ship who were cabin companions of COVID-19-positive patients subsequently tested positive.[4] This justifies the frequent observation of different results among family members or people sharing the same household with COVID-19-infected persons.

Almost all published studies are based on the collection of nasopharyngeal samples, wherein the sensitivity varies between 78% (for 1 test) and 86% (for 2 tests), with a specificity of about 99%. Some authors have highlighted the increase in sensitivity for COVID-19 diagnosis when RT-PCR is used in combination with chest computed tomography (sensitivity of 91.9%).[5]

A positive RT-PCR test probably means the actual presence of viral infection (true positivity), since false-positive results are very rare. False-positive results are most likely due to a processing error with the contamination of the tested sample. Test results that are not false-positive effectively identify viral genetic materials; however, it is necessary to consider the possibility of the occurrence of these materials even in the absence of viral replication, since the virus could be in an inactive state.[6]

The main limitation of this examination is the significant number of false-negative results because of diverse reasons such as:

- The viral load in secretions and excretions depends on the stage of infection; it is lower in samples collected less than 3 and more than 10 days after the onset of infection (Table 1).[3]
- The classical collection sites (nose and oropharynx) tend to show less positive tests than those collected in the lower respiratory tract (such as bronchoalveolar lavage); however, the collection technique of the latter is more complex and not available in most laboratories (Table 2).[7]
- Degradation of the sample during transportation and storage before analysis.

Many studies have reported the detectable presence of viral particles in the saliva, blood, feces, and urine; however, there are no routine protocols for diagnosis using these viral particles.[8] Viral particle detection in the urine is very rare.[9]

A possible explanation for the difference in positivity between these different samples is the time taken to express viral replication. Most of the current knowledge comes from RT-PCR test results from the analysis of samples collected from the nasopharynx. Wölfel et al.[10] described that sputum samples show positivity even after the virus is no longer detected in nasopharynx samples on RT-PCR. Zhang et al.[9] demonstrated that the RT-PCR test results for fecal samples remain positive for a longer period than those for nasopharyngeal samples.

The reason why viral material tarries for more than 14 days in some samples and its relationship with the possibility of disease transmission is unclear.

Generally, since the occurrence of false-positive results is very rare, a positive RT-PCR test result can be considered a case of SARS-CoV-2 contamination. However, when analyzing a strongly suspected COVID-19 case (high pre-test probability) that presents a negative RT-PCR test result, it is recommended to repeat the test at least once, and if possible, it is recommended to collect the sample from the lower respiratory tract.[1,11] Even if the results are negative, considering the high pre-test probability, it is better to isolate the patient.[11]

Watson et al.[11] calculated pre-test and post-test probabilities using the sensitivity (70%) and specificity (95%) data for RT-PCR in the literature (Table 3).

TABLE 1. PREVALENCE OF FALSE-NEGATIVE RT-PCR TEST RESULTS FOR SARS-COV-2 BASED ON THE TIME OF SYMPTOM ONSET

Time	False-negative results
4 days prior to symptom onset	100%
1 day after symptom onset	67%
3 days after symptom onset	20%
4 days after symptom onset	21%
16 days after symptom onset	66%

Kucirka et al., 2020[3]

TABLE 2. SENSITIVITY BY COLLECTION SITE IN SARS-COV-2 CARRIERS

Collection site	Sensitivity
Bronchoalveolar lavage	93%
Sputum	72%
Nasopharynx	63%
Oropharynx	32%

Wang et al., 2020[7]

TABLE 3. POST-TEST PROBABILITY OF POSITIVE AND NEGATIVE RT-PCR RESULTS

Pre-test probability	Post-test probability of 1 negative result	Post-test probability of 2 negative results	Post-test probability of 1 positive result
5%	1.6%	0.5%	42%
15%	5%	2%	71%
25%	10%	3%	82%
50%	24%	9%	93%
75%	49%	23%	98%
90%	74%	47%	99%

Watson et al., 2020[11]

Reverse transcriptase loop-mediated isothermal amplification polymerase chain reaction

Although less common than RT-PCR, the RT loop-mediated isothermal amplification PCR (LAMP) technique has some advantages such as faster execution time, simpler reading (visual), the possibility of measurement at the point of care, and the possibility of running more tests simultaneously.[12] Moreover, it presents a high specificity for SARS-CoV-2, and no cross-reaction with other coronaviruses (such as HCoV-229E, HCoV-NL63, HCoV-OC43, and MERS-CoV), influenza viruses (such as type B, H1N1pdm, H3N2, H5N1, H5N6, H5N8, and H7N9), or other respiratory viruses (such as RSVA, RSVB, ADV, PIV, MPV, and HRV).[13]

However, this technique needs higher viral loads, which reduces its detection limit, and is qualitative.[6]

At first, this would be the technique of choice for population use.

Serological diagnosis

Serological tests are based on the detection of antibodies produced against viral antigens. These tests can detect total or specific antibodies (IgM, IgG, and less commonly, IgA).

Antibody detection depends on the time elapsed since infection onset. IgA and IgM antibodies are usually detectable in the first 7 to 10 days of infection, whereas IgG can be detectable after about 10 to 15 days of infection. Ideally, these antibodies peak after the third or fourth week of illness.[14]

Patients whose clinical presentations are compatible with suspected COVID-19 and those with RT-PCR-confirmed COVID-19 present a positive antibody testing rate after 14 days of disease onset ranging from 50% to 100% (mean 72%) for IgM and 64.7% to 100% (mean 91%) for IgG. In other words, even in patients positive for COVID-19 on RT-PCR, about 10% do not present positive IgG; they constitute the so-called "false negatives" (Table 4).[15]

The reason why these patients present no seroconversion is unknown. These patients seem to show a similar tendency to that of those who develop no anti-HB antibodies even after repeated immunization attempts with a hepatitis B vaccine.

The percentage of positive antibody tests does not seem to depend on clinical severity.[15]

In addition to false-negative results, false-positive results can occur by cross-reaction with other viruses. This is more common when IgM or IgG titers are very close to the cutoff point. In the case of false-positive results, it is recommended to repeat serum tests after 2 weeks. In the case of a true-positive reaction, a significant increase in IgG titers (double or more) is expected. In false-positive cases, IgG tends to be negative in the second sample analysis.

TABLE 4. PERCENTAGE OF IGG SEROCONVERSION BASED ON THE TIME FROM INFECTION

1st author	Technique	IgG		
		Yes	No	%
Gao	CLIA, ELISA, GICA	14	0	100%
Jiang	Proteome microarray	29	0	100%
Yong	GICA	35	3	92%
Liu	In-house kit	131	2	98%
Long	MCLIA	285	0	100%
Lou	ELISA, LFIA, CMIA	75	5	94%
Pan	ICG strip	65	2	97%
To	EIA	16	0	100%
Zhao	ELISA	112	61	65%
TOTAL	TOTAL	762	73	91%

Flodgren et al., 2020[15]

Remote Laboratory Tests (rapid tests)

Remote laboratory tests (RLTs) or point-of-care tests, also known as rapid tests, are performed outside the laboratory setting. They are aimed at rapidly screening for the presence of antibodies and do not require the expertise of trained personnel. Generally, they are based on immunochromatography techniques using whole blood on substrates assembled in molded plastic (soap type).

Their simple interpretation, easy technique, and rapid performance make them, at least theoretically, a very useful diagnostic tool (Figure 3). However, the performance characteristics reported by the manufacturers during validation are not standardized. Moreover, they do not provide information on the characteristics of the population tested. Many samples have presented inappropriately low-reliability data, resulting in tests with low accuracy and making their usefulness in clinical practice unfeasible.[16]

For these reasons, the World Health Organization recommends the use of RLTs for research purposes only, including public health surveys. Rapid tests are used to estimate disease seroprevalence in a given population.[17]

Automated laboratory tests

Automated laboratory tests are performed inside the laboratory using automated analytical equipment

by trained personnel and ideally under the supervision of an experienced clinical pathologist. These tests are usually quantitative and have higher quality (accuracy, reproducibility, sensitivity, and specificity) than rapid tests.

Several methodologies have been used, but the most common are enzyme-linked immunosorbent assay (ELISA), chemiluminescence (CLIA), and electrochemiluminescence (ECL).

A comparison between these techniques shows that ECL is faster to implement, more sensitive, and more specific than the other automated techniques.[18] However, the technique identifies total antibodies, without distinguishing between IgM or IgG classes.

ELISA and CLIA, although less sensitive, distinguish between antibody classes. Therefore, it would be ideal to initially use ECL, and in positive cases, perform the ELISA or CLIA.

Immunological status evaluation

Typically, the presence of antibodies is interpreted as follows. IgM: These are usually interpreted as indicators of the initial phase of the immune response and, therefore, of recent infection. However, with the introduction of increasingly sensitive tests, it has become common to detect IgM antibodies weeks and even months after infection. It is also necessary to consider that antibodies of this class are less specific than IgG. Therefore, IgM positivity may be related to a cross-reaction with antigens of other viruses. IgG: These are usually interpreted as indicators of patient immunization; however, with COVID-19, this interpretation has been questioned. In addition to the large number of patients who are not IgG positive, it has been described that infected asymptomatic patients produce less-lasting antibodies.[19] The implications of these findings regarding the nature or duration of immunity and the efficacy of response to new viral attacks in the future is unclear.

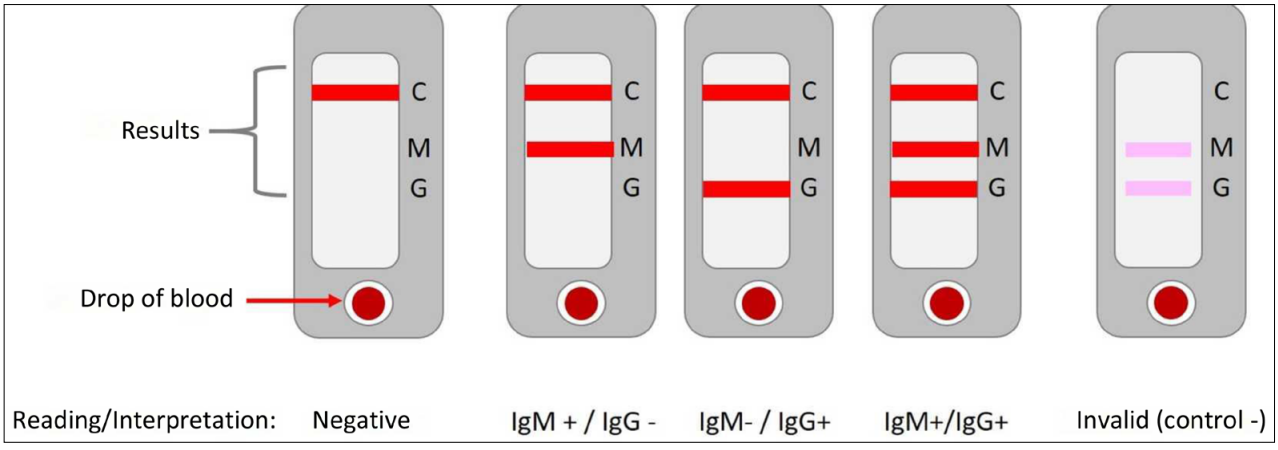
A possible explanation for differences between IgG positivity and immune status, in Covid-19, is the type of target antigenic determinant the antibodies produced. Sethuraman et al.[14] stated that most antibodies produced (and detected in assays) are directed toward the most abundant viral protein—the N protein. Thus, tests detecting the N protein are more sensitive; nonetheless, these are possibly not neutralizing antibodies and they may not indicate immunity. Conversely, antibodies directed toward the receptor-binding domain of the S protein are more specific and possibly neutralizing.

Atypical behaviors of anti-SARS-CoV-2 antibodies have been reported by Brazilian clinical pathologists[20]:

- IgM antibodies tarry for more than 7 weeks, with no set time for negativity
- False-negative or indeterminate IgG results up to 50 days after the symptom onset with RT-PCR positivity
- IgG results that become positive 20 days after symptom onset, with slow growth and no prediction of reaching the IgG concentration plateau
- Some patients present no IgM positivity even in the active phase of infection

In addition to the clinical diagnosis of recent infection (IgM positivity) or immunization (IgG positivity) commonly used by doctors to follow-up cases, the Brazilian Society of Clinical Pathology/Laboratory Medicine[21] highlights the following situations wherein these tests may be useful:

FIGURE 3



- The diagnosis of hospitalized patients with late clinical presentation (after the seventh day of symptom onset) as the first option before the PCR reaction. However, a negative result in this context does not rule out the diagnosis of COVID-19 and specific molecular testing (RT-PCR) is recommended.
- Return to work evaluation for health professionals from the seventh day of symptom onset. As previously stated, a negative result does not exclude the diagnosis of COVID-19 and RT-PCR is recommended.

Severity and/or prognosis indicators

Ruan et al. described mortality predictors in 150 patients with COVID-19. Some of the clinical predictors were mean age (67 vs. 50 years), presence of comorbidities (especially cardiovascular disease, renal failure, respiratory failure, and associated infections), and disease severity (need for ICU admission and life support). The laboratory parameters associated with a fatal outcome described were leukocytosis (10,620 vs. 6,760 /mm^3), lymphopenia (600 vs. 1,420 /mm^3), thrombopenia (173,600 vs, 222,100 /mm^3), evidence of renal failure (increased blood urea nitrogen and serum creatinine levels), changes in muscle enzyme levels (myoglobin and cardiac troponin levels), and changes in inflammatory response markers (decreased albumin levels and increased C-reactive protein, ferritin, and IL-6 levels).[22]

Coagulopathy is a recognized risk factor for COVID-19 mortality and expressed by significantly increased levels of D-dimer and fibrin degradation products.[23]

CONCLUSION

Table 5 shows the different clinical and epidemiological situations that physicians might encounter while treating patients with COVID-19 and the procedures that are most supported by current knowledge.

The beginning of the interpretation is based on the elapsed time since symptom onset.

In the absence of symptoms, the interpretation is based on the time elapsed since close contact with a SARS-CoV-2 carrier, evidenced by molecular testing. The more intimate and prolonged the contact, the more significant the history. However, the concept of close contact with a SARS-CoV-2 carrier can be quite broad, and includes the following[24]:

TABLE 5. CHOICE OF TESTS IN DIFFERENT CLINICAL EPIDEMIOLOGICAL SITUATIONS

Patient clinical and epidemiological	Reason for test	First-choice test	What to do if the result is negative
Asymptomatic with no history of contact with carriers	Find out if the patient has been contaminated before	No indication for testing	-
	Assess the population incidence of virus infection/ immunization	RLT "Rapid Test" (immunochromatography)	-
Asymptomatic after contact with COVID-19 patient	Define quarantine requirement	RT-PCR	Serology after 15-30 days of contact
Patient with early symptoms suggestive of COVID-19 (up to 7 days from symptom onset, ideally between 3-4 days)	Diagnosis of the disease	RT-PCR	Serology (after 15 days of symptom onset)
Patient with symptoms suggestive of COVID-19 with late symptom onset (after day 7 and before 14 days of symptoms)	Diagnosis of the disease	RT-PCR + chest CT	Serology (after 15 days of symptom onset)
Symptomatic or recovered patient, more than 15 days after symptom onset		Serology	
The patient recovered from COVID-19, after 7 days	Evaluate return to work of health personnel	RT-PCR	Serology
Patient recovered from COVID-19, more than 14 days after the first symptom onset	Diagnosis (retrospective) of the disease	Serology	

- A person who had direct physical contact (e.g., shaking hands).
- A person who had unprotected direct contact with infectious secretions (e.g., cough droplets, unprotected contact with used tissue or tissues containing secretions).
- A person who had face-to-face contact for at least 15 min and at a distance of at least 2 m apart.
- A person who was in an enclosed environment (e.g., classroom, meeting room, hospital waiting room, etc.) for at least 15 min and at a distance of at least 2 m apart.
- A health care professional or another person directly handling a COVID-19 case or laboratory workers handling samples from a COVID-19 case without recommended personal protective equipment (PPE) or with a possible PPE violation.

- An aircraft passenger seated within a radius of two seats (in either direction) from a confirmed case of COVID-19, his companions, or caregivers, and the crew members who worked in the aircraft section where the patient was seated.
- A person who lives in the same house/environment. Residents of the same house, dormitory, nursery, and accommodation should be considered.

Finally, we hope that this text will be useful and contribute to the better use of the laboratory in the diagnosis of SARS-Cov-2. Much of the knowledge about this condition will continue to evolve in the coming months, adding or changing part of what we have reviewed today.

PALAVRAS-CHAVE: *síndrome respiratória aguda grave - coronavírus 2, COVID-19, patologia clínica, diagnóstico clínico, laboratório.*

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REVIEW ARTICLE

Ultrasonographic evaluation of gastric content and volume: a systematic review

César Antonio Tavares da Rocha[1]
Lia Mayumi Kubota Kamada[1]
Pedro Hilton de Andrade Filho[1,4]
Isabela Araujo Villaverde[2]
Jacqueline Yamahata Barbosa Shiro[2]
João Manoel Silva Junior[3,4]

1. Hospital do Servidor Público Estadual de São Paulo, São Paulo, SP, Brasil.
2. Acadêmica de Medicina da Universidade Cidade de São Paulo, São Paulo, SP, Brasil.
3. Chefe do departamento de anestesiologia do Hospital do Servidor Público Estadual de São Paulo, São Paulo, SP, Brasil.
4. Programa de Pós-Graduação em Anestesiologia, Ciências Cirúrgicas e Medicina Perioperatória – FMUSP, São Paulo, Sp, Brasil.

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SUMMARY

OBJECTIVE: *Bronchoaspiration of gastric content is associated with high morbidity and mortality, but evaluating this complication is a difficult task. However, gastric ultrasonography can safely assess gastric content and prevent bronchoaspiration. Therefore, a systematic review was performed in order to verify the efficacy of ultrasonography in the qualitative and quantitative analyses of gastric content.*

METHODS: *A literature review of articles published between 2009 and 2019 in the PubMed and LILACS databases was conducted using combinations of the keywords "gastric ultrasound," "gastric emptying," and "gastric content."*

RESULTS: *Of the 20 articles found, 19 chose the antral region as the best site for qualitative analysis of the gastric content. Regarding quantitative measurement, the most commonly used method to calculate the gastric volume in eight articles was the formula "Gastric Volume = 27 + (14.6 × ATAG) − (1.28 × Age)," in which the area of the transverse section of the gastric antrum (ATAG) could also be calculated by the largest antral diameters or by free tracing.*

CONCLUSION: *An efficient evaluation of the gastric content can be performed by ultrasonography of the antral region, contributing to greater safety in the clinical management of patients with increased risk for bronchoaspiration during airway management.*

KEYWORDS: *gastric ultrasonography, gastric content, gastric emptying*

INTRODUCTION

Bronchoaspiration (BCA) of gastric content is a serious complication, and mortality increases with higher aspirated volumes or gastric content of pH <2.5.[1] Adequate gastric emptying contributes to greater safety in procedures such as orotracheal intubation and endoscopic examinations, in which the incidence of BCA varies from 0.1-19%, reaching mortality rates of 30%.[2-4]

Gastric content assessment is usually performed by invasive and/or high-cost techniques. Currently, the gold standard is gastric scintigraphy, which is a high-cost examination involving radiation exposure; hence, gastric ultrasonography (USG-G) is used as an alternative.[5]

Although there are reviews on the use of USG-G, none evaluated the qualitative, quantitative, and

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CORRESPONDING AUTHOR: Pedro Hilton Andrade Filho
Rua Pedro de Toledo, 1800, Vila Clementino, SP, Brasil – 04029-000
E-mail: pedrohaf07@gmail.com

risk analyses, and this review is the most recent. A literature review was performed to summarize the current knowledge about the assessment of gastric content by ultrasonography and measure the risk of perioperative BCA. This review aims to validate the methodology already described and evaluate its efficacy in determining the risk of complications in airway management.

METHODS

A systematic review was conducted in the CENTRAL (PubMed), MEDLINE (OvidSP), and EMBASE (OvidSP) databases for articles published between 2009 and 2019. The following keywords were used in the search: "antrum" or "gastric" and "sonography;" "antrum" or "gastric" and "ultrasound;" "antrum" or "gastric" and "emptying;" and "antrum" or "gastric" and "content." Studies involving the use of USG-G in humans were included when they contained specifications of the qualitative method of gastric content analysis (empty, clear, pasty, or solid-liquid), and/or method for quantification of gastric volume and/or volume considered as a risk for respiratory complications. Studies in animals and in special populations (pregnant women, children, obese, and diabetics) who present gastric motility different from the general population were excluded. The main purpose was to analyze the volume of gastric content (VCG) as a complication risk. In order to avoid bias in the selection of studies, two independent researchers conducted the search for the articles. After the initial selection, a discussion was held to define the most appropriate studies to be included in this review. For the articles to be considered, the following topics were observed: year of publication, the position of the patient during the examination, the portion of the stomach analyzed, existing gastric content, scanning technique, type of transducer, and type of research.

RESULTS

Selected articles

Initially, 426 articles were found, of which 20 were selected. The clinical trials included were evaluated for risk of bias, as shown in Figure 2, which resulted in a low to moderate risk in the selected articles.

The analyzed population included 1631 patients in several clinical settings such as emergency rooms (n = 39), intensive care units (n = 154), surgical procedures (n = 921), and high digestive endoscopy procedures (n = 108), as well as healthy volunteers (n = 409).

Anatomical portion

The gastric antrum was the anatomical region of choice for gastric content evaluation in 19 studies.

FIGURE 1. FLOWCHART OF ARTICLE SELECTION

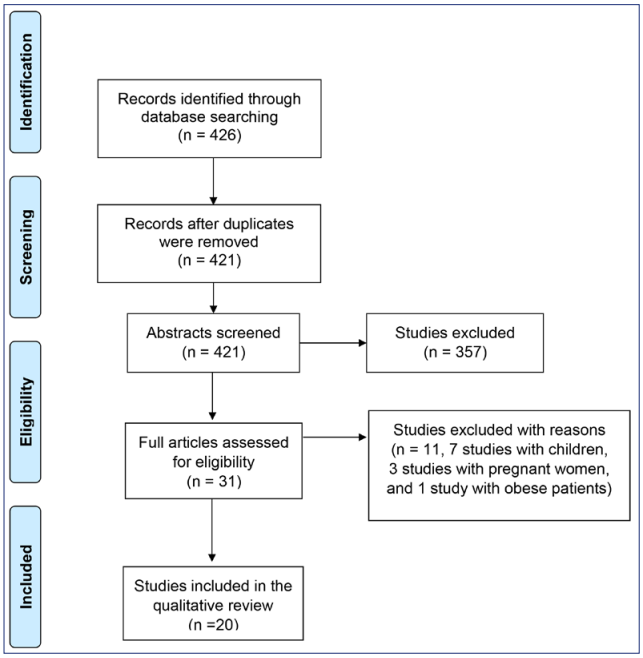
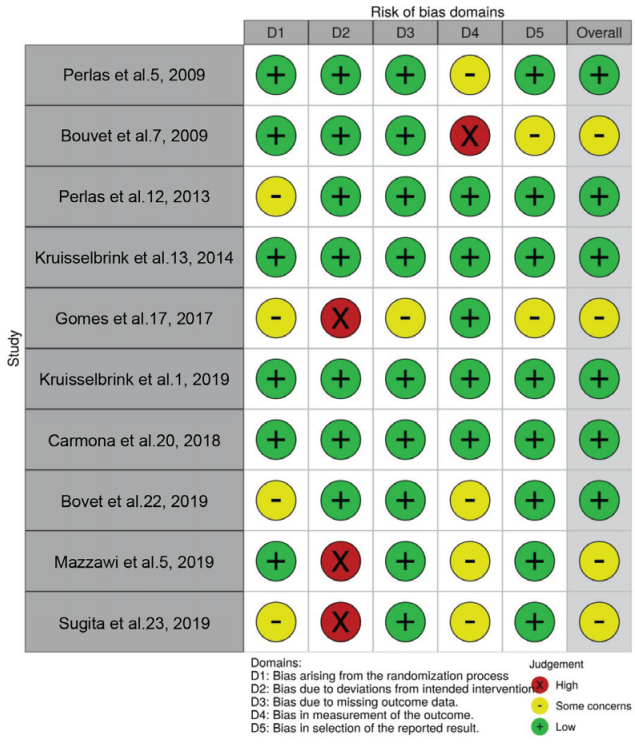


FIGURE 2. RISK OF BIAS ANALYSIS FOR CLINICAL TRIALS



Patient position

The patients were most commonly in the supine and right lateral decubitus (DLD) positions. Twelve studies used the DLD position, either alone or associated with the supine position.

Scanning and measures

Nineteen studies recommended the use of low frequency (1-5 MHz) curvilinear transducers, and only one of them additionally used a high-frequency linear transducer (8-13 MHz).

To locate the gastric antrum, most studies recommended initiating parasagittal scanning in the left subcostal region. The probe was then slid to the right in search of the following anatomical references: the left lobe of the immediately cranial liver, used as a reference in 14 studies; the immediately posterior pancreas, used as a reference in seven studies; and the more deeply abdominal aorta, used as a reference in 10 studies. The other structures reported less frequently as anatomic references were the vena cava and superior mesenteric vein.

METHODS OF GASTRIC CONTENT ANALYSIS

Qualitative evaluation

The evaluation of the type of gastric content was described in 11 articles, analyzed by differentiating between solid, liquid, and empty stomach contents.[1,7-11,16,18-21]

The imaging findings according to gastric content were as follows:

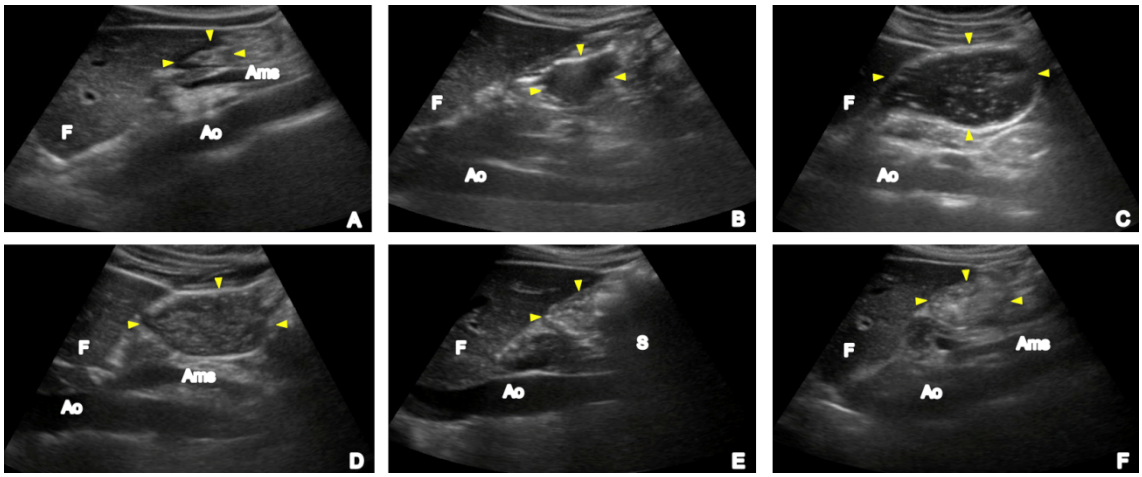
- Empty stomach: the gastric antrum was visualized with its walls juxtaposed with a "target" or "bull's eye" appearance (Figure 3A).[6,11]

- Liquid content: the distance from the antrum walls and filling of hypoechoic liquid content without residues was observed (Figure 3B). Immediately after ingestion or after a gastric contraction, the liquid content mixed with the air and formed a "star-filled sky" appearance (Figure 3C). Liquids with residues (e.g. milk or juices) showed a more echogenic content (Figure 3D).[6,11]

- Solid content: Solid food mixed with air, previous liquid, and gastric juices, formed a "frosted glass" appearance that could be accompanied by acoustic shading later (Figure 3E). After digestion, the gastric content presented a hyperechoic and more heterogeneous aspect, similar to that of hepatic parenchyma (Figure 3F).

A risk stratification model for the qualitative assessment of gastric content was proposed by Perlas, Davis:[9] grade 0, empty gastric antrum in supine and DLD positions (suggesting empty stomach); grade 1, liquid content visualized only in DLD position (suggesting liquid in small quantity); and grade 2, liquid content visualized in supine and DLD positions (suggesting liquid in large quantity). It was observed that 75% of grade 1 patients had <100 mL of gastric residue, 75% of grade 2 patients had >100 mL, and 50% of grade 2 patients had >250 mL.[9]

FIGURE 3. ULTRASONOGRAPHY OF GASTRIC CONTENT IN THE RIGHT LATERAL DECUBITUS POSITION



A: empty stomach; B: stomach with liquid without residues; C: stomach with liquid and air showing "sky of stars" appearance; D: stomach with liquid with residues; E: stomach with solids showing "frosted glass" appearance and acoustic shadow; F: stomach with solid late-stage digestion.

(F = liver; Ams = upper mesenteric artery; Ao = aorta; S = posterior acoustic shadow; arrowheads = gastric antrum)

TABLE 1. TECHNICAL ANALYSIS OF THE STUDIES

Author	Study design	Population studied	Number of participants	Patient position	Stomach portion	Scanning	Quantitative Evaluation	VCG risk for BCA (mL/kg)	Semi-quantitative evaluation	Probe
Perlas, Chan[6]	Clinical trial	Volunteers	Stage 1: 18 Phase 2: 36	Supine	A/C/F	Parasagittal	Yes	NA	NA	Curvilinear 2-5 MHz
Bouvet, Miquel[7]	Clinical trial	Volunteers	22	Semi-sitting	Antrum	Sagittal	Yes	NA	NA	Curvilinear 2-5 MHz
Koenig, Lakticova[8]	Observational	ICU patients	80	Supine	C/F	Sagittal and parasagittal in LAM	No	NA	NA	Curvilinear 1-5 MHz
Perlas, Davis[9]	Observational	Surgical patients	200	Supine/DLD	Antrum	Sagittal	Yes	NA	Yes	Curvilinear 2-5 MHz
Bouvet, Mazoit[10]	Observational	Surgical patients	183	Semi-sitting	Antrum	Sagittal	Yes	>0.8	NA	Curvilinear 2-5 MHz
Cubillos, Tse[11]	Observational	Volunteers	6	DLD	Antrum	Axial/Sagittal	No	NA	NA	Curvilinear 2-5 MHz/ linear 8-13
Perlas, Mitsakakis[12]	Clinical trial	EDA patients	108	DLD	Antrum	Sagittal	Yes	>1.5	Yes	Curvilinear 2-5 MHz
Kruisselbrink, Arzola[13]	Clinical Trial	Volunteers	22	Supine/DLD	Antrum	Sagittal	Yes	NA	NA	Curvilinear 2-5 MHz
Hamada, Garcon[14]	Observational	ICU patients	55	Semi-sitting	Antrum	Sagittal	Yes	>0.8	NA	Curvilinear 2-5 MHz
Bisinotto, Pansani[15]	Observational	Volunteers	67	Supine/DLD	Antrum	Sagittal	Yes	>1.5	NA	Curvilinear 2-5 MHz
Sharma, Gudivada[16]	Observational	ICU patients (enteral nutrition)	19	Semi-sitting	Antrum	Sagittal	Yes	>500	NA	Curvilinear 2-5 MHz
Gomes, Caporossi[17]	Clinical trial	Volunteers	20	DLD	Antrum	Sagittal	Yes	NA	NA	Not Informed
Bisinotto, Naves[18]	Observational	Volunteers	80	Supine/DLD	A/C	Parasagittal	Yes	NA	Yes	Curvilinear 2-5 MHz
Van de Putte, Vernieuwe[19]	Observational	Surgical patients	538	Supine/DLD	Antrum	Sagittal	Yes	>1.5	Yes	Curvilinear 2-5 MHz
Carmona, Almeida[20]	Clinical trial	Volunteers	17	DLD	Antrum	Sagittal	Yes	>0.8	NA	Curvilinear 2-6 MHz
Kruisselbrink, Gharapetian[1]	Clinical trial	Volunteers	40	Supine/DLD	Antrum	Sagittal	Yes	>1.5	Yes	Curvilinear 2-5 MHz
Okada, Toyama[21]	Observational	Emergency patients	39	Supine	Antrum	Sagittal	Yes	>1.5	NA	Curvilinear 2-5 MHz
Bouvet, Barnoud[22]	Clinical trial	Volunteers	25	Semi-sitting at 0, 30º, and 45º, and DLD	Antrum	Sagittal	Yes	>1.5	Yes	Curvilinear 2-5,5 MHz
Mazzawi, Bartsch[5]	Clinical trial	Volunteers	32	Sitting	Antrum	ND	Yes	NA	NA	Curvilinear 4 MHz
Sugita, Matsumoto[23]	Clinical trial	Volunteers	24	DLD	Antrum	Parasagittal	Yes	NA	NA	Curvilinear 2-5 MHz

DLD: right lateral decubitus; EDA: upper-digestive endoscopy; A: antrum; C: body; F: gastric fundus; ND: not described; NA: not evaluated; LAM: middle axillary line; VCG: volume of gastric content; BCA: bronchoaspiration; ICU: intensive care unit.

Quantitative Evaluation

Eighteen studies correlated the measurement of the area of the transverse section of the gastric antrum (ATAG) and the VCG. The ATAG measurement was calculated in two ways: measurement of the area by free tracing including the serous layer; and the technique used in 16 studies involving the anteroposterior (AP) and craniocaudal (CC) diameter measurements, measured from serous to serous by the formula: ATAG = (CC × AP × π)/4.[24]

A comparative study between these ATAG measurement techniques showed a small difference in the predicted VCG value of up to 20 mL in 96% of cases.[13]

Another study developed a mathematical model capable of estimating the VCG according to the measurement of ATAG in the DLD position using the age of the patient as the only covariable in the formula: VCG (mL) = 27+ (14.6 × ATAG) – (1.28 × age). This mathematical model was used by eight of the studies analyzed.[6,12] Okada Toyama,[21] using computed tomography as the

USG-G control group, showed that ATAG measurements >3.01 cm^2 in the supine position were indicative of either gastric content >1.5 mL/kg or presence of solid content, with a sensitivity of 85% and specificity of 53%.

Combined assessment and correlation with bronchoaspiration risk

High-risk predictors for BCA include the presence of solid residues in any amount or large liquid content, defined as VCG >1.5 mL/kg in six analyzed studies.[10,12]

The main fasting management protocol and risk classification for BCA proposed by Van de Putte and Perlas[25] advocate scanning in the supine and DLD positions and classifying BCA risk according to the USG-G findings as follows:

- Low risk: Empty antrum
- High risk: Solid waste
- Presence of liquid waste calculated by ATAG: After calculation, correlate the ATAG measurement with the VCG according to the formula: VCG (mL) = 27 + 14.6 × ATAG (cm^2) – 1.28 × age (years). If VCG is >1.5 mL/kg, it is considered high risk for BCA, and if <1.5 mL/kg, it is considered low risk.

DISCUSSION

This review found several studies using USG-G for qualitative and quantitative assessments of gastric content. Regarding the methodology, most of the articles opted for the analysis of the antral region, which has a better relationship with the measurement of gastric content and volume. Although a small divergence occurred in the image acquisition of the gastric antrum, all studies preferred sagittal/parasagittal scanning.

The antral ultrasound visualization is complicated by the presence of air and the depth of the anatomical structures, with the curvilinear transducer of low frequency (2-5 MHz) being the best to perform the exam.

The supine position followed by the DLD position seems favorable to measure the volume and classify the risk, since DLD not only allows better visualization of the antrum in its lower portion and a better correlation with ATAG as compared to the supine position but also provides greater sensitivity and specificity in the examination.[11,22]

The method of quantitative evaluation regarding the measurement technique and its accuracy, reliability, and reproducibility is still debatable. The measurement of ATAG through free tracing, though technically easier, still requires further studies for its validation, because it has only been used in two studies. ATAG measurement should be performed by involving the entire serous layer of the stomach and outside periods of peristaltic contractions. For greater accuracy of the test, it is recommended to use the mean of three ATAG measurements in clinical practice, since the mean of multiple measurements to calculate VCG has a good correlation with gastric scintigraphy measurements.[26]

Thus, USG-G could be an efficient method for airway management in cases of uncertain fasting, risk factors for gastric emptying delay, or unreliable history (cognitive dysfunction, delirium).[14]

This systematic review had the limitation of analyzing studies of different designs performed in controlled environments, single centers, and with homogeneous populations. Moreover, the number of selected articles may have been insufficient, despite the estimated time for the study to be conducted over 10 years. Therefore, studies with a larger scope are necessary to generalize these recommendations in other populations.

CONCLUSION

The bedside use of USG-G has been consolidated in clinical practice. Currently, the USG-G of the antral region is the most practical, non-invasive, and easy imaging method to characterize gastric content and volume.

Author's Contribution

César Antonio Tavares Da Rocha – conceptualization and design of the study, search and analysis of articles, writing of the original manuscript, elaboration and revision of the graphic components (tables and figures); Lia Mayumi Kubota Kamada – study design, search and analysis of articles, writing of the original manuscript, elaboration and revision of graphic components (tables and figures); Pedro Hilton de Andrade Filho – study design, review of the selected articles, elaboration and review of graphic components (tables and figures), review of the original manuscript, and writing of the final manuscript; Isabela Araujo Villaverde and Jacqueline Yamahata Barbosa Shiro – review of the selected articles, preparation and review of graphic components (tables and figures), writing of the original manuscript; João Manoel da Silva Junior – conceptualization and design of the study, revision and orientation of methodology, revision of graphic components (tables and figures), revision of the original and final manuscripts.

RESUMO

OBJETIVO: *A broncoaspiração do conteúdo gástrico associa-se `a alta morbimortalidade, porem a avaliação desta complicação é tarefa difícil. Por outro lado, a ultrassonografia gástrica avalia o conteúdo gástrico com segurança, podendo evitar a broncoaspiração. Portanto, foi realizada revisão sistemática com objetivo de verificar a aplicabilidade da ultrassonografia na análise qualitativa e quantitativa do conteúdo gástrico.*

MÉTODOS: *Revisão de literatura de artigos publicados entre 2009 e 2019 nas bases de dados PubMed e LILACS usando combinações das palavras chave: "Gastric ultrasound", "gastric emptying" e "gastric content".*

RESULTADOS: *Foram encontrados 20 artigos. A região antral foi escolhida em 19 artigos como melhor local do ponto de vista qualitativo para analisar o conteúdo gástrico. A respeito da mensuração quantitativa, o método mais utilizado para cálculo do volume gástrico, escolhido em 8 artigos, foi através da fórmula Volume gástrico = 27 + (14,6 x ATAG) – (1,28 x Idade), em que a Área da Secção Transversa do Antro Gástrico (ATAG) pode ser igualmente calculada pelos maiores diâmetros antrais ou pelo seu traçado livre.*

CONCLUSÃO: *A ultrassonografia da região antral permite boa avaliação do conteúdo gástrico, trazendo maior segurança ao manejo clínico de pacientess com risco aumentado para broncoaspiração no manejo da via aerea.*

PALAVRAS CHAVE: *Ultrassonografia gástrica, conteúdo gástrico, esvaziamento gástrico.*

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REVIEW ARTICLE

Abnormal uterine bleeding: hysterectomy versus resection

Wender Soares Santiago[1]
Jordan Barros da Silva[2]
Renato Martins Pereira[3]
Cintia do Couto Mascarenhas[1]

1. Escola de Saúde e Medicina, Curso de Medicina, Universidade Católica de Brasília, Brasília, DF, Brasil
2. Laboratório de Micologia do Núcleo de Parasitologia e Micologia (NPM) do Laboratório Central de Saúde Pública do Distrito Federal (LACEN-DF), Brasília, DF, Brasil
3. La Femme Ginecologia de Excelência Ltda, Brasília, DF, Brasil

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PALAVRAS-CHAVE: *Hemorragia Uterina. Menorragia. Histerectomia. Técnicas de Ablação Endometrial. Endométrio/cirurgia.*

INTRODUCTION

Abnormal uterine bleeding (AUB) is one of the most common clinical conditions in gynecology. It may compromise alterations in menstrual cycle duration, frequency, and regularity of flow[1]. It is estimated that about 10% to 30% of women will be affected by AUB during their lifetime, and more than 10 million American women are affected by it each year[2,3]. It influences the quality of life affecting physical, emotional and social well-being[3,4]. Menorrhagia mostly affects women of childbearing age and is more common in menarche and perimenopause, often causing excessive uterine bleeding, fatigue, and difficulty performing daily activities[5,6].

The diagnose of AUB is a big challenge in medical practice, requiring thorough clinical analysis and careful observation of inconclusive parameters. Concomitantly, there are suggestive diagnosis aspects that should be ruled out upon examination such as anemia, hypothyroidism, polycystic ovary syndrome, coagulation disorders, and clonal diseases such as endometrial cancer, colon, and thyroid tumours[3]. To reduce the difficulties of the diagnostic, a classification system for menorrhagia was created to be used worldwide, the Palm-Coein classification system[5]. Developed by the International Federation of Obstetrics and Gynaecology (FIGO), its letters represent an acronym with the possible problems associated with AUB (polyps, adenomyosis, leiomyomas, malignancy, ovulatory dysfunction, endometrial disorders, iatrogenic factor)[5,7].

Initially, the treatment for abnormal uterine bleeding consists of clinical attention using drugs or a levonorgestrel-releasing intrauterine device. The surgical procedure is best suited for women who have no

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CORRESPONDING AUTHOR: Wender Santiago
QS 07 – Lote 01, EPCT - Taguatinga, Brasília - DF, 71966-700 – Taguatinga, DF – Brasil – 71966-700
E-mail: wender13ws@gmail.com

intention to become pregnant and is usually preceded by an unsuccessful clinical treatment, normally is performed using hysterectomy or endometrial ablation resection techniques[8].

The surgery in which the uterus is totally or partially removed, hysterectomy, can be done by abdominal, vaginal, laparoscopic, or robot-assisted laparoscopic surgery, and abdominal hysterectomy is the most invasive approach[8]. Endometrial ablation, in turn, is a surgical method aimed at reducing uterine bleeding by removing a portion of the endometrial tissue layer. Among the ablation possibilities are the hysteroscopic or 1st generation ablation techniques, which are more suitable for abnormal uterine cavities, leiomyomas, or polyps. This surgical class is characterized by endometrial resection that uses electrosurgical or laser tools. On the other hand, there is non-hysteroscopic or 2nd generation ablation that is recommended for benign bleeding. The treatments for those cases include radiofrequency, hot fluid, cryotherapy, microwave, and laser or thermal balloon ablation techniques[9,10]. In general, newer endometrial ablation techniques are less invasive and are alternatives to hysteroscopic techniques.

There are few retrospective clinical studies or randomized controlled trials comparing hysterectomy and endometrial ablation techniques. Thus, current analyses are inconclusive as to which treatment options are the best. However, it is inferred that both techniques are effective in treating dysfunctional uterine bleeding, thus each clinical context must be considered.

METHODS

A literature review covering publications from 2007 to 2018 was conducted to select controlled clinical trials or retrospective studies that compared hysterectomy and resection with endometrial ablation techniques used to treat abnormal uterine bleeding (AUB). We searched the PubMed - NCBI, SciELO, BIREME, Cochrane, LILACS and MEDLINE databases using the words “abnormal uterine bleeding”, “hysterectomy”, “resection versus hysterectomy”, “endometrial ablation and abnormal uterine bleeding”, “endometrial”, “ablation and hysterectomy”, “endometrial ablation versus hysterectomy”, “heavy menstrual bleeding”, “dysfunctional uterine bleeding”, “heavy menstrual bleeding”, “menorrhagia”, “laparoscopy” and “endometrial resection and ablation” as well as their respective translations in Portuguese databases. Case studies, reviews, articles that compared surgical techniques with pharmacological treatments along with book chapters, theses, dissertations, and course completion papers were excluded from the study. We obtained 2,375 articles from the searches. Of the articles identified, only eight articles, five clinical trials (including multicentric studies) with recommendation grade A (levels 1A and 1B), and three retrospective studies of recommendation grade B public databases (level 2C) according to the Oxford Centre classification for evidence-based medicine, fit the research. These articles deal with the positive and negative aspects of each surgical technique.

RESULTS

Regarding pain in the postoperative period, data from controlled studies vary; two controlled studies reported no significant differences in the 24-month period under review[6,7]. Two controlled studies state that pain was higher in patients undergoing hysterectomy than in those undergoing endometrial ablation[11,12]. A randomized study that focused its analysis on the reoperation rate and quality of life did not analyze pain[13]. Articles with pain data analyzed post-surgery did not reach a consensus, yet they were favorable to endometrial ablation techniques.

Analyzing the quality of life after surgery, three controlled studies corroborate that both techniques are effective in treating abnormal uterine bleeding leading to significant improvements in patient well-being[6,7,12]. A randomized clinical trial concluded that thermal balloon endometrial ablation may replace vaginal hysterectomy for perimenopausal women with uterine leiomyomas by demonstrating good rates of response[11]. This study claimed all women undergoing amenorrhoeic endometrial ablation had reduced menstrual flow volume over a 12-month period. Another randomized trial concluded that laparoscopic supracervical hysterectomy resulted in a better quality of life when compared to hysteroscopic endometrial ablation[13]. The clinical trial of Sesti et al.[12], in which the SF-36 score was satisfactory in both groups, obtained a low result for emotional function in patients treated with laparoscopic supracervical hysterectomy, which was associated with the removal of the uterus. The Medical Outcomes Short-Form Health Survey (SF-36) was the most widely used instrument in controlled studies to assess the quality of life. In a randomized clinical trial, in addition to the SF-36, the abbreviated

form of Health Metric's Health Shot form (SF-12v2), shortened from the SF-36, was used[13]. Finally, a randomized clinical trial used Symptom and Quality of Life (UFS-QOL) scores in its analysis before and after surgical procedures to conclude that there were improvements in the quality of life of patients treated for abnormal uterine bleeding when both techniques were compared[11].

When comparing reoperation rates, studies show that endometrial ablation has considerable relapse rates[2,6,7,13-15]. A randomized controlled trial focusing on reoperation rates and quality of life comparing laparoscopic supracervical hysterectomy with hysteroscopic endometrial ablation yielded a reoperation rate of 13.4% (12/89) after 24 months (12/89) for ablation and 1% (1/92) for those who underwent hysterectomy[13]. One randomized clinical trial had a 5% (1/20) reoperation rate for patients who underwent thermal balloon ablation against none (0/20) of those who underwent vaginal hysterectomy[11]. Another randomized clinical trial achieved a 24-month reoperation rate of 24.5% (27/110) among women who underwent endometrial ablation, with the rate increasing to 31% (34/110) at 60 months[6]. In further studies and clinical analyses, another trial found reoperation rates of approximately 29% within 48 months for the group of women undergoing endometrial ablation[7]. A randomized clinical study did not report present data in this article[12]. Three retrospective studies were also in line with data studies regarding findings that there are greater rates of reoperation for women undergoing ablation for AUB[2,14,15].

Regarding bleeding, the literature concedes that hysterectomy is the definitive procedure to contain menorrhagia since this technique produces very high percentages of amenorrhea patients. Three randomized clinical trials have obtained significant results not only in solving the problem of excessive bleeding but also claim to have achieved considerable increases in hemoglobin levels for both surgical techniques[7,11,12]. One of the studies claims that in 24 months, 75% (15/20) of women underwent a second-generation ablative technique with amenorrhea, compared to 20% (4/20) who had mild bleeding and only 5% (1/20) of the patients in this group were reoperated for the same issue[11]. A multicentre, controlled study states that hysterectomy is more effective in stopping bleeding, but both groups performed well and were effective[6].

Analyzing postoperative complications, a randomized clinical trial reported nearly six times more infections and four times more adverse effects in the hysterectomy group[6]. One randomized clinical study reports greater adverse effects for hysterectomy, and three other trials did not find significant post-operative complications[6,11-14]. Among the retrospective studies, two confirm data from the literature that hysterectomy is associated with an increased incidence of pelvic organ prolapse and promotes higher urinary incontinence rates[2,4,15]. One study, a retrospective cohort study based on national Scottish hospital data from 1989 to 2006, points out that while there are few ablation complications, one should consider the fact that approximately a quarter of all cases require future surgery for AUB[15]. Finally, another retrospective cohort study showed that hysterectomy was associated with twice the chance (36% versus 15%; p <0.001) of postoperative complications than endometrial ablation[14].

A retrospective study assessing the risks of further surgery and gynecological cancer did not find any relevant incidence of postoperative cancer (<1.6%)[15]. Another retrospective study compared the costs between second-generation endometrial ablation and hysterectomy in the period 2006-2010 from the US database and reported that the cost of hysterectomy is approximately twice as high as that of endometrial ablation ($12,147 vs. $5,837) as well as demonstrates that the likelihood of short-term disability claims is four times higher (84% vs 21%; p <0.001)[14]. Finally, a retrospective, longitudinal, observational study compared the clinical and economic benefits of radiofrequency ablation versus hysterectomy[2]. This study used the German health database as a source from January 2008 to September 2013 and concluded that the second-generation techniques save around €1,844 per case in the country and are less associated with adverse effects.

DISCUSSION

Abnormal uterine bleeding (AUB) is one of the most frequent menstrual cycle disorders worldwide, generally affecting women at the end of their fertile lives. It accounts for about one-third of disturbance cases and is characterized by excessive blood loss by volume, frequency, regularity, or duration of flow. AUB compromises the quality of life of many women, including their ability to perform daily activities, and the emotional, social, and physical aspects of their lives[1]. Surgical techniques for treating AUB favorably reduce anxiety and depression that may have existed

pre-surgery. However, excluding the first year after surgery hysterectomy is associated with a better quality of life and sexuality results when compared to endometrial resection[16]. There is a treatment based on the clinical history; however, diagnosis can be challenging due to the thorough clinical analysis required, and doctors are often only guided by patient reports[3]. In this context, hysteroscopy is an important diagnostic technique to assess the uterine cavity[17]. The possible visualization, biopsy, and confirmation of abnormal findings reveal the importance of this practice, which has several forceps that can be used[17,18]. Lin's biopsy forceps are flexible in the management of endometrial abnormalities, effectively helps hysteroscopy with both the biopsy and removal of uterine lesions[17]. Furthermore, the Vitale biopsy forceps were developed with the capacity to grab tissue and cut at the same time, thus making it useful in the field of ambulatorial hysteroscopy[18]. All of this technology involved in the diagnostic hysteroscopy technique provides greater accuracy for professionals when viewing and evaluating the interior of the uterus and finding the possible causes of the SUA[17].

It is possible to choose one of the hysterectomy or resection techniques with endometrial ablation, and hysteroscopic techniques include abdominal, vaginal, laparoscopic, and robot-assisted laparoscopic hysterectomy[8]. Abdominal hysterectomy is achieved by an incision in the lower abdomen, and this technique is the most invasive. Vaginal hysterectomy is performed with no need for incisions and has a lower morbidity rate and recovery time than the abdominal option. Laparoscopic hysterectomy is less invasive, performed making fewer incisions or vaginally assisted by video accessories, and can also be performed by a robotic mechanism. Also, there is the resection with endometrial ablation, which is a technique used to dissect the endometrium layer, inducing tissue regeneration and the reduction of uterine bleeding. They are divided into 1st generation hysteroscopic resections of the endometrium with electrosurgical tools, and as such more suitable for abnormal uterine cavities, leiomyomas or polyps, and non-hysteroscopic cavities (2nd generation), recommended for women in whom bleeding is of benign cause. The 2nd generation techniques are: radiofrequency ablation; thermal flask; hot fluid; cryotherapy; microwave; laser. Surgical options are usually considered after the patient is refractory to clinical treatment and should only be chosen after case analysis and detailing of uterine characteristics in order to determine the most appropriate technique.

The present article aims to compare hysterectomy and resection with endometrial ablation from a literature review. As a real clinical implication, those studies have shown that maybe the definitive technique for obtaining amenorrheic women is hysterectomy[6,7,11-13]. There was no consensus that one of the procedures was definitely the best but hysterectomy techniques were more resolutive and with fewer recurrences[2,6,7,11-15]. Taking into account the viability of each method, both surgical classes can be used in clinical practice, taking into account the indications and contraindications when choosing.

There are several possible surgical techniques to be chosen, and the choice of the most appropriate method in each case must consider the age, the choice of the patient, the desire to become pregnant, anatomical changes, and the contraindications for the use of a surgical procedure. The patient's choice is influenced by variables such as recovery time, the fact that hysterectomy techniques are more invasive, the simplicity of endometrial ablation, and the achievement of amenorrhea when using hysterectomy. When comparing two classes of surgical techniques, it is notable that there are many variables to be paired and outlined in future studies in order to achieve greater accuracy on the best indication or even reach a consensus.

CONCLUSION

Even though all techniques have shown to be effective in treating this gynecological disorder, there is no consensus in the literature as to which one of the surgical techniques is definitively the best treatment option for abnormal uterine bleeding. In regard to improved quality of life, both are visibly beneficial. Endometrial ablation was related to a higher percentage of refractoriness as it preserves much of the uterine structure, whilst hysterectomy has been shown to be more effective in generating amenorrhea in patients than the others techniques. However, hysterectomy techniques were associated with higher rates of adverse effects such as pelvic organ prolapse and urinary incontinence. Among the hysteroscopic techniques, vaginal hysterectomy has been shown to cause greater adverse effects. There was no evidence of a relationship between gynecological cancer and treatments and there was no consensus on which technique promotes greater pain during the postoperative period.

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REVIEW ARTICLE

Eating disorders and psychosis: a review and case report

Rita Almeida Leite[1]
Tiago Santos[1]
Patrícia Nunes[2]
Isabel Brandão[2]

1. Department of Psychiatry and Mental Health, Baixo Vouga Hospital Centre, Aveiro, Portugal
2. Psychiatry and Mental Health Clinic, São João University Hospital Centre, Porto, Portugal

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SUMMARY

INTRODUCTION: *The interplay between eating disorders and psychosis is a challenging field to which little attention has been paid. Its study raises conceptual and methodological questions in both areas, making the diagnosis and management of patients difficult. Such questions are addressed and illustrated with a review and case report.*

METHODS: *The authors present the case of a woman with Anorexia Nervosa and with comorbid Shared Psychotic Disorder, based on a literature review regarding the comorbidity between eating disorders and psychosis. The authors conducted a non-systematic review by searching the PubMed database, using the Mesh Terms "anorexia nervosa", "bulimia nervosa", "comorbidity" and "psychotic disorders".*

RESULTS: *The findings suggest that studies on the subject are limited by issues regarding data on the prevalence of comorbidities, phenomenological aspects of eating disorders, and the interface and integration with psychotic symptoms.*

CONCLUSIONS: *The case presented illustrates the difficulties in managing a patient with a comorbid eating disorder and psychosis. In order to ensure a rigorous assessment of both psychotic and eating disorder symptoms, the focus should be on the pattern of appearance or emergence of symptoms, their phenomenology, clinical and family background of the patient, and clinical status on follow-up.*

KEYWORDS: *Anorexia nervosa. Comorbidity. Schizophrenia, paranoid. Psychotic disorders. Feeding and eating disorders. Shared paranoid disorder.*

INTRODUCTION

The comorbidity between eating disorders (ED) and psychosis remains a subject of insufficient study. Reports exist since Bleuler in 1911, who described cases of schizophrenia with disordered eating related to delusional ideas. Particular disturbances, such as potomania, merycism, and pica have been often described in these patients, with variable degrees of severity[1]. Psychotic episodes are reported in 10-15% of ED patients, most of them transient, although 3-10% will suffer from schizophrenia[2,3]. However, reports of comorbid schizophrenia and anorexia nervosa (AN) are scarce, and a significant number has been questioned on the basis of insufficient descriptions or lack of rigorous diagnostic criteria[4,5]. More recently, other authors have reported cases of comorbid AN and schizophrenia[6-12]. Studies reporting comorbid bulimia

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CORRESPONDING AUTHOR: Rita Almeida Leite
Avenida Doutor Artur Ravara, 3810-193 Aveiro, Portugal – 3810-193
E-mail: rita.almeidaleite3@gmail.com

nervosa and schizophrenia are even more scarce. The existing literature refers to case reports or studies with small series of patients[4,5,13-15]. However, questions remain regarding the nature of the symptoms described and the diagnoses proposed.

METHODS

The authors report the case of a woman with a clinical history of AN previously treated at the age of 14, who developed a new episode at the age of 32, with comorbid Shared Psychotic Disorder. This study was based on a literature review regarding the comorbidity between eating disorders and psychosis. The authors conducted a non-systematic review by searching the PubMed database, using the Mesh Terms "anorexia nervosa", "bulimia nervosa", "comorbidity" and "psychotic disorders". The reviewed articles were selected according to their relevance. Some articles were also included from the references of the previously selected bibliography.

DESCRIPTION

This is the case of a 33-year-old single woman, who will be addressed as Mrs. S.

Mrs. S had no psychiatric background up until the age of 13, when she started to show an excessive concern with her body weight. She restricted her diet until it only included fruit and yogurts. Mrs. S. lost weight gradually, and at the time of her first admission, she presented severe restriction of caloric intake due to a morbid fear of getting fat, with a Body Mass Index (BMI) of 12.1 kgs/m², without binge eating or compensatory behaviors such as self-induced vomiting or use of laxatives or diuretics. She was diagnosed with AN, of the restrictive type. She was admitted to an eating disorder treatment program (first being hospitalized, and then followed-up in an outpatient clinic) and became fully recovered with a BMI of 19 kgs/m². Then, Mrs. S. suspended follow-up until one month prior to the current hospital admission. She was admitted again due to a BMI of 11,7 kgs/m². Both Mrs. S. and her family reported a normal weight until two years previously.

Her educational and family histories were assessed. Mrs. S. attributes her first episode of AN to her cousins since she was perfectly convinced she had been through some kind of spell, a perspective corroborated by her mother.

Mrs. S. went to Law School, as "I suffered from injustice throughout my life."

After finishing university, Mrs. S. applied to an elite estate institute for judges and public prosecutors. Before the final evaluation, she was told that she had failed due to an unsatisfactory performance during training. Mrs. S. believes that this was due to the fact they had discovered her disease when she was 14 "they probably secretly investigated my Hospital records..."

After leaving the institute, Mrs. S. refers that the problems continued "they broke into my house and searched my computer..." She believes that apparently ordinary people with whom she crosses on the street are involved in her surveillance "they look at me in a different way..."

Mrs. S's mother supports every statement, adding that "there are people disguised as tourists taking pictures..." Her mother looks insecure and avoids giving further details because of what may happen if she does. Mrs. S's father is less sure of this conspiracy theory, although stating that "it became necessary to change her house locks..."

During her developmental and social history assessment, Mrs. S. refers that she never had close friends, partners, or even family beyond the nuclear family since she feels insecure and mistrusted. Her mother always supported her. On the contrary, her father doesn't believe in that interpersonal mistrust, keeping friendly contacts.

There are no significant psychiatric antecedents in the family. Mrs. S's mother doesn't agree to her own psychiatric examination, neither to any kind of treatment.

As an inpatient, Mrs. S. remains reserved, circumspect, and expresses doubts about being watched: "there are cell phones... and we don't know if they are recording our conversations at this time..." She doesn't accept any antipsychotic medication.

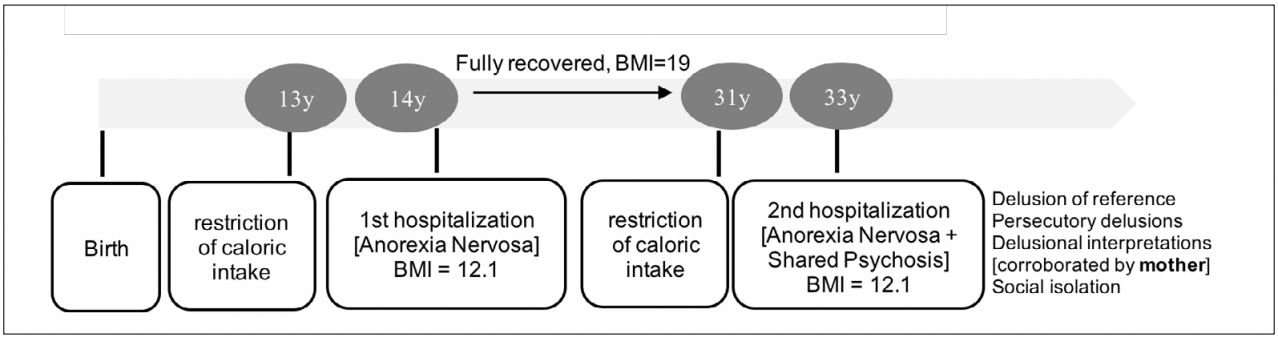
The clinical case is resumed in Figure 1.

DISCUSSION

Concerning her eating behavior, the patient presents a restrictive type, with the first episode at the age of 13 and a recurrence when she was 31 years old. In the inter-critic period, the BMI was within normal range and no disturbed eating behavior was noticed.

Besides disturbances in eating behavior, the patient shows traits of a sensitive paranoid personality type,

FIGURE 1. CLINICAL CASE SUMMARY. BMI=BODY MASS INDEX. Y=YEARS



with a constant pattern of suspicion and distrust towards others. A peculiar sense of justice built on the idea that people around the patient are set to harm and conspire against her determined her path to law school.

The distrust and the scrutiny of the loyalty of those surrounding the patient progressively narrowed her social and family network. During the first episode of AN, the family reaction was to restrict contact with other members, as if they were part of the illness. The patient describes this movement as a crucial part of the recovery.

In the current episode, a type of rigid process of thinking evolves into a clinical picture compatible with a diagnosis of Delusional Disorder. The patient presented a delusional belief that she was the victim of a conspiracy with the intent of stopping her law career. With time and new experiences, these delusional beliefs became more solid and structured, in a clear continuum with previous personality traits, evidencing the constitutional organization of the delusional thought. Despite the direct impact of the delusional ideas, the patient maintained her previous levels of functioning, without any strange or bizarre behavior.

The delusional ideas were shared in full by the patient's mother, suggesting a Shared Delusional Disorder. This entity was first described by Laségue and Falret, under the designation of "folie à deux", in which there is a transference of delusional ideas from one individual to one or more people of close relations. People sharing delusional beliefs are usually relatives (close relatives and spouses in 97% of cases) who lived together for long periods of time, often in relative social isolation[16].

In the case presented, all these premises are present, with a highly fusional mother-daughter relationship. This pattern is also common among families of patients with AN, along with the agglutination of their members, exaggerated repercussions of events in the family system, overprotection, rigidity, and difficulty in conflict resolution. Such characteristics, present in the patient's family, equally provide fertile ground to the development of a shared delusional disorder.

The case presented illustrates the difficulties in the diagnosis of these patients. Issues concerning the

TABLE 1. THE PREVALENCE OF PSYCHOSIS IN SAMPLES OF PATIENTS WITH EATING DISORDERS (ED)

Author	Year of publication	Number of Subjects	Comorbidity between psychosis and ED	Other comments
Foulon[1]	2003	N=75	Below 10%	Reaches 35% in males, the most frequent form being hebephrenia
Hudson et al.[4]	1984	N=130 (anorexia or bulimia)	12%	16 patients had psychotic symptoms attributed to an affective disorder or a schizoaffective disorder, none strictly responding to schizophrenia
David et al.[13]	1986		Below 10%	
Ferguson and Damluji[17]	1988	N=200	6%	Reported other references such as Dally (1969) 1%; Theander (1970) 1%; Hsu et al (1979) 3% and (1981) 6% (3% acute and transient during weight gain, 3% schizophrenic); Farquharson and Hyland (1966) 7%; and Silverman (1977) 29%.
Steinhausen[18]	2002	119 series, N=5590	4.6%	Reported that schizophrenia was rarely observed at follow-up
Shiraishi et al.[19]	1992	N=55	14%	
Striegel-Moore et al.[20]	1999	N=98 (ED, men)	36% (AN) 18% (BN)	Men with AN were at high risk for comorbid psychosis, men with BN were at risk for comorbid personality disorder.

interplay between symptoms of the psychotic spectrum and disordered eating are particularly challenging, as well as the nature of symptoms of ED.

Prevalence of comorbid Psychosis and Eating Disorders

Studies addressing the relationship between psychosis and ED show conflicting results, which are addressed in Table 1.

One of the factors contributing to these differences is that little is known about the comorbidity of psychiatric disorders among each other, of the accurate comorbidity with eating disorders specifically, and about sequential patterns across time[18]. Another aspect that confuses the numbers on prevalence is the lack of rigorous diagnostic criteria and symptom assessment, which can contribute to a higher number of cases found[2,3].

Comorbid Psychosis Versus Comorbid Eating Disorder

The study of the sequential pattern of appearance of both psychotic and ED on each patient may contribute to the disclosure of some of the diagnostic difficulties.

Little doubt remains about the diagnosis of schizophrenia when cases refer to patients with a prior ED who develops full-blown psychotic symptoms[3,13]. A rupture in the personal biography occurs, conditioned by delusional ideas, which may partially overrun symptoms of the pre-existing ED.

On the other hand, two main studies conducted with samples of chronic schizophrenic patients show that they often demonstrate maladaptive eating attitudes[8,21]. However, these attitudes are substantially different from the symptoms presented by typical ED patients. They are frequently seen in the context of delusional ideas or hallucinatory experiences, and the BMI is usually within the normal range. Although patients may show a fear of becoming fat and other concerns regarding body image, they seldom take notice of the caloric content of food or manifest the obsessional pattern of behavior characteristic of ED patients[21]. These aspects lead to the conclusion that patients with chronic schizophrenia may present disordered eating of an AN-like quality, although a distinct form compared with classic ED patients[8,21].

Adding to these findings, the elderly are susceptible to demonstrating nutritional deterioration and substantial changes in eating habits, a condition often described as "anorexia tarda", associated with living conditions and cognitive decline, and in any case related to the pattern seen in ED patients[8,22].

Other studies present case reports of disordered eating in schizophrenic patients[3,6]. The symptoms presented have a clinical background of delusional ideas and disordered behavior.

However, questions emerge in studies describing cases of young patients that present both psychotic and ED symptoms. We support that these difficulties are due to an erroneous attribution of a psychotic nature to ED symptoms, and to the use of ED diagnostic criteria regardless of the context of each particular case.

Eating Disorder Symptoms Versus Psychotic Symptoms

Several authors have proposed different hypotheses for comorbid psychotic and ED symptoms, resumed in Table 2.

One possibility is that ED and psychosis may represent different phenotypes of the same illness process[13,23]. This hypothesis is supported by cases in which the symptoms of the pre-existing ED are overrun by the psychotic process, or in which the psychotic symptoms fluctuate inversely over time with the ED symptoms. The conclusion is that the ED symptoms can be attributed to the cognitive impairments resulting from the schizophrenic process and that would eventually lead to manifestations of psychosis[23]. In the same way, concerns about eating and body image may be seen as overvalued ideas, and therefore a relevant component of the psychotic process[17]. However, as referred earlier, studies available show no overrepresentation of schizophrenia in patients with ED or their families.

Curiously, the same arguments which are used to sustain the phenotype hypothesis are also used to support the hypothesis of disordered eating as a defense against psychosis[3,5,13]. ED symptoms are considered to produce marked psychological stress and therefore can give rise to psychotic symptoms. This rationale presents several conceptual and methodological limitations.

TABLE 2. DIFFERENT HYPOTHESES PROPOSED FOR COMORBID PSYCHOSIS AND EATING DISORDERS (ED)

ED and Psychosis may represent different phenotypes of the same illness process.
ED may be a defense against Psychosis.
Psychosis recurs because of the distress induced by the treatment of ED symptoms.

On the other hand, the compensatory fluctuation of psychotic and ED symptoms seen in some cases is also regarded as a dynamic interaction in which psychosis recurs because of the distress induced by the treatment of ED symptoms. These considerations are sustained despite the fact that in the cases mentioned the fluctuation of symptoms was observed in parallel with the discontinuation of antipsychotics[3,5,13].

Contents related to eating behavior are frequently present in delusional and hallucinatory activity, and hypothetically more in cases with a previous or comorbid ED, although the few cases described are insufficient to confirm this. However, the assumption that psychotic symptoms may be protective against the disorganization caused by the ED symptoms is just as accurate as affirming the exact opposite. On the other hand, ED symptoms in the context of a psychotic syndrome do imply a clinical and management profile of a different nature compared to typical EDs[8,21].

Psychotic Disorders Versus Eating Disorders

An important issue in the discussion of co-morbid ED and psychotic symptoms, besides the possibility of co-occurrence of fluctuation over time, is the correct distinction between the two entities. Descriptions offered in studies of patients with comorbid psychotic and ED features raise questions about the nature of the symptoms presented[2,3,9,11,17].

Patients with ED, in particular with low weight, may present with a pressure of speech, circumstantiality, and other forms of disordered speech, as well as labile or angry affect, among others. The ways these patients describe their bodily experiences and the constant focus on food and body weight, particularly with lower weights, are easily taken for somatic delusions. Authors such as Ferguson and Damluji[17] in a series of 12 patients, in the absence of consistent delusional or hallucinatory activity, classified them as suffering from AN and schizophrenia of the disorganized type. The symptoms present were interpreted as evidence of the negative dimension of schizophrenia and related to deficits of functioning.

Another author also describes two cases of patients diagnosed with AN who manifested psychotic symptoms[10]. The author makes equal assumptions regarding symptoms that, to our view, are not to be excluded as features of an ED. It is striking how psychotic symptoms do not seem to be consistent, remit in a short period of time without the use of antipsychotics, and are focused on bodily experiences.

Cinemre and Kulaksizoglu[11] describe a patient diagnosed with AN who later developed schizophrenia. Some questions arise regarding the diagnostic proposal. The authors describe a 18-year-old male patient with a 4-year period prior to a full-blown schizophrenic crisis characterized by disordered eating and odd behavior regarding weight and body image, without reaching an abnormal BMI.

CONCLUSIONS

The comorbidity between eating disorders and psychosis is infrequent and raises several conceptual and methodological questions. Epidemiological and family studies show that there is no more significant association between schizophrenia and ED, although these results are somehow limited by the lack of rigorous data regarding ED.

The existence of ED symptoms in schizophrenic patients and in elderly patients with cognitive deterioration is well documented, as well as the organization of symptoms, which makes it distinct from the classic ED diagnosis.

It is important to determine the sequential pattern of appearance of the symptoms and to make a rigorous assessment of their nature. Many ED symptoms, in particular with a low and very low BMI, may resemble psychotic features. Symptoms must be contextualized while simultaneously avoiding tempting interpretations of ED or psychotic features.

The case presented described a patient with AN and comorbid delusional disorder and shared psychotic disorder. The patient's delusional thought was coherent, with a developmental course and susceptible personality traits. In addition, her parent's involvement, in particular her mother, in full-blown shared psychosis, is consistent with what is known about the characteristics of families of patients with AN. In fact, the reaction and the solution found within the family to the first episode of AN may have accentuated the pre-existing personality and functioning features of the patient. Later in life, an intense distressful event, included in the main theme of her delusional thought, triggered a new stage of AN. This process took place without any degree of disorganization or deficit and exhibiting all features of AN. The sharing of psychotic beliefs is a demonstration of the level of agglutination among family members, overprotection, and of the high sensitivity and repercussion events have in the family system, simultaneously present in ED patients' families.

The case presented and the review on the subject point out the importance of defining the context of symptom presentation, its rigorous phenomenology, and the course of the illness for a correct assessment and diagnosis, as well as further investigation of the relationship between psychotic and eating disorders.

Author's Contribution

Rita Almeida Leite: Planning of the publication, drafting of the project, definition of strategies and steps, bibliographic search in medical databases, and drafting of the paper.

Tiago Santos: Supported the planning of the publication and drafting of the project, guided the bibliographic search, drafted the clinical case, and revised the text in English.

Patrícia Nunes: Participated in the drafting of the clinical case, revised the bibliographic search, the draft of the papers, and the text in English.

Isabel Brandão: Revise the entire text of the clinical case and the final text of the paper.

RESUMO

INTRODUÇÃO: *A interface entre perturbação do comportamento alimentar e psicose é um campo desafiador para o qual pouca atenção foi direcionada. O seu estudo levanta algumas questões conceituais e metodológicas em ambas as áreas, dificultando o diagnóstico e o manejo dos pacientes. Essas questões são abordadas e ilustradas neste trabalho com uma revisão e um relato de caso.*

MÉTODOS: *Os autores apresentam o caso de uma mulher com anorexia nervosa e perturbação psicótica partilhada comórbida, com base numa revisão da literatura sobre a comorbilidade entre perturbação do comportamento alimentar e psicose. Os autores realizaram uma revisão não sistemática, por meio de pesquisa no banco de dados PubMed, utilizando os termos "anorexia nervosa", "bulimia nervosa", "comorbilidade" e "perturbações psicóticas".*

RESULTADOS: *Os resultados sugerem que os estudos sobre o tema são limitados por questões inerentes a escassos dados sobre prevalência de comorbilidades, aspectos fenomenológicos das perturbações alimentares, e sua interface e integração com sintomas psicóticos.*

CONCLUSÕES: *O caso apresentado ilustra as dificuldades no manejo de uma paciente com perturbação alimentar e psicose. A fim de garantir uma abordagem rigorosa dos sintomas psicóticos e alimentares, a avaliação do paciente deve focar o padrão de emergência dos sintomas, a sua fenomenologia, antecedentes clínicos e familiares e o seu* ***status*** *clínico.*

PALAVRAS-CHAVE: *Anorexia nervosa. Comorbidade. Esquizofrenia paranoide. Transtornos psicóticos. Transtornos da alimentação e da ingestão de alimentos. Transtorno paranoide compartilhado.*

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REVIEW ARTICLE

Pelvic floor parameters in women with gynecological endocrinopathies: a systematic review

Júlia Ferreira Fante[1]
Cristine Homsi Jorge Ferreira[2]
Cassia Raquel Teatin Juliato[1]
Cristina Laguna Benetti-Pinto[1]
Glaucia Miranda Varella Pereira[1]
Luiz Gustavo Oliveira Brito[1]

1. Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas
2. Department of Health Sciences, Ribeirão Preto Medical School, University of São Paulo

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SUMMARY

There is no pooled information about pelvic floor parameters (muscle assessment, disorders) of women with gynecologicaL endocrinopathies (eg. polycystic ovary syndrome, congenital adrenal hyperplasia, premature ovarian insufficiency). Given that, a systematic review was performed on the Pubmed, Scopus, Google Scholar, Scielo and PEDro databases regarding the main gynecological endocrinopathies [polycystic ovary syndrome (PCOS), premature ovarian insufficiency (POI), congenital adrenal hyperplasia (CAH) and hyperprolactinemia (HPL)] since their inception to April 2020. Data quality assessment was made by the Newcastle-Ottawa Scale (NOS) adapted for cross-sectional studies. A total of 4,272 results were retrieved from all databases. After excluding duplicate results and screening by title and abstract, nine studies were selected for quantitative analysis. Seven studies were performed with women with PCOS and two studies with POI. Women with PCOS presented a higher prevalence of urinary incontinence (UI) among obese women, a higher thickness of the levator ani muscle, and higher levels of muscle activity measured by surface electromyograph when compared to the control women. Regarding POI, there was no association with UI, FI, and POP. NOS found that the quality assessment for these selected studies ranged from 5 to 8. We concluded that higher pelvic muscle activity and volume were found in women with PCOS, with further studies needed to confirm this data. Literature was scant about POI, CAH, and HPL.

KEYWORDS: *Systematic review. Urinary incontinence. Pelvic floor. Polycystic ovary syndrome. Menopause, premature.*

INTRODUCTION

The female endocrine axis is complex and connects the hypothalamus, hypophysis, ovaries, and uterus[1]. Estrogen and progesterone are the most important hormones and any interruption on the hypothalamus-hypophysis-ovarian (HHO) axis may cause chronic anovulation or other menstrual cycle disorders, each one with its specificities[2].

The female pelvic floor is composed of muscles, ligaments, and fascia that act as support for the bladder, reproductive organs, and rectum. It is related to micturition, defecation, sexual function, and has the same embryologic origin as the vulva, vagina, and inferior urinary tract[3]. Similarly, it presents estrogen and progesterone receptors[4]. Given that sexual steroids stimulate the proliferation, differentiation, and maturation of target organs and/or glands, and its

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CORRESPONDING AUTHOR: Luiz Gustavo Oliveira Brito,
Rua Alexander Fleming, 101 – Cidade Universitária, Barão Geraldo, Campinas – 13083-881 – São Paulo – Brasil – Tel:: 55-19-3521-9595
E-mail: lgobrito@gmail.com

deficit may cause a disintegration of tissue-dependent hormones, we do not know whether the hormonal variation found within some gynecological endocrinopathies may influence the inferior urinary tract or pelvic floor muscles[5].

Some endocrinopathies are related to the reduction of hormonal levels and others, with the increase. Thus, women with premature ovarian insufficiency (POI) characterized by hypoestrogenism and increased FSH levels and women with hyperandrogenism or other endocrine diseases such as hyperprolactinemia, may present different local hormone levels, and we do not know whether this hormonal microenvironment may influence the female pelvic floor.

The higher presence of pelvic floor dysfunctions in middle-aged women is usually related to age-related modifications; however, hypoestrogenism may be related to these disorders as well. Furthermore, hyperandrogenism may activate receptors in the pelvic floor and generate different behaviors. The relationship between the PF and hormonal status is still unknown. Thus, we sought to understand if women with these disorders may present different levels of urinary, vaginal, and fecal symptoms or different pelvic muscle measurements in comparison to control women, by performing a systematic review of the literature.

METHODS

Review registration, eligibility, and inclusion/exclusion criteria

This systematic review was submitted to registration at the PROSPERO database for systematic reviews (CRD42019128540). We included all studies, containing women with gynecological endocrinopathies (premature ovarian insufficiency, polycystic ovary syndrome, hyperprolactinemia, congenital adrenal hyperplasia), with no restriction to language and publication date. Since the term gynecological endocrinopathy is not standardized, we have chosen this group of endocrine disorders whose hormonal disbalance may play a role in women's pelvic floor. We have excluded: experimental studies, studies that analyzed general muscle groups, male subjects, and pelvic dysfunction after surgeries.

Study search and selection/data extraction

Study search was performed by two authors (J.F.F and L.G.O.B.), on the following databases: PubMed/MEDLINE, Scopus/EMBASE, Google Scholar, SciELO, and PEDro. The last search data was July 6 2020. We used the following strategy:

- PubMed/MEDLINE - ("pelvic floor muscle" OR "pelvic floor" OR electrostimulation OR biofeedback OR incontinence OR "pelvic organ prolapse" OR prolapse) (PCOS OR "polycystic ovary syndrome" OR polycystic OR prolactin OR hyperprolactinemia OR "congenital adrenal hyperplasia" OR hirsutism OR "primary ovarian insufficiency" OR "premature ovarian failure") NOT (man OR animal);

- Scopus/EMBASE - (("pelvic floor" OR electrostimulation OR biofeedback OR incontinence OR "pelvic organ prolapse") (pcos OR "polycystic ovary syndrome" OR "primary ovarian insufficiency" OR hyperprolactinemia OR "congenital adrenal hyperplasia"));

- Google Scholar - "primary ovarian insufficiency" "premature ovarian failure" ("pelvic floor" OR incontinence OR prolapse); "polycystic ovary syndrome" "hirsutism" ("pelvic floor" OR incontinence OR prolapse), "congenital adrenal hyperplasia" ("pelvic floor" OR incontinence OR prolapse); "hyperprolactinemia" ("pelvic floor" OR incontinence OR prolapse) - for each item, an independent search was performed.

- SciELO - (Primary Ovarian Insufficiency, Hirsutism, Polycystic Ovary Syndrome, Hyperprolactinemia, Congenital adrenal hyperplasia) - for each item, an independent search was performed in English and Portuguese languages.

-PEDro - (Primary Ovarian Insufficiency, Hirsutism, Polycystic Ovary Syndrome, Hyperprolactinemia, Congenital adrenal hyperplasia) - for each item, an independent search was performed.

Outcomes

The following variables were investigated during data extraction prior to study selection: urinary incontinence, pelvic organ prolapse or fecal incontinence by self-report or measured by specific questionnaire; voiding or other irritative symptoms (urinary frequency, nocturia, bladder or pelvic pain) by self-report or measured by specific questionnaire; pelvic floor muscle assessment (strength, tone, thickness, maximum voluntary contraction - MVC) by PERFECT and Oxford modified scales, vaginal manometer (Peritron™), surface electromyography (sEMG) and transperineal ultrasound.

Quality assessment

We used the Newcastle-Ottawa Scale adapted for cross-sectional studies. This tool was initially

developed to assess the quality of observational studies and contains eight items divided into three domains (selection, comparability, and outcome). The score was divided into good quality (3-5 points for selection, 1-2 points for comparability, and 2-3 for outcomes), moderate quality (2 points for selection, 1-2 for comparability, and 2-3 for outcomes), and poor quality (0-1 points for selection, 0 for comparability and 0-1 for outcomes)[6,7].

RESULTS

Characteristics of the selected studies and NOS quality assessment

Figure 1 depicts the retrieved results and details for excluding studies for each step of the analysis. A total of 4,272 studies were found. After excluding the duplicates and studies that did not match the title and/or abstract, and studies with no eligibility criteria, only 14 studies were potentially eligible for final analysis. Nine manuscripts were included for quantitative analysis. It was not possible to perform a metanalysis due to the heterogeneity of the instruments used to assess outcomes among the studies. All of them had a cross-sectional design[8-16], and most of them were performed in Brasil (n=6), followed by China, Iran, and Turkey (n=1), comprising 1,132 women. Four of them[9,11,12,15] did not inform the study period, and five presented a variation of 4 to 24 months of data collection (Table 1). Seven studies addressed polycystic ovary syndrome and two analyzed premature ovarian insufficiency. We did not find any studies investigating hyperprolactinemia or congenital adrenal hyperplasia. Regarding the primary outcomes (pelvic floor disorders), we did not find any studies connecting PCOS directly with urinary incontinence, fecal incontinence, or pelvic organ prolapse. One study within POI women[16] focused on PFDs. Basically, our secondary outcomes were the main results of this review.

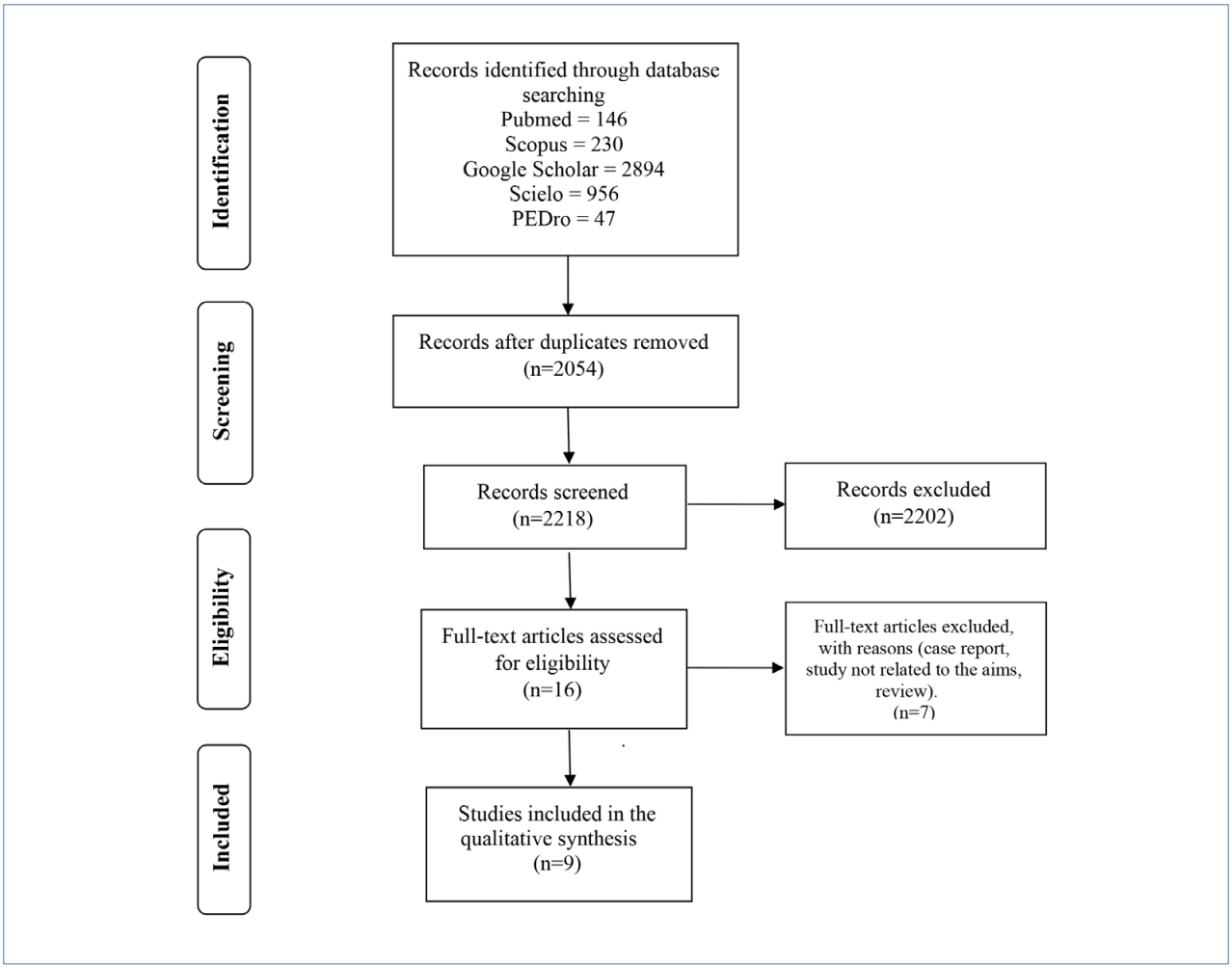

FIGURE 1. PRISMA FLOWCHART FOR STUDY SELECTION.

Regarding the NOS for quality assessment, half of the studies (n=4) presented scores equal to six (good quality)[9,11,12,14]. Three studies[8,13,15] scored five (good quality), one study[3] scored seven, and one[16] scored eight (Table 2). None of the studies presented an analysis of the non-responders since no studies were surveys or cohort databases; thus, they lacked this item within the selection domain.

Diverse instruments were applied to these studies. Most commonly, the questionnaires were self-administered and created by the authors: International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ-UI-SF), Short Form Health Survey 36 (SF-36) Pelvic Floor Disability Index 20 (PFDI-20), Pelvic pain and urgency/frequency questionnaire (PUFq). Other instruments were the transperineal 3D ultrasound (US), surface electromyography (EMG), and manometer (Table 2).

Premature ovarian insufficiency

We found two studies[8,16] that sought to investigate the association between POI and SUI. One[8] was performed in China, with 149 women with POI treated with hormone therapy (HT) and 303 women from the control group, matched by age, BMI, and parity. The main objective was to investigate the prevalence of urinary incontinence within this population, as well as to assess the effects of the temporal, individual, and therapeutic factors on SUI for POI women. They had a higher SUI prevalence (20.9%) than the control group (16.2%), with no statistically significant difference (p=0.297). The duration of POI and HT use did not have any effect on the incidence of SUI. The risk factors that were associated with SUI in POI women were: age over 40 years (p=0.001), primiparity (p=0.001), and vaginal delivery (p=0.023).

The other study[16] was performed in Brasil, and 150

TABLE 1. GENERAL CHARACTERISTICS OF THE SELECTED STUDIES.

References	Study Design/Period	Country	Female related endocrinopathy	Sample Case + control	Mean age ±SD Case/Control	Objectives
Fante et al.[16], 2020	CS/Aug-2017/Nov 2018	Brasil	POI	150/150	35.41±8.58/33.19±8.47	To investigate the prevalence of self-reported UI, POP, and FI and its associated factors within POI women and a control group
Tan et al.[8], 2018	CS/ Jan 2016-Aug 2017	China	POI	451	33.56 ±5.49/33.06±5.06	To investigate the prevalence of SUI in patients with POI and evaluate the effect of temporal, individual, and therapeutic factors on SUI in patients with POI.
Melo et al.[9], 2018	CS/NI	Brasil	PCOS	20	25.3±4.5/26.2±4.0	To assess pelvic floor muscle thickness using ultrasound imaging in women with PCOS and compare it to those with a normal menstrual cycle.
Taghavi et al.[10], 2017	CS/ May-September 2014	Iran	PCOS	202	28.76±5.33/28.48±4.87	(a) To compare the prevalence of PFD in patients with and without PCOS; and (b) to test the hypothesis that PFD varies according to the different PCOS phenotypes.
Micussi et al.[12], 2016	CS/NI	Brasil	PCOS	55	25.3±4.5/26.2±4.0	To compare the electrical activity of PFM in a group of women with PCOS and premenopausal women during the follicular phase of the normal menstrual cycle.
Vassimon et al.[13], 2016	CS and CC/2008-2010	Brasil	PCOS	72	27±5.5/30±4.9	To assess PFM thickness and neuromuscular activity among hyperandrogenic women with PCOS and controls.
Antônio et al.[14], 2013	CS and CC/2008-2010	Brasil	PCOS	79	27.2±5.5/37±5.3	To assess PFM strength and UI among hyperandrogenic women with PCOS and a control group for comparison.
Montezuma et al.[15], 2011	CS controlled/NI	Brasil	PCOS	113	24.78 and 28.75/ 31.69 and 30.61	To compare reports of urine leakage and quality of life between women with and without polycystic ovary syndrome.
Sahinkanat et al.[11], 2011	CS/NI	Turkey	PCOS	140	26.1±5.2	To examine the relationship between bladder symptoms and serum testosterone levels in women with polycystic ovary syndrome.

Abbreviations: CS (cross-sectional); NI (not informed); POI (premature ovarian insufficiency); PCOS (polycystic ovary syndrome); SUI (stress urinary incontinence); PFD (pelvic floor dysfunction); PFM (pelvic floor muscle).

TABLE 2. METHODOLOGICAL QUALITY (NEWCASTLE-OTTAWA SCALE), INDEPENDENT VARIABLE(S), INSTRUMENTS, AND THE MAIN RESULTS OF SELECTED STUDIES.

References	Quality(NOS)	Variables	Instruments	Results
Fante et al.[16], 2020	Selection: **** Comparability: * Outcome: **	PFD (IU, POP, FI) and PFM assessment (PERFECT scale)	Self-reported UI, POP, and FI symptoms PFDI-20 KHQ	The prevalence of self-reported UI was 27.33% and 37.33% for, respectively, POI and control women, with no statistically significant differences. Similarly, no differences were perceived between the groups with regard to POP and FI prevalence. The PERFECT scale presented similar scores on power, endurance, repetitions, and fast contractions between POI and control groups.
Tan et al.[8], 2018	Selection: ** Comparability: * Outcome: **	SUI	Self-administered questionnaire	The prevalence of SUI in the POI group tended to be higher than that in the control group (20.9%, 30/149 vs. 16.2%, 49/303), although not significantly (p = 0.297). About 41.6% (62/149) of patients with POI received HT. Patients with POI and SUI were older (p = 0.018) and had higher BMI (p = 0.007) than women with POI without SUI (p = 0.007).
Melo et al.[9], 2018	Selection: *** Comparability: * Outcome: **	Pelvic Floor thickness	Transperineal 3D ultrasound	There was no difference between the thickness of the pelvic floor muscles (PCOS group: right 1.12 ± 0.5, left 1.0 ± 0.6 and control group: right 0.89 ± 0.6 and left 0.94 ± 0.4). However, the PCOS group exhibited a tendency to a greater thickness. This may be due to the state of hyperandrogenism or abdominal overload.
Taghavi et al.[10], 2017	Selection: **** Comparability: * Outcome: **	PFD (UI, POP, FI))	PFDI-20	Briefly, the reported pelvic organ prolapse (POP) symptoms were higher in the PCOS group (p=0.05). The mean PFD score in the HA+M+PCO group was higher compared to other phenotypes, although the difference did not reach a significant level (p>0.05). However, there was a significant positive correlation between the luteinizing hormone (LH) level and the POP symptom portion of the PFDI-20 (p<0.05).
Micussi et al.[12], 2016	Selection: *** Comparability: * Outcome: **	Electrical activity of PFM	sEMG	There was a difference in muscle tone (PCOS=59.9 mV and Control group=25.5 mV; p<0.0001) and MVC (PCOS=159.7 mV and Control group=63.7mV; p<0.0002) between the groups. The concentration of estradiol and testosterone showed a strong correlation with tone (r=0.9, r=0.8, respectively) and MVC (r=0.9, r=0.9 respectively) in women with PCOS.
Vassimon et al.[13], 2016	Selection: ** Comparability: * Outcome: **	PFM (electrical activity and pelvic floor thickness)	sEMG/US	There were no significant differences in PFM sEMG activity between PCOS and controls in any of the contractions: quick contraction (73.23 mV/ 71.56 mV; p=0.62), 8 s (55.77 mV/ 54.17 mV; p=0.74), and 60 s (49.26 mV/47.32 mV; p=0.68), respectively. There was no difference in PFM thickness during contractions evaluated by US between PCOS and controls (12.78 mm/ 13.43 mm; p=0.48).
Antônio et al.[14], 2013	Selection: *** Comparability: * Outcome: **	PFM strength and UI	Manometer Peritron; ICIQ-UI-SF	There was no statistically significant difference in mean PFM strength between the PCOS and the control group: 2.7 cm H2O (95 % CI −6.2–11.6) p=0.55. The prevalence of UI was 18.6 % in the control group compared with 0 % in the PCOS group p<0.01.
Montezuma et al.[15], 2011	Selection: *** Comparability: * Outcome: *	UI symptoms	ICIQ-SF, SF-36	The answers to the ICIQ-SF revealed a significant difference in urinary function between the groups, with 24% of the subjects in the control group with BMI > reporting urinary incontinence. The mean scores for the SF-36 questionnaire revealed that group II had the lowest quality of life.
Sahinkanat et al.[11], 2011	Selection: *** Comparability: * Outcome: **	Bladder storage symptoms	PUFq	A significant positive correlation was found between serum testosterone level and total, symptom and bother scores of the PUFq, symptoms of dyspareunia, urgency, nocturia, and bladder/pelvic pain. There was no correlation between the serum testosterone level and ultrasonographic findings such as bladder capacity, postvoid residual volume, and symptom of frequency.

Abbreviations: POI (premature ovarian insufficiency); PCOS (polycystic ovary syndrome); SUI (stress urinary incontinence); PFD (pelvic floor dysfunction); PFM (pelvic floor muscle); UI (urinary incontinence); PFDI-20 (pelvic floor distress inventory-20);sEMG (surface electromyography); PUFq (pelvic pain and urgency/frequency questionnaire); US (ultrasound); SF-36 (Short-Form Health Survery); ICIQ-SF (international consultation on incontinence questionnaire-short form); ICIQ-UI-SF (international consultation questionnaire - urinary incontinence short form; NOS (Newcastle-Ottawa scale); KHQ (Kings Health Questionnaire)

patients with POI were control-matched for age and body weight (n=150). There was no difference between the groups with regard to self-reported UI, POP, and FI (p>.05). Moreover, pelvic floor muscle assessment by the PERFECT scale did not differ between the groups.

Polycystic ovary syndrome (PCOS)

Seven studies were retrieved[9-15]. Five of them aimed to assess the relationship between hyperandrogenism and some variables about muscle activity (tone, strength, neuromuscular activity, and thickness) and urinary symptoms. The other two studies aimed to investigate or compare the prevalence of pelvic floor dysfunctions between women with and without PCOS.

Montezuma et al.[15] investigated the prevalence of urinary incontinence (UI) and quality of life within obese and non-obese women with and without PCOS, and found a higher UI prevalence in overweight women of the control group (24%) with a statistical difference among the groups (p=0.04); however, when the groups were stratified by BMI, no difference was noted. Furthermore, there was no difference regarding urine loss among the groups, and the quality of life was lower in overweight PCOS women. Taghavi et al.[10] assessed the prevalence of pelvic floor dysfunctions in patients with and without PCOS according to the clinical phenotypes (menstrual disorders/hirsutism) and found that PCOS women presented more pelvic prolapse symptoms (p=0.05). Also, women with menstrual disorders and hirsutism presented higher scores for pelvic floor dysfunctions when compared with each phenotype occurring individually, but with no statistical difference (p>0.05).

Regarding pelvic floor muscle within PCOS women, studies were not confluent with their findings. One study did not confirm its hypothesis that PCOS women with hyperandrogenism would generate higher muscle strength in PFM with control women[14]. Vassimon et al.[13] did not find any significant difference between PCOS and control women regarding fast (p=0.62) and sustained pelvic muscle contractions (p=0.74) measured by surface electromyography. Perineal muscle thickness was not different between the groups during contractions when measured by ultrasound (p=0.48). Another study specifically measured the levator ani muscle, and the PCOS group presented a higher muscle thickness when compared to the control group (PCOS right side: 1.2 ±0.5 cm; PCOS left side: 1.8 ±0.6 cm vs Control right side: 0.89±0.6 cm; Control left side: 0.94±0.4 cm), with no significant difference between the groups[9].

One study[11] analyzed the association between testosterone levels and bladder symptoms by using the Pelvic pain and urgency/frequency (PUFq) questionnaire, and they found a positive correlation between testosterone levels with total PUFq score (p=0.000), symptoms (p=0.000), bother (p=0.000), nocturia (p=0.008), dyspareunia (p=0.000), pain frequency (p=0.024), urgency (p=0.006), pain severity (p=0.016), and urgency severity (p=0.000); however, there was no correlation between testosterone and ultrasonographic findings related to bladder capacity (p=0.345) and postvoiding residue (p=0.061).

Finally, Micussi et al.[12] found significant differences between PCOS and control groups with regard to obesity when analyzing the electrical activity by surface electromyography; PCOS patients presented higher activity with or without obesity (obese PCOS – 69.3 µV versus control – 25.5 µV; overweight PCOS – 54.6 µV versus control – 25.5 µV; non-obese PCOS – 47.7 µV versus control – 25.5 µV). Furthermore, PCOS women presented higher maximum contraction volumes versus the control group (obese PCOS – 202.8 µV versus control – 63.7 µV; overweight PCOS – 140.9 µV versus control – 63.7 µV).

DISCUSSION

This review has found nine studies addressing pelvic floor parameters in gynecological endocrinopathies. Two of these studies analyzed PFDs and POI, and the other seven investigated PFDs with PCOS. It seems that the connection between the pelvic floor and female related endocrinopathies is not so well investigated. Estrogen receptors are present in the genital area and within the urinary tract. They are also related to the increase of collagen synthesis within connective tissue from the pelvic floor[17], as well as muscle regenerative markers[18]. Thus, hypoestrogenism will have effects within these areas, with vulvovaginal and urinary symptoms[19]. However, we cannot ascertain whether estrogen, by itself, is responsible for all these symptoms or plays a main role in pelvic floor dysfunctions.

Primary ovarian insufficiency is an important topic that needs further studies. Two studies were found, and we did not find any association with UI[8,16]. However, one of the studies[8] observed that even with no statistically significant difference, POI women presented higher UI prevalence than control women. Women with POI, especially patients that did not

have the chance to start hormone therapy and present a longer POI duration, represent a good model to understand whether hypoestrogenism may cause pelvic floor dysfunction. The other study had a smaller group of patients with POI not using HT and the reason for that is because these patients were the ones who were in their first consultations ready to start using it. It would be unethical not to prescribe HT for these women just to see the effect of hypoestrogenism on PFM function and the incidence of PF disorders.

Tan et al.[8] did not find any significant differences between HT usage by POI women and the presence of SUI. Furthermore, the association between HT usage and urinary continence has not yet been established, and according to some data, oral HT impairs urinary symptoms[20]. Some authors have found that estrogen improves collagen production[17], whereas others suggest that exogenous estrogen may be related to collagen degradation in the bladder neck region, altering bladder support and favoring symptoms related to SUI. It was observed that women with SUI presented a decrease of collagen production and that its degradation was augmented after six months of using HT[21]; there is a lack of evidence about whether the role of HT on POI women and PFDs would be general. There is a case report from a 25-year nulliparous patient, with the diagnosis of POI presenting constipation and localized scleroderma with an advanced uterine prolapse; this patient sheds some light about the etiology of hypoestrogenism associated with other factors that increase intra-abdominal pressure (eg. constipation) causing POP[22].

Another relationship that should be explored is between androgens and the pelvic floor. Skeletal muscles present receptors for estrogens and androgens, and it is believed that the variation of these hormones affects endurance and muscular strength[23]. As androgens may increase muscle mass, this might happen with PCOS women with clinical and/or laboratorial hyperandrogenism[24-26]. Some studies, despite not presenting significant differences among the groups, show that the presence of circulating androgens may generate more muscle thickness[9] and strength[14]. On the other hand, PCOS women may present a worse quality of life, more prolapse, and urinary symptoms[10,11,15]. Central obesity caused by PCOS may increase bladder pressure and urethral mobility, contributing to urinary incontinence[27]. However, Micussi et al.[12], have found that hyperandrogenism in PCOS women may be a protective factor for the pelvic floor since women with high BMI presented better muscle electric activity. This information may be related to high levels of estrogen and testosterone in the PCOS group. There is evidence that estradiol regulates the growth and regeneration of the skeletal muscle fibers through satellite cells and modulates immune responses[17]. Testosterone stimulates protein synthesis, recruits satellite cells by the anabolic effect, and inhibits protein degradation, increasing muscle fiber recruitment[28]. Regarding PCOS studies, obesity seems to play a role in a more meaningful way than the endocrinopathy itself.

No studies were found with regard to hyperprolactinemia or congenital adrenal hyperplasia. As we know that high levels of prolactin, in a chronic fashion, may decrease muscle mass in non-treated patients, studies analyzing the pelvic floor of patients with this disorder would be interesting to see its effect.

The strengths of this review were the fact that, to our knowledge, there is no previous compilation of the literature looking for women with gynecological endocrinopathies that could influence pelvic floor muscles and other structures and the development of these disorders. The limitations were related to the scant number of retrieved results, and since these studies are cross-sectional, it is not possible to infer a causality relationship. Other factors are certainly influencing this connection. The quality assessment for these studies showed moderate to good quality. We did not use a filter to exclude studies with low quality, but this did not impact the number of retrieved results.

Further studies with diseases such as premature ovarian insufficiency are needed so that we may have a deeper understanding of how estrogen (and its absence) impact the pelvic floor. Similarly, androgen seems to play a role in increasing muscle strength and thickness. However, PFM anatomy differs from PFM function, and this should be the goal for future studies.

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Author's Contribution

J.F. Fante: data curation, investigation, methodology, project management, writing of the original draft, review, editing; L.G. Brito: conceptualization, methodology, project management, supervision, review, editing; C.H. Ferreira: formal analysis, visualization; C.R. Juliato: formal analysis, supervision, visualization; C.L. Benetti-Pinto: formal analysis, visualization, review, editing; G.M. Pereira: methodology, visualization.

RESUMO

Existe informação não organizada sobre a avaliação do assoalho pélvico de mulheres com endocrinopatias ginecológicas (ex. síndrome dos ovários policísticos - SOP, hiperplasia adrenal congênita - HAC, insuficiência ovariana prematura - IOP). Dessa forma, objetivamos realizar uma revisão sistemática foi realizada nas bases Pubmed, Scopus, Google Scholar, Scielo e PEDro sobre as endocrinopatias ginecológicas (SOP, HAC, IOP e hiperprolactinemia (HPL) desde a origem a abril de 2020. A avaliação da qualidade de dados foi realizada pela escala de Newcastle-Ottawa Scale (NOS) adaptada para estudos transversais. De 4,272 resultados encontrados em todas as databases, após exclusão por duplicatas, triando por título e resumos, nove estudos foram selecionados para análise quantitativa. Sete estudos foram realizados para mulheres com SOP e dois estudos com IOP. Em suma, mulheres com SOP apresentados uma alta prevalência de incontinência urinária (IU) em mulheres obesas, alta espessura do músculo elevador do ânus, altos níveis de atividade muscular aferida por eletromiografia de superfície quando comparadas com mulheres do grupo controle. Sobre a IOP, esta não foi associada com IU, IF e POP. A escala NOS evidenciou que a qualidade dos estudos selecionados variou de 5 a 8. Concluímos que uma alta atividade e volume muscular foi encontrada em mulheres com SOP, com estudos posteriores sendo necessários para confirmar estes achados. Literatura foi escassa para IOP, HAC e HPL.

PALAVRAS-CHAVE: *Revisão sistemática. Incontinência urinária. Diafragma da pelve. Síndrome do ovário policístico. Menopausa precoce.*

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REVIEW ARTICLE

Diastolic dysfunction for nephrologists: diagnosis at the point of care

Marcus G Bastos[1,2,3]
Ana Luisa Vieira[4]
José M Pazeli Jr[4]
Arise GS Galil[1]

1. Faculdade de Medicina da Universidade Federal de Juiz de Fora, MG, Brasil.
2. UNIFAGOC, Ubá, MG, Brasil.
3. Faculdade de Ciências Médicas e da Saúde (SUPREMA), Juiz de Fora, MG, Brasil.
4. Serviço de nefrologia, Santa Casa de Misericórdia de Barbacena, Faculdade de Medicina de Barbacena, Barbacena, MG, Brasil.

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SUMMARY

Cardiovascular diseases are important causes of morbidity and mortality in the course of chronic kidney disease (CKD). Diastolic dysfunction (DD) may progress with the clinical manifestation of heart failure, known as heart failure with preserved ejection fraction, a condition that precedes systolic dysfunction. The early identification of DD by echocardiography at the point-of-care before the appearance of symptoms and signs of pulmonary congestion and the implementation of appropriate treatment can improve the prognosis of CKD. This review article briefly addresses DD in kidney disease and presents a practical approach to the echocardiographic diagnosis of DD at the point of care.

KEYWORDS: *Heart Failure, Diastolic. Echocardiography. Heart failure. Renal Insufficiency, Chronic.*

INTRODUCTION

Patients with chronic kidney disease (CKD) associated with cardiovascular disease (CVD) have a higher risk of death than the general population[1]. About 40% of patients with stage 4-5 CKD have a diagnosis of heart failure, as seen in the USRDS Annual Data Report of 2015[2]. Consequently, in patients with CKD, heart failure (HF) must be diagnosed and treated early. HF with preserved ejection fraction (HFpEF) describes HF due to diastolic dysfunction (DD) occurring with normal or slightly reduced ejection fraction, and without the attributable valvular disease[3]. HFpEF is still underdiagnosed and has been implicated as a significant contributor, if not the leading cause, of congestive HF in such patients[4]. The importance of identifying HFpEF is its association with poor prognoses. For example, when 4,550 patients in the general population were followed-up for five years, 2,126 with HFpEF and 2,424 with heart failure with reduced ejection fraction [HFrEF], the survival rate was almost the same regardless of whether the ejection fraction was reduced or not (risk-standardized for death, 0.96; p = 0.03)[5]. In this short review, we discuss DD succinctly and present a practical approach to identifying it at the point of care, essential when the comprehensive

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CORRESPONDING AUTHOR: Marcus Gomes Bastos
Rua Ivan Soares de Oliveira, 234, Parque Imperial – 360-36350 – Juiz de Fora (MG), Brasil – Tel: 55 32 991293935
E-mail: marcusbastos7@gmail.com

echocardiography cannot be obtained or is not immediately available to the nephrologist.

Heart failure with preserved ejection fraction in kidney disease

HFpEF is a disease that affects the elderly and patients who have, on average, at least four comorbid conditions. The prevalence of CKD is high (and rising) in part due to the aging population who frequently present multiple comorbidities[6]. It is not surprising that HFpEF is frequently diagnosed in these patients[7]. The occurrence of DD in CKD can be identified in patients in conservative and dialytic treatments, as well as in transplant recipients and critically ill renal patients. In patients with CKD not yet on dialysis and complaining of dyspnea, HFpEF was diagnosed in 73%, and in 44,6%, the HFpEF was the only explanation for the dyspnea[8]. Also, in patients on dialysis subjected to transthoracic echocardiography (TTE), left ventricular hypertrophy was found to be the primary anatomical abnormality and DD, present in 81% of cases, the main functional alteration[9]. Notably, DD preceded systolic dysfunction, which makes this study's findings even more interesting for nephrologists.

In renal transplant, DD is also a frequent and dangerous complication, since the vast majority of these patients present left ventricular hypertrophy due to volume overload and hypertension during their dialysis treatment. Therefore, it is not surprising that transplant patients are also diagnosed with HFpEF[10].

Nephrologists looking after patients in the intensive care unit may also deal with clinical conditions associated with left ventricular DD, which is associated with mortality in critically ill patients[11]. Thus, for example, in critically ill patients who need fluid resuscitation, if the assistant nephrologist can assess DD, the fluid replacement could be promptly implemented even before the availability of comprehensive echocardiography by cardiologists.

When present, the symptoms and signs of HFpEF are indistinguishable from those observed in HFrEF: neurohumoral activation with sodium and water retention (edema); paroxysmal nocturnal dyspnea; orthopnea; jugular vein distension; third heart sound; hepatomegaly; and cardiomegaly on X-ray[12]. Due to the limitations of myocardial relaxation and compliance during diastole, the left ventricular end-diastolic pressure (LVEDP) in patients with DD is already high previously to physical activity. Consequently, the cardiac output does not increase, which makes these patients highly intolerant to physical exercise. Similarly, these patients do not tolerate large volume fluctuations, making them more susceptible to intradialytic hypotension[13].

Pathophysiology of diastolic dysfunction

Although a detailed description of DD's pathophysiology is beyond the scope of this review, a basic comprehension of the various grades of DD is essential for nephrologists to understand the echocardiographic findings that will allow them to diagnose DD at the point of care.

Hypertension is considered the leading risk fac- tor for the development of HFpEF. Other precipitating factors such as diabetes mellitus, obesity, and coronary artery disease, also common in patients with CKD, may explain the high prevalence of DD observed in kidney patients[14]. Potential explanations for this increased prevalence of DD include aortic stiffness and volume overload, which are frequently observed in CKD, particularly in the more advanced stages of the disease[15].

The left ventricle could be compared to a powerful propulsion pump, in systole, and suction pump, in diastole. Diastole comprehends the period between the closure of the aortic valve (end of systole) and the closure of the mitral valve (end of diastole), and it comprises four distinct and consecutive phases: isovolumic relaxation, early rapid diastolic filling, diastasis, and atrial contraction[16]. The first two phases of diastole are active and require adenosine triphosphate (ATP) production from the myocardium. During isovolumetric relaxation, LV pressure decreases with the closure of the aortic valve but without a change in volume. When the pressure drops below the atrial pressure, the mitral valve opens. Then, the initial early rapid diastolic filling period occurs, with the blood being suctioned by the LV from the left atrium. Several parameters, including myocardial relaxation, LV compliance, and the atrioventricular pressure gradient, influence the velocity of blood flow through the mitral valve. As the amount of blood in the LV increases, gradual pressure equalization occurs with the left atrium, which characterizes diastase, a period in which transmitral blood flow is minimal. The final phase of diastole occurs with atrial contraction, which transiently increases left atrial pressure and promotes late LV filling. The phases of diastole in the right ventricle are similar to those described for LV, except for the

total duration, which is shortened due to a more extended systolic ejection period[17].

HFpEF, when DD becomes clinically evident, results from different mechanisms, such as ventricular-arterial coupling, chronotropic incompetence, and endothelial dysfunction, but without a doubt, DD is the most significant[18]. DD results in increased LVEDP, increased pressure in the left atrium, and may lead to the development of symptoms and signs of pulmonary congestion.

Assessment of diastolic dysfunction

TTE is the most commonly used propaedeutic tool in the diagnosis of DD. The American Society of Echocardiography (ASE) has published guidelines for the assessment of diastolic dysfunction[17]. The parameters suggested for the diagnosis of DD include: 1. transmitral blood inflow by pulsed-wave Doppler (PWD); (2) mitral annulus downward velocity (septal and lateral walls) using tissue Doppler imaging (TDI); (3) left-atrial volume index; and (4) peak tricuspid regurgitation velocity. DD is present if more than half of the available parameters meet the cutoff values. However, all these measurements are challenging to obtain, time-consuming, and impractical at the point of care. Besides, the ASE guidelines do not categorize DD of a third of septic patients and rate 41% of patients with elevated E/e' as normal[19].

Point-of-Care evaluations of DD in everyday nephrology, whether in office, ward, or intensive care unit are used mainly to answer two fundamental questions: (1) is DD present?; and (2) is the LVEDP high?

In this context, DD can be assessed qualitatively and quantitatively. Qualitatively, DD can be inferred by comparing the linear size of the left atrium (LA) with the size of the aorta and the right ventricle outflow tract in an image obtained in the cardiac parasternal long-axis view at the end of diastole. The size of LA does not change acutely, and in the absence of significant mitral regurgitation or stenosis, its enlargement represents chronically elevated LVEDP[20]. Under normal conditions, the size of the left atrium is similar to that of the aorta and right ventricle outflow tract, and this evaluation is known as the rule of thirds (Figure 1). A left atrium-to-aorta diastolic diameter ratio >1 correlates with LA enlargement and may be useful as a quick bedside technique that suggests DD[21]. Besides, the assessment of the left atrium has prognostic importance. For instance, a left atrial volume index (LAVi) lower than 32 mL/m^2, adopted as a normal superior limit, was associated with a higher survival rate compared to values >32 mL/m^2 and was shown to be an independent predictive value of prognosis in patients subjected to hemodialysis[9].

Moreover, DD can be assessed qualitatively by the visual analysis of the amplitude of the basal displacement of the septal annulus of the mitral valve during diastole. In conditions of normality, this displacement is large, while in DD, it is limited in amplitude[17].

Quantitatively, DD can be analyzed through PWD and TDI[19]. For instance, in septic patients subjected to TTE, a simplified definition of DD, based on transmitral

FIGURE 1. RULE OF THIRDS: PARAESTERNAL LONG-AXIS VIEW SHOWING THE RELATIONSHIP BETWEEN THE LEFT ATRIUM, AORTIC ROOT, AND RIGHT VENTRICLE OUTFLOW TRACT DIAMETERS IN DIASTOLE IN A HEALTH SUBJECT (A) AND IN A PATIENT WITH AN INCREASED LEFT ATRIUM (ASTERISK) WITH DIATOLIC DYSFUNCTION (B)

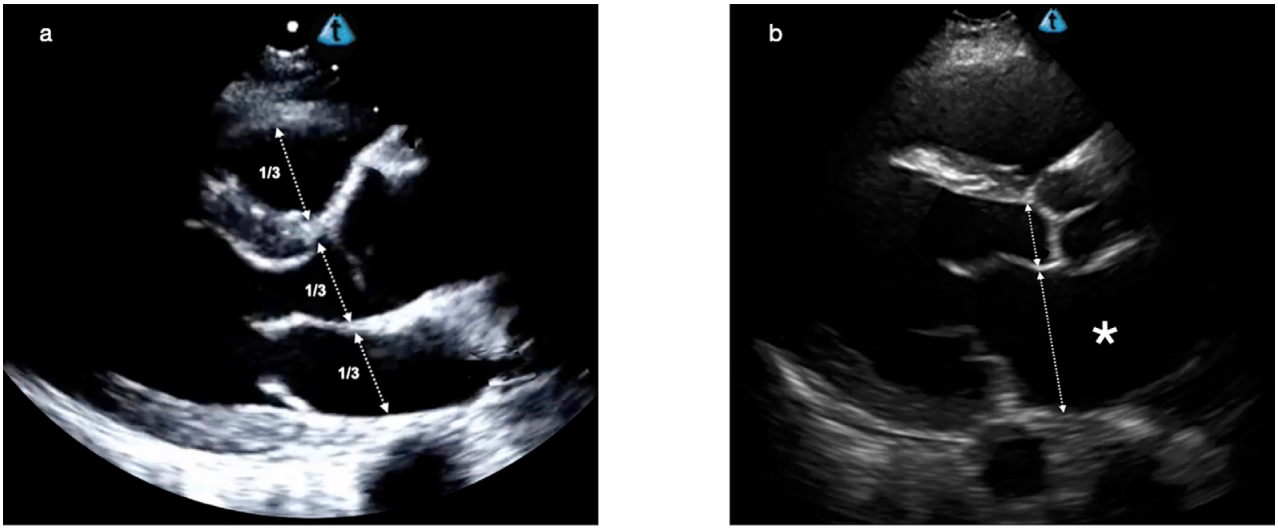

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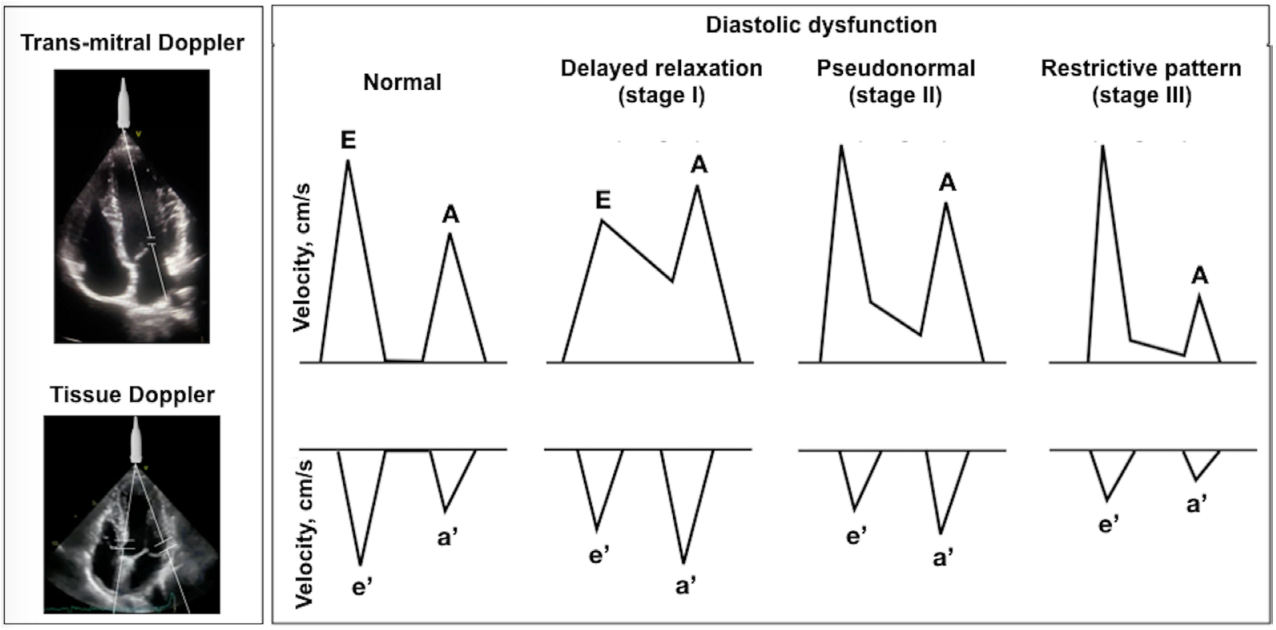
FIGURE 2. DEGREES OF DIASTOLIC DYSFUNCTION BASED ON TRANSMITRAL AND TISSUE DOPPLER IMAGING SHOWING BLOOD FLOW VELOCITIES THROUGH THE MITRAL VALVE (E AND A WAVES) AND THE VELOCITY OF THE BASAL DISPLACEMENT OF THE MITRAL VALVE ANNULUS TOWARDS THE BASE OF THE HEART (E' AND A' WAVES); NORMAL AND DIFFERENT STAGES OF DIASTOLIC DYSFUNCTION.



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blood inflow and mitral annulus downward velocity, categorized more patients than the American Society of Echocardiography 2016 definition (78% vs. 71%, p= 0.035) and showed reasonable correlation with comorbidities (hypertension, diabetes, and myocardial infarction)[19].

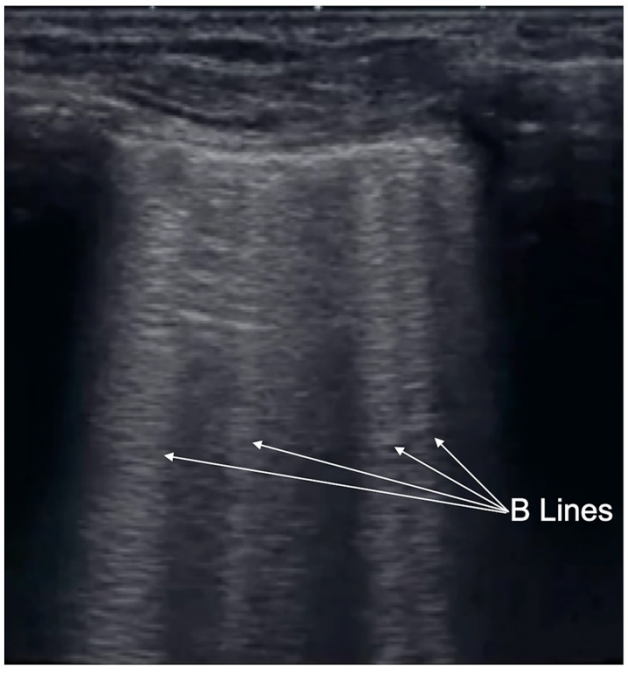
The assessment of transmitral blood flow velocity by PWD is performed with phased-array transducer (1-5 MHz), placing the sample gate on the free-edge of mitral valve leaflets in the apical 4-chamber view of the heart. The blood flow velocity from the left atrium into the LV during the early rapid diastolic filling is measured. Since the direction of the blood flow is towards the ultrasound probe positioned at the tip of the heart, the signal obtained is an upward deflection, denominated E wave. The final phase of diastole, which results from atrial contraction, also presents an upward deflection and is represented by the A wave.

The PWD patterns allow categorizing the DD (Figure 2). In normal diastole, the majority of diastolic filling occurs during the early phase of the cardiac cycle (passive pull of LV relaxation), graphically represented by an E wave higher than the A wave (E/A >0.8). In grade I DD, the impairment in myocardial relaxation results in a reduced rate of decrease of LV pressure. As a consequence, there is a decrease of the normal "pull" during early LV diastole and a "push" of the remaining blood into the LV during the atrial contraction. Grade I DD has a very distinct mitral inflow pattern with an E/A ratio <0.8. This is the most frequent DD; however, because LVEDP is normal, symptoms of heart failure are not yet apparent. In grade II DD, there is a transition from abnormal relaxation to an impairment of both ventricular relaxation and compliance. The E wave is again higher than the A wave (resulting in the term pseudonormal), but now due to a concurrent increase in the left atrial pressure, which pushes the blood flow across the mitral valve (E/A >0.8). Grade III DD represents a restrictive filling pattern, in which relaxation and compliance continue to worsen, leading to severe increased left atrial pressure and size, which masks underlying abnormalities. The E wave is also higher than the A wave (E/A rate >2). In grades II and III DD, the ventricular diastolic filling is compromised, the LVEDP increases, and the patient develops symptoms and signs of pulmonary congestion[17,19].

TDI evaluates the slower speed of tissue, and it measures the velocity of the longitudinal displacement of the mitral valve annulus towards the base of the heart (Figure 2)[17]. The sample gate is positioned at the intersection of the mitral annulus and the septum and/or the LV lateral wall. As TDI assesses the velocity of myocardial tissue movement, the values measured are much lower than the transmitral blood flow velocity. Because the analysis of the mitral annular movement is towards the base of the heart, i.e., the movement of

the tissue is in the opposite direction to the probe positioned at the tip of the heart (in the apical four-chamber view), the graphical representation of the wave is negative. In normal diastole, the movement of the mitral annulus during the early rapid diastolic filling is represented by the e' wave (normal velocity is ≥8 cm/s for septal e' and ≥10 cm/s for lateral annulus e'), which is higher than the a' wave, a representation of the atrial contraction. Fortunately, only the e' wave is usually needed to assess DD, which explains its frequent use as a surrogate for the LV relaxation rate. The higher the e', the faster the ventricular relaxation and the better the diastolic filling. As the relaxation and myocardial compliance worsen, expressed as e' wave abnormally low in amplitude (<8 cm/s for septal e' and <10 cm/s for lateral annulus e'), the LV diastolic filling raises, and the atrial pressure consequently increases[17]. As shown in Figure 2, in grade I DD, the e' wave is <8 cm/s (septal e') or <10 cm/s (lateral annulus e') and shorter than the a' wave. In grade II DD, the e' wave is also <8 cm/s for septal e' and <10 cm/s for lateral annulus e' and shorter than the a' wave, a finding that allows differentiating normal diastole from pseudonormal DD. Finally, in grade III DD, the e' wave is even smaller, and the a' wave sometimes almost does not exist[17].

FIGURE 3. B-LINES ARE DEFINED AS DISCRETE LASER-LIKE VERTICAL HYPERECHOIC REVERBERATION ARTIFACTS THAT ARISE FROM THE PLEURAL LINE, EXTEND TO THE BOTTOM OF THE SCREEN WITHOUT FADING, AND MOVE SYNCHRONOUSLY WITH LUNG SLIDING



The importance of answering the second question, "Is the LVEDP high?" is because elevated left ventricular filling pressure correlates with increased pressure in the left atrium and pulmonary capillary wedge pressure, therefore, with symptoms and signs of HFpEF[17,22].

LVEDP determination by echocardiography is easy and obtained by dividing the transmitral blood flow velocity by the velocity of the mitral valve towards the base of the heart (E/e' ratio). The LVEDP is normal when the E/e' ratio is ≤8 and considered increased when it is >15. Values between 9 and 15 are considered indeterminate[22]. Additionally, the E/e' ratio can be used to estimate pulmonary capillary wedge pressure (PCWP) by this simple formula: PCWP = 1.9 + (1.24*E/e')[23].

In cases of indeterminate LVEDP, two other assessments can be used: 1. visual evaluation of the left atrium size, which, if increased, suggests elevated LVEDP; and 2. assessment of B lines by lung ultrasound. B lines are lung artifacts seen as vertical lines that originate at the visceral pleura, move with the respiration, and erase the A lines (Figure 3)[24]. In the right clinical context, three or more B lines, in two or more intercostal spaces in both lungs, should be considered a diagnostic for pulmonary edema, with 94% sensitivity and 92% specificity[25,26]. The assessment of B lines does not require sophisticated software or a prolonged learning curve and is performed at the bedside in just a few minutes with the same probe used for echocardiography. It has been shown that B lines exhibit a good correlation with lung congestion diagnosed by chest radiography and brain natriuretic peptide levels[25] and correlates with non-invasively and invasively measured LVEDP[27,28].

In CKD, particularly in patients undergoing pre-dialysis treatment, the usefulness of the E/e' ratio as a predictive factor for CVD and mortality has not been thoroughly studied but may be promising. For example, the echocardiographic assessment with PWD e TDI in patients of both genders, with a mean age of 61 years, and CKD stages 3 (25 patients), 4 (22 patients) and 5 (89 patients), followed-up for five years, showed that patients with E/e' ratio >15 had lower survival rates and higher cardiovascular events[8].

However, it is important to recognize that there are cases where the point of care echocardiography cannot provide diagnostic certainties. For instance, in AF, atrial contraction is lost. Consequently, there is no transmittal A wave, and hence the E/A ratio cannot

be used. In this case, the use of the E/e' ratio, which is independent of atrial influence, correlates well with elevated LVEDP and can be used as a marker for DD in AF. Other conditions that may demand comprehensive echocardiography are abnormalities of basal left ventricular motion, mitral annular disease, and improper cardiac windows. However, if the echocardiologist's assessment also proves inconclusive, invasive evaluation of the left ventricular filling pressure remains the gold standard modality of choice[27].

CONCLUSION

DD is a cardiovascular complication in CKD and is potentially associated with undesirable outcomes. Determining DD and the LVEDP through the E/e' ratio obtained by PWD and TDI is a non-invasive method that allows predicting mortality and cardiovascular events at the point of care. Although we realize that DD is a complex condition, the simplification of its assessment, as shown in this review, can attract nephrologists to incorporate it into their everyday practice. However, we recognize that further prospective studies are warranted to validate and extend this simplified definition of DD, particularly in nephrology.

Conflicts of interest

The authors declare there are no conflicts of interest that could have influenced the work presented herein.

Author's Contribution

Conceptualization, structuring of the manuscript, critical review, and final approval of this version: All authors; Drafting of the manuscript: Marcus G Bastos

SUMÁRIO

As doenças cardiovasculares são causa importante de morbidade e mortalidade no curso da doença renal crônica (DRC). A disfunção diastólica (DD) pode evoluir com insuficiência cardíaca manifesta clinicamente, denominada insuficiência cardíaca com fração de ejeção preservada, e precede a disfunção sistólica. A identificação precoce da DD pela ecocardiografia "point of care", antes do aparecimento dos sintomas e sinais de congestão pulmonar, e a implementação de tratamento adequado podem melhorar o prognóstico da DRC. Este artigo de revisão aborda brevemente a DD na doença renal e apresenta uma abordagem prática para o diagnóstico ecocardiográfico da DD à beira do leito

PALAVRAS-CHAVES: *Insuficiência Cardíaca Diastólica. Ecocardiografia. Insuficiência cardíaca. Insuficiência renal crônica.*

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