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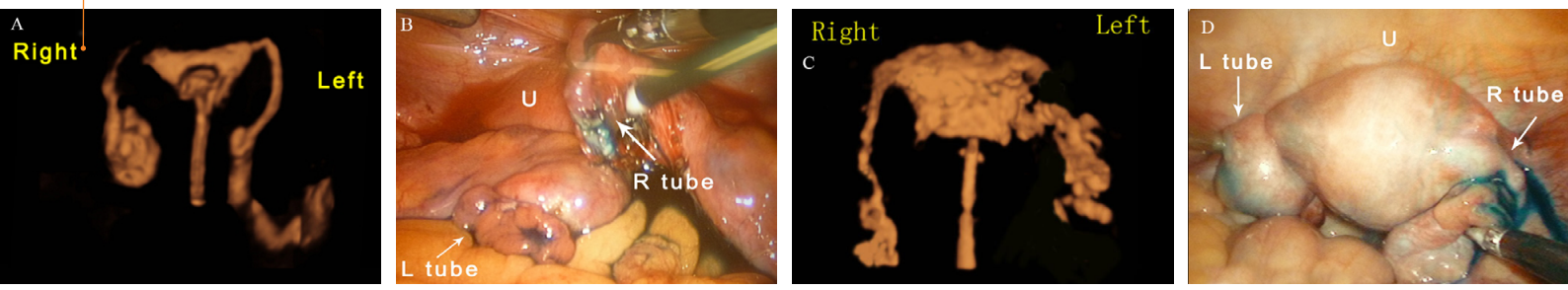
SECTIONS

ARTICLES



RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
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SUMMARY

SECTIONS

ARTICLES

REVIEW ARTICLES

Transcatheter implantation of aortic valve bioprosthesis: changing paradigms

Fábio Sândoli de Brito Junior[1]
Flávio Tarasoutchi[1]

1 - Codiretores do Núcleo de Intervenção em Cardiopatia Estrutural do Instituto do Coração (InCor), Faculdade de Medicina da USP, São Paulo, SP, Brasil
fabio.cardiol@gmail.com

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The surgical replacement of the aortic valve was, for decades, the treatment of choice for patients with symptomatic aortic stenosis, providing relief of symptoms and increased survival. On 16 April 2002, Alain Cribier began to change history by conducting, in Lyon, France, the first transcatheter aortic valve implantation (TAVI) in a 57-year-old patient with severe aortic stenosis and advanced heart failure. Since then, there have been thousands of TAVIs around the world, all based on strong scientific evidence that stimulated, beyond doubt, the progressive expansion of indications for this procedure. In 2019 alone, it is estimated that approximately 140,000 TAVIs will be performed, mostly in Europe and the United States.

There is no doubt that patients with advanced age and clinical comorbidities, who are considered inoperable or at high surgical risk, are the ones who will benefit the most from this innovative and less invasive treatment. However, currently, there is no longer any doubt regarding the effectiveness of TAVI; it is the best treatment for patients with symptomatic aortic stenosis, regardless of the level of surgical risk. Recent randomized studies Partner 3 and Evolut Low Risk, published in 2019 in the same issue of the renowned *The New England Journal of Medicine*, showed that the TAVI is superior to conventional surgery also in patients with low surgical risk, with lower rates of death and cerebrovascular accident.[1,2] The national and international guidelines still need to be updated, which certainly will be done in the near future.

In Brasil, aortic valve implantations by catheter started being used in January 2008 and, despite the lack of coverage by public and private health systems, we have seen a significant increase in the use of this treatment in the country. It is estimated that, in 2019, approximately 2,000 TAVIs will be conducted in Brasil. This number, which represents approximately ten TAVIs for every million inhabitants per year, is still minimal compared to some European countries or the United States, where 100 to 150 for every million inhabitants take place per year. This represents a considerable challenge, since the vast majority of institutions in Brasil have less than ten procedures per year and, therefore, are still learning the method. In a recent international multicenter publication that included Brazilian data, we found that for an institution can achieve excellence, at least 50 cases per year are required.[3] Less than five medical centers in Brasil have that number.

With that regard, the role of the Brazilian Society of Hemodynamics and Interventional Cardiology (SBHCI) deserves to be mentioned. In 2010, they created the Brazilian Registry of aortic valve bioprosthesis implantation via catheter to monitor the results of this new modality of treatment in Brasil.[4] The results of this registry, presented in various scientific meetings and publications, demonstrate the progressive improvement of results in Brasil, similarly to what was found by other important international records. The progressive improvement of results is due to a greater experience of operators, the implementation of procedures under the supervision of medical tutors (proctors) certified by SBHCI and the enormous progress of the transcatheter devices, with smaller circumference, the possibility of repositioning, and design to minimize periprosthetic aortic regurgitation. Nowadays, approximately half of the procedures in our country already use the minimalist strategy, i.e., conscious sedation, percutaneous femoral arterial access, with no vesical catheter, and with monitoring by transthoracic echocardiogram. In general, hospital discharge can happen two to three days after the TAVI, and patients can soon resume their activities. The challenge is still how to obtain uniformity in results in Brasil, a country of continental dimension and with important regional differences.

TAVI is still a new procedure and, as such, full of future prospects. The advancements reached so far are, without a doubt, irreversible. There still remains some doubt in relation to the durability of transcatheter devices, since their short history does not allow to prove, definitively, that their durability will be equivalent to that of surgical prostheses. For this reason, there is a consensus that, in patients younger than 70 years old, conventional surgery is still indicated. There is, however, much more to come. Ongoing studies are assessing the results of the technique in asymptomatic patients and patients with moderate aortic stenosis associated with left ventricular dysfunction. New paradigm shifts are sure to come from these studies. All we have to do is wait and see!

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Emergency neurosurgery for traumatic brain injury: the need for a national and international registry study

Davi J. Fontoura Solla[1]
Danilo Gomes Quadros[1]
Angelos G. Kolias[2,3]
David J. Clark [2,3,4]
Peter J. Hutchinson[2,3]
Manoel Jacobsen Teixeira[1]
Almir Ferreira de Andrade[1]
Wellingson Silva Paiva[1]

1. Divisão de Neurocirurgia, Departamento de Neurologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil
2. Division of Neurosurgery, Department of Clinical Neurosciences, Addenbrooke›s Hospital and University of Cambridge, Cambridge, UK
3. NIHR Global Health Research Group on Neurotrauma, University of Cambridge, Cambridge, UK
4. Division of Neurosurgery, University Teaching Hospital, Lusaka, Zambia

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Traumatic Brain Injury (TBI) continues to be a global public health issue, affecting millions of lives and with an incidence that varies from some tens to many hundreds of cases per 100.000 inhabitants yearly, depending on the country. Its impact, however, is not homogeneous among high and low Human Development Index (HDI) countries, which have faced different recent transformations on TBI epidemiology [1]. Low-income countries, with less resource availability, observe TBI incidence increase partially due to an expansion on the number of motor vehicles. On the other side, populational aging poses new challenges to high-income countries, along with the proportional increment on standing height falls and victims' basal frailty [2].

Brasil is situated at the upper-middle-income stratum and deals simultaneously with both sides of the aforementioned spectrum of transformations. It is estimated that more than one million Brazilians are victims of TBI annually, of which 20-30% are classified as moderate or severe [3]. What was once regarded as an epidemic situation, can no longer be classified as such since it is already part of the quotidian of the large metropolis and small towns in Brasil. According to data from the Hospital Information System of the Brazilian Unified Health System Informatics Department (*Sistema de Informações Hospitalares do Departamento de Informática do SUS - SIH /DATASUS*), there was a more than 10% increase on the number of hospitalizations due to TBI over the last 10 years – currently, more than 100.000 / year. Despite a sensitive reduction on hospital lethality from 10.5% to 9.5% (including any TBI severity), it was observed an increase on the mean length of hospital stay (less

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CORRESPONDING AUTHOR: Davi J. Fontoura Solla
Rua Sílvio Sacramento, n· 73, apt. 502, Pinheiros, São Paulo-SP, CEP 05.408-040. Telefone: +55 11 94234-1989
Email: davisolla@hotmail.com

than 6 days to approximately 6.5 days), as well as on the direct mean cost per hospitalization (40% higher, totaling around R$1750) [4]. As all secondary databases, the DATASUS has inherent limitations, particularly underreporting and cost underestimation. Still, there are few studies on this matter at a national level, none with primary data, which seems limited to some investigations on restricted cities or regions [5,6].

We should recognize that the tendencies above are not homogeneous throughout the Brazilian territory and an even more extreme discrepancy is expected regarding the management of TBI patients and Neurosurgical practice [7]. Although there are some publications on the experience and results of some Neurosurgery services in our country, one should know that these are isolated commendable efforts, and the national neurosurgical prospect and its regional heterogeneities and specificities are unknown. For this specific lack of information, the available secondary databases are even more limited, and the Neurosurgical community responsibility on filling this gap grows.

Considering that these knowledge gaps are also observed in other low-and-middle-income countries, an initiative by the Global Health Research Group on Neurotrauma, funded by the United Kingdom National Institute for Health Research (NIHR) / National Health Service (NHS), was developed: The Global Neurotrauma Outcomes Study (GNOS). It is the first global registry study about the outcomes of patients submitted to emergency neurosurgery for TBI [8].

This is a prospective, multi-center, international cohort study that aims to provide a comprehensive international picture of the management and outcomes of patients undergoing emergency surgery for TBI worldwide - the first of its kind endorsed by the World Federation of Neurosurgical Societies (WFNS) and all continental neurosurgical societies. It will be possible to describe differences in patient demographics, clinical characteristics, surgical choices, and postoperative care as well as compare the global practice to the currently accepted standards of care for the surgical management of TBI. The typical resources available for essential and emergency neurosurgical care may greatly differ between high and low HDI countries. In addition, this initiative will establish a platform and clinical network to facilitate future research in neurotrauma and neurosurgery.

Any Neurosurgery service that performs emergency surgery for TBI is welcome to collaborate. More information is available at https://globalneurotrauma.com. Let us move forward!

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PALAVRAS CHAVE: *Lesões Encefálicas Traumáticas. Neurocirurgia. Emergencies.*

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Urinary lithiasis: diagnostic investigation

Participants:
Ernesto Reggio[1]
Alexandre Danilovic[1]
Francisco Tustumi[1]
Wanderley Marques Bernardo[2]

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1. Sociedade Brasileira de Urologia, Rio de Janeiro, RJ, Brasi
2. Coordenador do Programa Diretrizes da Associação Médica Brasileira, São Paulo, SP, Brasil
I

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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.

Urolithiasis is a disease prevalent worldwide that affects approximately 15% of the world population. We performed a systematic review of the literature, with no time restrictions, in the Medline database, using the PICO methodology (patients with ureterolithiasis, pregnant, imaging exams, ESWT, radiography, ultrasonography, MRI, computed tomography, sensitivity, specificity, and accuracy, benefit, damage). We selected 18 papers to answer the questions clinics. The details of the methodology and the results of this guideline are set out in Annex 1.

INTRODUCTION

Urolithiasis is a disease prevalent worldwide that affects approximately 15% of the world population[1]. Urinary stones can deposit in renal calyces, the renal pelvis, ureter (proximal, middle, and distal), urinary bladder, and urethra. The risk factors are the male gender, third or fourth decade of life, exposure to heat, genetic factors, metabolic factors, dehydration, among others. The stones are constituted of pure calcium oxalate, calcium oxalate and phosphate, pure calcium phosphate, struvite, uric acid, or cystine[2].

RESULTS

What is the protocol for the radiological investigation of urolithiasis in pregnancy?

When ureterolithiasis is suspected in pregnant women, the method of radiological investigation of choice is the ultrasonography[3](D). It is an exam without adverse effects inherent to the method. But, as inconvenient, it has low sensitivity in pregnancy, it is an operator-dependent examination, and physiological hydronephrosis can be misinterpreted as urinary obstruction[4-6](A) Magnetic resonance imaging can be used as a second option, although it does not have the same diagnostic accuracy that computed tomography[4,7,8](A). Com-

puted tomography, with low doses of radiation, should be used only in selected cases because of the risk of carcinogenesis, especially in the first trimester of pregnancy[9,10](C).

What are the radiological diagnostic methods (accuracy, dose of irradiation, anatomical detailing) for patients in emergency care with clinical manifestations suggestive of renal colic?

Computed tomography

Computed tomography allows a detailed identification of anatomic structures, also enabling the identification of differential diagnoses for renal colic. It also allows the evaluation of the density of the stones. Its sensitivity is approximately 95%, and the specificity about 98%, in cases of renal colic[11,12](B). For computed tomography using low-doses of radiation, a meta-analysis[13](A) has shown a sensitivity of 93.1% (CI 95%: 91.5 to 94.4), specificity of 96.6% (CI 95%: 95.1 to 97.7%), positive predictive value of 19.9 (CI 95%: 12.7 to 31.2), negative predictive value of 0.05 (CI 95%: 0.02 to 0.10) and accuracy of 0.9877.

Ultrasonography

Ultrasonography is an exam with no adverse effects inherent to the method, but it is operator-dependent. It can evaluate the degree of hydronephrosis, absence of ureteric jets, and increased resistivity of the renal artery if done with the help of a Doppler[14](B). In the emergency room, ultrasonography allows for a sensitivity of 72% (CI 95%: 59 to 83%), specificity of 73% (CI 95%: 52 to 88%), positive predictive value of 85% (CI 95%: 71 to 94%), negative predictive value of 54% (CI 95%: 37 to 71%), accuracy of 72% (95% CI: 61 to 82%)[15] (B).

Simple radiography

In relation to computed tomography, it is an exam with much lower ionizing levels. Its sensitivity is 57% and the specificity 76%[16](B).

Magnetic resonance imaging

It provides a detailed assessment of associated anatomic structures. The sensitivity of magnetic resonance imaging is 66 to 72%, with a specificity of 96 to 100%, positive predictive value of 95 to 100%, negative predictive value of 71 to 75,5% accuracy of 80 to 85%[17](B).

What evaluation is needed for better diagnosis and planning of patients with complex renal lithiasis who must be submitted to surgical treatment, such as percutaneous kidney lithotripsy?

For the best therapeutic planning, patients who are candidates to percutaneous kidney lithotripsy must be submitted to computed tomography for detailing of the anatomical structures, the collecting system, and the stone, in order to plan the puncture pathway[18](D).

The use of intravenous contrast-enhanced computed tomography should be considered in complex anatomical situations, such as malformations or previous kidney surgery, in which it is desirable to know the anatomy of the renal collecting system. Alternatively, excretory urography can be used to evaluate the anatomy of the renal collecting system, but it does not replace the computed tomography since it does not allow the visualization of neighboring organs and their relationships with the kidney.

How should the radiological follow-up of patients submitted to surgical treatment for urinary lithiasis be conducted?

Ultrasound or computed tomography should be performed during the follow-up period to evaluate residual stones or relapse[18](D).

What radiological parameters are necessary to improve indications of eswt for urinary lithiasis?

The main tomographic parameters for the indication of ESWT are size and density of the stone, the distance between the stone and the skin, and location[19](A)[20](B).

SYNTHESIS OF EVIDENCE

The exams for diagnostic investigation of urolithiasis most frequently used are ultrasonography, computed tomography, x-ray of the abdomen and, less frequently, magnetic resonance imaging. Each has advantages and disadvantages regarding diagnostic accuracy, ability to identify anatomical structures, radiation dose, among others. The choice of method of radiological investigation depends on the characteristics of the patients and the purpose of the exam.

APPENDIX I

Clinical question

What is the protocol for the radiological investigation of urolithiasis in pregnancy?

What are the radiological diagnostic methods (accuracy, dose of irradiation, anatomical detailing) for patients in emergency care with clinical manifestations suggestive of renal colic?

What radiological parameters are necessary to improve indications of ESWT for urinary lithiasis?

Structured clinical question

P - Patients with ureterolithiasis and pregnant women
I - Imaging exams
C - Does not apply
O - Does not apply

P - Patients with ureterolithiasis
I - Imaging exams (X-Ray, USG, MRI)
C - Computed tomography
O - Sensitivity and specificity/Accuracy

P - Patients with ureterolithiasis
I - ESWT
C - Does not apply
O - Results of the treatment

Search strategy

("Urolithiasis/diagnosis"[Mesh] OR "Urolithiasis/diagnostic imaging"[Mesh]) AND (pregnant*)

("Urolithiasis/diagnosis"[Mesh] OR "Urolithiasis/diagnostic imaging"[Mesh]) AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields])

("treatment outcome"[MeSH Terms] OR ("treatment"[All Fields] AND "outcome"[All Fields]) OR "treatment outcome"[All Fields]) AND ("lithotripsy"[MeSH Terms] OR "lithotripsy"[All Fields] OR ("extracorporeal"[All Fields] AND "shock"[All Fields] AND "wave"[All Fields] AND "lithotripsy"[All Fields]) OR "extracorporeal shock wave lithotripsy"[All Fields]) AND Clinical Trial[ptyp]

Studies retrieved

The scientific database searched was Medline via PubMed. A manual search was conducted on reviews in references (narrative or systematic) and on the selected papers.

Date of last search: 25/03/2019

248 studies
689 studies
441 studies

Eligibility criteria

The selection of the studies and the evaluation of the titles and abstracts obtained from the search strategy in the database consulted were independently and blindly conducted by two researchers with expertise in the development of systematic reviews, in total accordance with the inclusion and exclusion criteria established and described in the PICO. The studies with potential relevance were separated.

According to the design of the studies

We included in our evaluation systematic reviews with meta-analysis of randomized clinical trials, and before and after studies, considering the best evidence available to answer the clinical questions. Narrative reviews were considered for full reading with the purpose of retrieving references which may have had been during the initial search strategy.

Language

We included studies available without restriction to the language.

According to publication

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

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.

Results application - External validity

The level of scientific evidence was classified per type of study according to Oxford[21] (Table 1).

TABLE 1: 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 case/study 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[22], considering Jadad trials < three (3) as inconsistent (grade B) and those with score ≥ three (3 , consistent (grade A), and according to the Grade[24] 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[23], which considered consistent cohort studies with scores ≥ 6, and inconsistent < 6.

TABLE 2. PROCESS FOR CRITICAL EVALUATION OF RANDOMIZED CONTROLLED TRIALS

Study data Reference, study design, Jadad, level of evidence	Sample size calculation Estimated differences, power, significance level, 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 effects, and controversy will be specifically defined whenever possible.

The results will be presented preferably in absolute data, absolute risk, 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

Application of evidence - Recommendation

The recommendations will be elaborated by the authors of the review, with the initial characteristic of synthesis of evidence 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[21]/ Grade[24]) 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.

Final declaration

The Guidelines Project, an initiative of the Brazilian Medical Association in partnership with the Specialty Societies, aims to reconcile medical information in order to standardize approaches that can aid the physician's reasoning and decision- making process. The information contained in this project must be submitted to the evaluation and criticism of the physician , responsible for the conduct to be followed, given the reality and clinical condition of each patient.

TABLE 3 - PROCESS FOR CRITICAL EVALUATION OF COHORT STUDIES

Representativeness of the exposed and selection of the non-exposed (Max. 2 points)	Exposure definition (Max. 1 point)	Demonstration that the outcome of interest was not present in the beginning of the study (Max. 1 point)	Comparability on the basis of the design or the analysis (Max. 2 points)	Outcome assessment (Max. 1 point)	Adequate follow-up time (Max. 2 points)	Scores and level of evidence

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ORIGINAL ARTICLE

Serum Annexin V and Anti-Annexin V levels and their relationship with metabolic parameters in patients with type 2 diabetes

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

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

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SUMMARY

BACKGROUND: *We investigated the serum annexin V and anti-annexin V levels and their relationship with metabolic parameters in patients recently diagnosed type 2 diabetic.*

METHODS: *A total of 143 patients recently diagnosed type 2 diabetes and 133 control subjects were included in the study. Body mass index (BMI), hs-CRP, HOMA-IR, carotid intima-media thickness, and serum levels of annexin V and anti-annexin V were investigated.*

RESULTS: *HOMA-IR, serum hs-CRP, and carotid intima-media thickness were found to be statistically significant. The Pearson correlation analysis revealed a statistically significant positive relationship between the carotid intima-media thickness and the annexin V level (r=0.29, p=0.006*). A statistically significant positive relationship was also detected between the Annexin V level and level of serum hs-CRP (r=0.29 p=0.006*).*

CONCLUSION: *A positive relationship was observed between the carotid intima-media thickness and annexin V at the end of our investigation. In this regard, we also believe that serum levels of annexin V may be increased for cardiovascular protection in the elevation of carotid intima-media thickness.*

KEYWORDS: *Diabetes Mellitus, Type 2. Annexin A5. Carotid Intima-Media Thickness.*

INTRODUCTION

Annexin V is a protein known to have potent anticoagulant effects. It is also known that Annexin V plays a role in apoptosis regulation and is effective for the prevention of excessive coagulation and inflammatory activity[1,2]. Anti-annexin V antibodies have been demonstrated in several autoimmune diseases[3-5]. Anti-annexin V antibodies are expected to lead to thrombotic and vaso-occlusive events by blocking the effects of annexin V.

Early detection of atherosclerosis is crucial, and ultrasound imaging of the carotid arteries is a non-invasive, reliable, and easily accessible method that can give characteristic information about the carotid artery. The evaluation of the carotid artery provides

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Corresponding Author: Oktay Bilgir
Izmir Bozyaka Training and Research Hospital, Department of Internal Medicine, 35380 Bozyaka, Izmir; Turkey
Tel: +902322505050/5022 - Fax: +902322614444
E-mail: oktaybilgir@gmail.com

information about the risk of coronary artery diseases such as stroke and cardiovascular disease. Many cardiovascular risk factors are known to affect the carotid artery wall. According to the American Heart Association guidelines, an evaluation of the carotid intima-media thickness is recommended as Evidence II A. Similarly, if the Framingham risk score is between 10% and 20%, an evaluation of the carotid intima-media thickness of the patients without known CAD, cerebrovascular disease, and peripheral arterial disease is recommended by other guidelines[6,7].

We aimed to investigate the serum annexin V and anti-annexin V levels and their relationship with metabolic parameters and carotid intima-media thickness in newly diagnosed diabetic patients. There are investigations conducted with serum annexin V and anti-annexin V levels in type 1 diabetic patients in the literature. In addition, annexin V and anti-annexin V levels have been investigated in patients with many autoimmune and inflammatory diseases, which were known to increase the tendency of thrombosis. There are also studies in which its levels were investigated in patients who had acute myocardial infarction. We aimed to investigate the relationship between serum annexin V and anti-annexin V levels and micro- and macrovascular complications of diabetes. We planned a study that would help to identify possible co-morbid conditions as well as determine the serum annexin V and anti-annexin V levels in diabetic patients at the time of diagnosis.

METHODS

Patients recently diagnosed with diabetes who were admitted to the internal medicine and endocrinology outpatient clinic were informed about the study and its objectives. A total of 143 diabetic patients were included after giving written consent. As a control group, a total of 133 healthy individuals were enrolled in the study after giving written consent. The study was designed as a cross-sectional case-control study.

The inclusion criteria were a recent diagnosis of diabetes, willingness to participate, and age between 30 and 65 years old. The exclusion criteria were pregnancy, a previous diagnose of diabetes, a history of malignancy, a history of steroid use, a history of drug use affecting the autoimmune system (Azathioprine, cyclosporine A, cyclophosphamide, quinine, TNF-alpha blockers, etc.), acute/chronic kidney failure, and acute/chronic liver disease.

Patients

Demographic information was recorded for the entire study group (age, sex, waist and hip circumference, body mass index values). Body mass index (BMI) was calculated as kg/height (m^2). It was obtained by dividing the weight in kg by the body surface area in m^2. Waist and hip circumferences were measured using a measuring tape (cm). The waist circumference was measured midway between the lower edge of the rib and the iliac spine. The blood pressure of the patients was measured at the brachial artery using Erka brand sphygmomanometer after resting for at least 10 minutes. The insulin resistance of the patients was calculated according to HOMA-IR (Homeostasis Model Assessment).

Laboratory

In both study and control groups, fasting and postprandial plasma glucose levels, as well as HbA1c levels, were measured to assess the glycemic status, fasting serum insulin, metabolic parameters as serum lipid levels (total cholesterol, HDL, LDL, triglycerides), and liver and kidney function tests (ALT, AST, urea, creatinine) to evaluate the pancreatic β-cell function. An additional 10cc of blood was drawn from the study participants to assess serum-annexin V and anti-annexin V levels in addition to blood samples taken for complete blood count, hs-CRP, serum electrolytes, and thyroid function tests.

Analysis of all blood samples was performed in the Biochemistry Laboratory of Izmir Bozyaka Training and Research Hospital. The venous blood of the patient and control groups was drawn into 8 ml vacuumed tubes with gel separator (Vacuette, Greiner Bio-One, Austria) after an average of 10 hours fasting. The blood samples were centrifuged at 3,000 rpm for 10 minutes at room temperature after waiting about 30 minutes for clotting. Some of the serums obtained were used for routine biochemical analysis. Routine biochemical tests were performed with biochemistry autoanalyzer using commercially available kits by standard methods (Olympus 2700, Olympus Optical Co. Ltd, Shizuoka-ken, Japan). The remaining serum was portioned and stored at -800C until the Human Annex V, and anti-Annexin V levels were studied.

Annexin V and Anti-Annexin V

A commercial kit (eBioscience, North America) which works with the ELISA method was used for serum Annex V and anti-Annexin V levels. Studies

were carried out according to the instructions in the kit. The spectrophotometric measurement was made with Thermo Scientific Multiskan GO models ELISA reader (Finland) at 620 nm wavelength. Anti-Annexin V and Annexin V concentrations of the samples were determined by the standard curve drawn using dilute standard absorbance. The results were expressed in ng/ml.

Carotid intima-media thickness was measured at the Radiology Clinic of our hospital, using Hitachi Ultrasound and by the same physician in order to ensure standardization. The patient was placed in the supine position with the neck slightly extended, and the head turned contralateral to the side for the measurement. A grayscale examination was carried out using a 13.6 MHz probe during the investigation. The grayscale investigation was started in the transverse projection. The examination was performed to include the entire cervical carotid artery, from the supraclavicular notch to the angle of mandible on both sides. In our study, the measurement of both carotid arteries was taken. Intima-media thickness was defined as the distance from the lumen intima interface to the media adventitia interface. B-mode US measurements of the extracranial vascular structures were performed in regions without plaque. Intima-media thickness was measured in the thickest part of the bilateral carotid arteries.

Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences software version 21.0 (SPSS Inc., Chicago, USA) software. The distribution of continuous variables was done using the Kolmogorov-Smirnov test, and all continuous variables were found to be normally distributed ($p>0.05$). The results of continuous variables were expressed as mean ± standard error. The demographic and laboratory data of diabetic individuals and individuals with normal glucose tolerance were obtained using an independent t-test. The relationship between Annexin V, Anti-Annexin V, and other demographic and laboratory data was analyzed using the Pearson correlation analysis. Multiple linear regression analysis was performed on the independent relationship between Annexin V, Anti-Annexin V, and other variables. The co-occurrence of Klotho and diabetes was also investigated with binary logic regression analysis. $P<0.05$ was considered statistically significant in our study.

RESULTS

A total of 143 patients, aged 30-65 years, with newly diagnosed diabetes who were admitted to the internal medicine and endocrinology outpatient clinic between October 2014 and January 2018 and a total of 133 healthy controls of equivalent age were enrolled in the study. The comparison of demographic, anthropometric, radiological, and laboratory findings of newly diagnosed DM and control groups are presented in the Table. No statistically significant difference was observed regarding age, BMI average, and sex distribution between two groups ($p>0.05$). The waist circumference was significantly higher in the group of the newly diagnosed diabetes (T2DM= 99.34 ± 10.54 cm, Control=91.37 ± 11.63 cm, p=0.001*). No statistically significant difference was observed in terms of systolic and diastolic blood pressures between the two groups (T2 DM= 116.97 ± 9.82 mmHg, Control=114.65 ± 16.08 mmHg, p=0.421 and T2 DM= 71.62 ± 6.42 mmHg, Control=70.46 ± 11.53 mmHg, p=0.566 respectively). The carotid intima-media thickness was significantly higher in the newly diagnosed group (T2 DM=0.81 ± 0.19, Control= 0.67 ± 0.11, $p<0.001$).

Serum hs-CRP (T2DM=6.62 ± 5.98 mg/dL, Control= 4.08 ± 4.29 mg/dL, p=0.027*) and HOMA-IR (T2DM=10.06 ± 16.04, Control= 1.70 ± 1.44, p=0.002*) levels detected were significantly higher in diabetic patients than in the individuals in the control group.

No statistically significant difference was observed in terms of renal functions (creatinine) between the two groups (T2 DM= 0.90 ± 0.16 mg/dL, Control=0.88 ± 0.17 mg/dL, p=0.642). The serum ALT level detected was significantly higher in diabetic patients (T2DM= 30.74 ± 30.73 U/L, Control=19.95 ± 12.12 U/L, p=0.037*). The serum HDL level detected was significantly lower (T2DM= 46.80 ± 21.36 mg/dL, Control=64.35 ± 33.61 mg/dL, p=0.005*), and the triglyceride level higher in diabetic patients than control group (T2DM= 242.88 ± 195.53 mg/dL, Control=138.97 ± 59.80 mg/dL, p=0.002*).

No statistically significant difference was observed regarding serum annexin V (type 2 diabetic group= 9.38 ± 8.17, control group= 11.42 ± 8.27 ng/ml, p=0.253) and anti-annexin V (type 2 diabetic group= 63.10 ± 43.13, control group= 54.08 ± 28.93 ng/ml, p=0.258) between the two groups.

Demographic and laboratory data of the patients in the two groups were compared using the t-test for independent variables. The gender distribution

between the two groups was compared with the chi-square test.

TABLE 1. COMPARISON OF DEMOGRAPHIC AND LABORATORY DATA OF PATIENTS NEWLY DIAGNOSED WITH TYPE 2 DIABETES AND NON-DIABETIC INDIVIDUALS IN THE CONTROL GROUP.

Variables	Tip 2 DM n=143	Control n=133	Pa
Age, year	50.41 ± 8.62	48.79 ± 7.25	0.346
Gender, F/M	20/23	26/17	0,280
BMI, kg/m2	28.80 ± 4.36	26.96 ± 4.84	0.068
Waist Circumference, cm	99.34 ± 10.54	91.37 ± 11.63	0.001*
Systolic Blood Pressure, mmHg	116.97 ± 9.82	114.65 ± 16.08	0.421
Diastolic Blood Pressure, mmHg	71.62 ± 6.42	70.46 ± 11.53	0.566
HbA1C, %	9.49 ± 3.15	5.42 ± 0.26	<0.001*
Fasting Glucose,mg/dL	203.95 ± 92.03	91.41 ± 9.39	<0.001*
OGTT 2. hour, mg/dL	246.36± 57.09	120.74 ± 20.92	<0.001*
Fasting Insulin,µIU/mL	23.47 ± 45.64	7.56 ± 6.50	0.031*
HOMA-IR	10.06 ± 16.04	1.70 ± 1.44	0.002*
CIMT, mm	0.81 ± 0.19	0.67 ± 0.11	<0.001*
hs-CRP, mg/dL	6.62 ± 5.98	4.08 ± 4.29	0.027*
Annexin-5, ng/ml	9.38 ± 8.17	11.42 ± 8.27	0.253
Anti-annexin-5, ng/ml	63.10 ± 43.13	54.08 ± 28.93	0.258
Creatinine, mg/dL	0.90 ± 0.16	0.88 ± 0.17	0.642
Urea, mg/dL	28.76 ± 9.80	28.11 ± 8.63	0.745
ALT, U/L	30.74 ± 30.73	19.95 ± 12.12	0.037*
AST, U/L	25.31 ± 17.56	21.28 ± 5.70	0.159
Total cholesterol, mg/dL	229.33 ± 36.66	223.93 ± 52.85	0.583
HDL, mg/dL	46.80 ± 21.36	64.35 ± 33.61	0.005*
LDL, mg/dL	156.13 ± 35.01	142.03 ± 45.69	0.112
Triglycerides, mg/dL	242.88 ± 195.53	138.97 ± 59.80	0.002*

*Data were expressed as mean ± standard deviation. P<0.05 was considered significant.

DISCUSSION

Annexin V deficiency has been shown to cause hypercoagulability and hence, cardiovascular events in newly conducted studies. Diabetes is a major health problem all over the World, and cardiovascular morbidity and mortality are also observed in more than half of these patients. We investigated the levels of annexin V in patients newly diagnosed with type 2 diabetes. We did not detect any statistically significant difference regarding the serum annexin V level between the Dm group and the control. Interestingly, although no difference was detected in terms of serum annexin V level, a statistically significant and positive correlation was found between CIMT and the level of serum annexin V.

Increased CIMT has been shown to be closely associated with various microvascular events such as MI and stroke, particularly in patients with Type 2 DM, in many studies in the literature. CIMT was found to be higher in Type 2 diabetics compared to control groups in a study of Temelkova-Kurktschiev et al.8, which was found to be associated with microvascular complications. CIMT, as expected, was found to be significantly higher in the diabetic group than in the healthy control group in our study. A significant relationship was also detected between CIMT and ANV level. As CIMT increased, serum ANV level increased. We have interpreted this relationship as a reactive ANV elevation for cardioprotection.

ANV and heparin have been compared in coagulation studies in rabbits. According to this, ANV inhibits fibrin production and platelet aggregation safely and quickly[9]. In another study, ANV was detected to be bound quickly to PSA positive platelet, red blood cells, and microparticles in a surprisingly formed clot[10]. The study conducted by Bakar et al. compares both serum levels of ANV and a ANVa of type 1 diabetic patients with normal healthy individuals[11]. The study reported that serum ANV levels were significantly lower in the type 1 diabetic patient group compared to the healthy control group, and serum a ANVa levels (both IgG and IgM) were significantly higher in the type 1 diabetic group than in the healthy control group11. In our study, when serum ANV and a ANVa levels of diabetic patients were compared with the control group, no statistically significant difference was found. The reason for this disparity in results between the two studies may be explained by antibody development against ANV caused by an autoimmune process. Based on the results of our study, we believe that there is no effect of ANV in increased atherosclerosis and hypercoagulopathy in type 2 diabetics.

ANV is known to have an anti-inflammatory role. A statistically significant relationship was found between ANV and hs-CRP, an inflammatory marker, in our study. Similarly, we assessed this relationship as the reactive increase of ANV in parallel to the increase of inflammation.

Hyperglycemia leads to the formation of free radicals, directly and indirectly, by increasing the load of free fatty acids. This is known as the initiator of a process that triggers many adverse metabolic pathways, increases the vascular permeability, reduces the fibrinolytic activity, and triggers inflammation. Inflammation has recently been associated with

atherosclerosis, type 2 DM, and cancer. Although inflammation is a normal response to infection and tissue damage, it may have a pathological nature if the response is excessive or continuous. Numerous studies have shown that there was a strong positive association between obesity and hs-CRP12,13 and weight loss resulted in the reduction of hs-CRP concentration[14,15]. A causal relationship between obesity and CRP has also been supported by recent laboratory evidence. Adipocytes and monocyte-derived macrophages in enlarged adipose tissue masses increase the synthesis of CRP in the liver by secreting proinflammatory cytokines, such as TNF-α and IL-6[16]. The increase in hs-CRP is also observed in the presence of metabolic syndrome, in which metabolic abnormalities, including glucose intolerance, hypertriglyceridemia, and hypertension, are seen together. This condition, in which the central obesity is the main component, increases cardiovascular disease as well as type 2 diabetes risks[17]. It has been shown that as the number of abnormal metabolic properties increases, the CRP level elevates progressively in different populations[18,19]. In our study, serum hs-CRP levels were found to be statistically significantly higher in the group of patients newly diagnosed with type 2 diabetes, in comparison to the normoglycemic control group. This result also supports the relationship between hs-CRP and clinical disorders such as diabetes, atherosclerosis, and metabolic syndrome.

CONCLUSION

No difference was found between patients newly diagnosed with type 2 diabetes and the normoglycemic individuals of the control group regarding serum annexin V levels. These data suggest that annexin V has no role in the high incidence of cardiovascular events and hypercoagulopathy in type 2 diabetic patients. A positive relationship was observed between CIMT and annexin V in our study. In this regard, we also believe that serum annexin V levels may be increased in CIMT increase for cardioprotection.

Author Contributions

Study conception and design: Oktay Bilgir; Acquisition of data: Huseyin Afsin Vural; Analysis and interpretation of data: Ismail Demir; Drafting of the manuscript: Ozden Yildirim Akan; Critical revision: Oktay Bilgir.

RESUMO

OBJETIVO: *Investigar os níveis séricos de anexina V e antianexina V e sua relação com os parâmetros metabólicos em pacientes diabéticos tipo 2 recém-diagnosticados.*

MÉTODOS: *Foram incluídos no estudo 143 pacientes e 133 controles com diabetes tipo 2 recém-diagnosticado. O índice de massa corporal (IMC), PCR-as, Homa-IR, espessura íntima média carotídea e níveis séricos de anexina V e antianexina V foram investigados.*

RESULTADOS: *O Homa-IR, a PCR-s do soro e a espessura média da carótida foram estatisticamente significantes. A análise de correlação de Pearson revelou uma relação positiva estatisticamente significante entre a espessura média da carótida e anexina V (r=0,29; p=0,006 *). Foi também detectada uma relação positiva estatisticamente significativa entre o nível de anexina V e o nível sérico de PCR-as (r=0,29, p=0,006*).*

CONCLUSÃO: *Também foi observada uma relação positiva entre a espessura média da carótida e anexina V no final da nossa investigação. A esse respeito, também pensamos que os níveis séricos de anexina V podem ser aumentados para proteção cardiovascular na elevação da espessura média da carótida.*

PALAVRAS-CHAVE: *Diabetes mellitus tipo 2. Anexina A5. Espessura íntima média carotídea.*

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ORIGINAL ARTICLE

Time management of Internal Medicine medical residents, São Paulo, Brasil

Rafael Augusto Tamasauskas Torres [1]
Frida Marina Fischer [2]

1. Médico, com residência em Medicina do Trabalho pelo Hospital das Clínicas da Faculdade de Medicina da USP, Mestre em Ciências pela Faculdade de Saúde Pública da USP, São Paulo, SP, Brasil
2. Professora, Doutora, Livre-docente do Departamento de Saúde do Trabalhador da Faculdade de Saúde Pública da USP.
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SUMMARY

INTRODUCTION: *Medical Residency is a recognized form of professional qualification, but there are criticisms regarding the overload of work activities. Given the length of the daily and weekly workdays, residents develop practices that enable them to reconcile the Residency with their personal life.*

AIM: *To describe time management strategies in the daily routine of Internal Medicine Medical Residents of a university hospital in São Paulo, Brasil.*

METHODS: *Eight interviews were conducted with resident physicians of the second year, addressing aspects of personal and family life, theoretical study, practical activities, and work bonds. Content analysis was carried out using the MaxQDA software.*

RESULTS: *Six thematic categories emerged from the reports: work organization at the Medical Residency; learning and/or professional activities; housing, financial planning, and household activities; time for leisure and interpersonal relationship; family planning/children; rest/sleep.*

DISCUSSION: *Several strategies are adopted for time management: residing near the hospital, domestic activities helped by housekeepers, postponement of maternity leave, and social support centered on interacting with other residents. There are paid activities not associated with the Residency, which lead to reduced time for rest, study, and leisure, with a greater loss during work at night shifts.*

CONCLUSIONS: *Residents experience a period of intense learning, which requires a high workload and complex work. The evaluation of the work organization of medical residents should include not only time for rest but also time management strategies for daily activities, which can reduce the negative outcomes associated with long working hours.*

KEYWORDS: *Internship and Residency. Shift Work Schedule. Time management.*

INTRODUCTION

A physician can participate in a graduate program called Medical Residency. This program, when fulfilled entirely in a single specialization, confers the title of specialist[1]. This stage is characterized by a period of "immersion" in the professional activity, with many hours of work and study, caring for patients in complex situations[2].

In 2017, there were 35,187 physicians enrolled in Residency, in 6,574 programs of 790 institutions; 40% of these were specializing in four areas (Internal Medicine, Pediatrics, General Surgery, and Obstetrics and Gynecology); 34.5% of the resident physicians were in São Paulo[3].

The bond with the hospital it is educational, but the

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CORRESPONDING AUTHOR: Rafael Torres
Av. Dr. Arnaldo, 715 - Cerqueira César – Sao Paulo – SP – Brasil – 01246-904
E-mail: rafaeltamatorres@yahoo.com.br

resident can work in other institutions in their free time, although this is not recommended[4]. In Brasil, the Law 6,932 of 1981 establishes a maximum of 60 weekly work hours, included 24-hour shifts, with at least one day of rest[5].

Working at night schedules can lead to worsened performance, increased risk of incidents in the workplace, and the worker's exposure to environmental stressors that can lead them to premature labor incapacity[6,7].

Time management can be understood as the ability to plan which activities should be carried out, how, and when. This planning involves establishing priorities and strategies to achieve them[8].

In view of the multiple activities carried out by residents, it is important to understand what are the time management strategies adopted, as well as the repercussions of long workdays.

METHODS

Eight physicians of the 2nd year of residency at the Medical Clinic of a public university hospital, located in São Paulo, Brasil, were interviewed. They reported their activities over the previous year and how they interfered in their lives. The interviews were recorded and transcribed.

The data were categorized and analyzed using the MaxQDA software[9]. This research obtained the consent of the Committee of Medical Residency of the Hospital studied and was approved by the Research Ethics Committee of FSP-USP. The participants signed the informed consent form, drawn up according to the 466/2012 resolution of the National Council of Research Ethics[10].

RESULTS

The average age of the participants was 27 years old, with no children; four were men. Three participants reported taking drugs for anxiety or depression (Table 1).

The activities included in the residency program are divided between outpatient internships (in which patients are treated on scheduled appointments), emergency internships (patients with acute conditions), internships in hospital wards (patients who are hospitalized in less serious conditions), and in Intensive Care Units or intermediaries (hospitalized patients in more severe conditions). The categories selected are described in Table 2.

Work organization

About the main reports on the ICU internship were: high workload and the occasional need to work night shifts after daytime work. There was a sharp reduction of performance at night and during the group discussions that followed the shift.

In the wards, the activities ceased at around 3 pm. One of the residents stayed until the 15 pm, and another was on duty during the night and was off-duty the following morning, after evaluating the patients with the other residents and the supervisor.

Emergency care was considered stressful. On these shifts, physicians reported an absence of rest time and the need to be constantly alert.

There are two months of elective internships, in which the residents choose where they wish to intern. During this period there are night shifts in case of any intercurrences in the hospital wards.

The Intensive Therapy Units, intermediaries, and emergency rooms presented high demands of time

TABLE 1. CHARACTERISTICS OF THE RESIDENTS WHO PARTICIPATED IN THE STUDY, SÃO PAULO, BRASIL, 2018.

Participant identification	Age	Gender	Participated in the first stage	Medications in use	Children?
P1	27	Male	Yes	No	No
P2	26	Female	Yes	No	No
P3	27	Female	Yes	Alprazolam	No
P4	28	Male	Yes	No	No
P5	26	Female	Yes	No	No
P6	27	Male	Yes	Venlafaxine	No
P7	28	Male	Yes	No	No
P8	25	Female	Yes	Fluoxetine	No

Source: Study data. São Paulo, 2018.

TABLE 2. SUMMARY OF THE CATEGORIES SELECTED FOR THIS STUDY, SÃO PAULO, BRASIL, 2018.

Categories	Context addressed by the category
Work organization	Explanations about the activities performed in the various stages of Internal Medicine Residency
Learning or professional activity?	Addressing the conflict between the educational bond and the work of the medical practice
Housing, financial planning, and domestic activities	Reports explaining how the choice of housing is made, how it is maintained, and how it affects financial planning
Time for leisure and interpersonal relationships	How the resident deals with the time available for leisure and with interpersonal relationships of everyday life
Family planning/children	What are the resident's plans regarding children and what governs these decisions
Rest/sleep	Reports about the time to rest/sleep and the perception of its quality

Source: Study data. São Paulo, 2018.

and complexity, which did not happen in the outpatient, elective, and hospital ward internships.

On the demands of complex situations and with critical patients: despite being an important stage of the training, the way it was done was considered inappropriate.

"[...] in the ICU, a stage that traumatized my whole group, we lost many patients... These were very complex cases [...] the worse one was when I had to tell the father of a 19-year-old patient that his son was brain dead! [...] the work hours are demanding, but the worst part was having to live with these stories, with these patients" (P6).

The work hours are often cited as a stressful and tiring factor, which can lead to inappropriate professional behavior. There is a clear perception of worsening quality of service, the longer the work hours. Tiredness appears as a crucial factor, impacting medical decisions and the doctor-patient relationship.

"[...] sometimes there is a moment you are not able to think anymore, you lose your reasoning ability! Sometimes, you give the wrong dose of a medication because of sleepiness. The night shifts usually last 24 hours, because you're on the Residency during the day and then you have the night shift, so the performance is affected for the worst [...]" (P7).

The day off after the night shift is usually respected (CNRM Resolution No. 4, 2011[11]). However, in some internships, the night shift is extended beyond the usual hours due to the evaluation of patients and discussion of cases. Due to the reduced free time at some internships, the time you would use to rest ends up being used for other activities.

"[...] after the night shift you have the day off, but you don't sleep because it is daytime and you have things to do... We are not able to rest much during the day" (P3).

"[...] the next day you have post-shift duties, so there was no time in between the night shift and other shifts at other places" (P5).

Learning or professional activity?

The boundaries between learning activities and the use of resident labor are thin.

"Often you feel like you are working... without the assistance that you expected, you feel like you are just working a regular job" (P6).

In an effort to support resident physicians, the Medical Clinic has a mentoring program, in which each tutor counsels some residents in monthly meetings in order to check how they are adjusting to the Residency, assist in the program progress or with personal issues. The residents reported this initiative positively, demonstrating an approximation of the institution/teachers with the student and generating opportunities for sharing experiences and talking.

"[...] we meet to discuss ethical and work issues and everything else. It ends up being an environment where we can talk about things that had an impact on us [...] it is an opportunity to talk things out [...]" (P6).

Housing, financial planning, and domestic activities

All the residents live near the hospital where they study due to the journey time between the workplace and their home. Meals during the internship periods are had almost exclusively in the hospital cafeteria, both due to cost and time. Usually receive aid for domestic activities in the form of housekeepers.

The Residency pay is approximately 1⁄6 of the

amount these professionals would get if they were hired under the CLT system. As an alternative, the residents fit other jobs into their free time, which compromises the use of that time for leisure, relaxation, or study activities.

"When you add up the shifts from other places, the workload gets heavy, it is particularly physically demanding, but also because we would like some free time to see friends, to go home... But we have to afford to live in São Paulo; we need to work in other places too [...]" (P7).

Time for leisure and interpersonal relationships

There are difficulties in reconciling the time between practical activities, study hours, leisure, domestic activities, interpersonal relationships, and other paid activities. In this stage, the intern's schedule undergoes a restructuring, which is generally followed by complaints of chronic fatigue.

"[...] Residency is about adapting to the lack of time. For most of the day you are in the hospital, so you have to adapt your schedule: decreasing gym time, sleeping later so you can spend some time with the boyfriend [...] you end up learning to accept and adapt" (P5).

Often, the time that was supposed to be rest time is sacrificed to allow some time for leisure, social obligations, or romantic relationships.

"It was a good month, but I had less interaction with the rest of my life, you know? [emergency internship]. I spent less time with my girlfriend, did less physical activity... it was one of the months when I saw my family the least ... A month of a lot of sacrifice in my personal life." (P12).

Family planning/children

In order to maintain relationships, residents reported the need to adapt to their routine. In many cases, the relationship with other physicians facilitates the understanding of this dynamic. Seven of the interviewees were unmarried and without children. One of the residents was married and was awaiting the birth of a son who, according to him, had not been planned. It is striking in the discourse of the four women the impossibility of having children due to the amount of study required for the specialization test or for the Residency.

"[...] I do not see myself studying for a difficult test with a small child at home. I know it would not work out." (P2, fem.).

There are reports of the only married resident, whose wife is also a resident in Pediatrics and was awaiting the birth of their son. He realized that there was, in fact, no support or institutional strategy for dealing with pregnant medical residents.

Rest/sleep

The time available for rest is scarcer in emergency care and Intensive Care Units. In hospital wards and outpatient clinic internships, this time was reported as being more appropriate.

Sleep deprivation and fatigue have on tasks, and the tension experienced in the internship is often externalized in dreams and as sleep difficulties. There are several reports of the use of coffee and energy drinks as stimulants.

"[...] there are many patients, and you are not able to see them properly, so I felt guilty. I would finish my shift and stay there dealing with things that I had not had time to do during the day. There were several nights when I left the emergency room at 11 pm. I sometimes got five hours of sleep, but I was stressed, worried. The stress of the internship affected the quality of my sleep for the worse [...] daytime naps were very frequent, which, in my opinion, are not very restful." (P12).

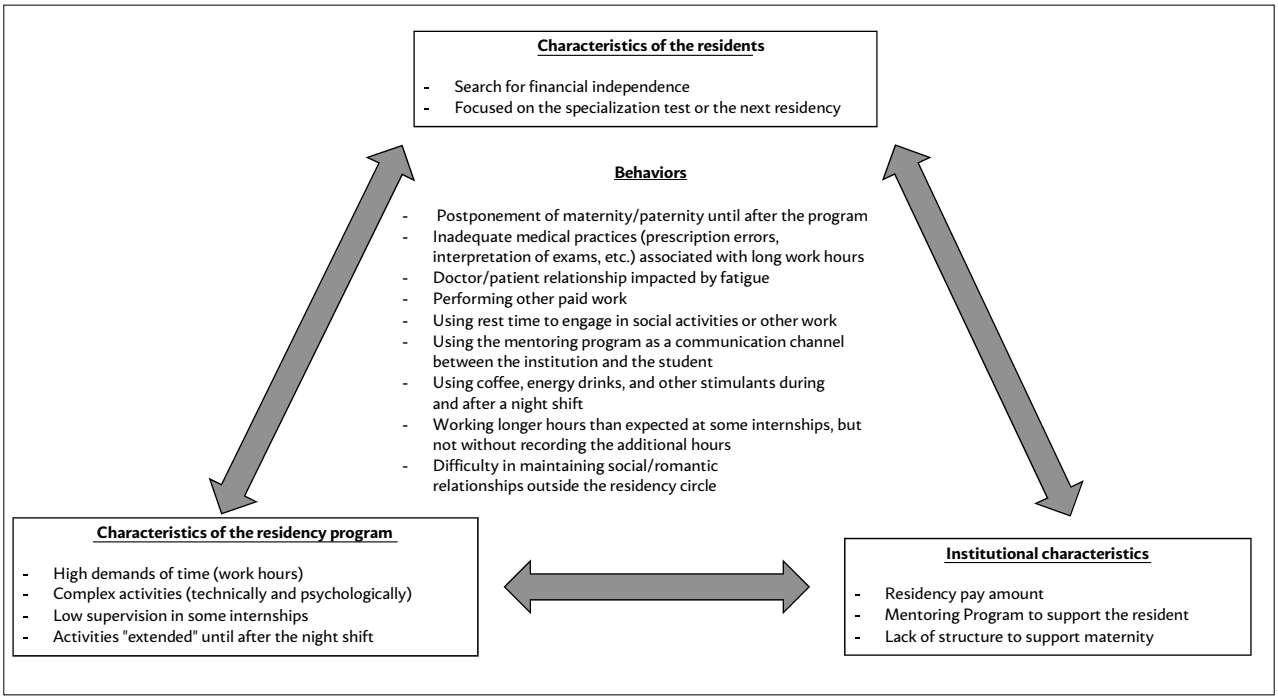
DISCUSSION

Reconcile work and personal life is the result of adopting time management strategies. These allow residents to handle more appropriately with the long work hours. This frequently happens because the activity extends beyond the planned time due to intercurrences and ward visitations. The main characteristics and behaviors reported by residents are described in Figure 1.

Several studies have shown a high incidence of mental disorders in residents, usually associated with highly demanding work in the face of complex situations[12-14], long work hours, lack of control over work processes, and patient consultations[15]. The mentoring program to residents works as a support for dealing with these demands.

Night work can lead to a worsened performance and increased risk of incidents, and it is also associated with various chronic diseases[6]. There were reports of worsened performance, including incidents that could compromise patient safety. In line with studies on sleep deprivation, the effects are mental psychological,

FIGURE 1



and physical exhaustion, with a decreased ability of reasoning, retention of information, problem-solving, including the interpretation of exams and errors in drug prescription[16-18].

A survey conducted in the United Kingdom[19] with Anesthesiology residents showed that fatigue resulting from the program, even with reduced work hours (from 56 to 48 hours per week), interfered in the psychological well-being, physical health, and personal relationships; these interferences were maximized on the night shift.

There were port-night-shift difficulties reported, like falling and staying asleep, in addition to the sensation of restless sleep and the desire to the time off for other activities[6,16,20].

All participants were involved in other paid activities unrelated to the Residency. There are several factors that cause this: time management strategies that increase the cost of living (housing near the education institution, food in restaurants, hiring housekeepers), need for financial independence, and the current Residency pay.

Shift workers, especially those who work the night shift, have a different routine from the rest of the community, leading to greater difficulties with friends and family[16,20,21] . This difficulty is described due to the number of hours and complexity of the internships, and it is worse in periods of night work.

Asaiag et al.[22] conclude that the residents realize their quality of life is worse during the Residency than in other periods; the same was found in this study.

There are difficulties concerning the limited free time to maintain relationships with relatives and friends, reports of friendships and support among the residents, and difficulties in reconciling romantic relationships. It is worth noting that social support is important to prevent mental disorders[23,24].

The complexity and severity of the patients, the management of delicate situations, and the feeling of powerlessness when faced with the lack of resources were mentioned as factors of psychological burden. These are added to the physical overload due to the long work hours[2,25]. These situations were mainly observed in the emergency room and intensive care unit internships.

Despite being contradictory, the residents acknowledge these factors of psychological burden as necessary for their training. The emergency room internship presents high overhead, but it is associated with a period of greater responsibilities, during which the residents feel useful and rewarded.

CONCLUSION

Internal Medicine residents experience a period of intense learning that demands long hours of complex activities.

The strategies reported for time management

were: living near the hospital; hiring housekeepers to assist in domestic activities; eat most of the meals at the hospital cafeteria; postpone motherhood/paternity for after the Residency; use of the time available for leisure activities, family and romantic relationships, at the cost of rest time; social support focused on relationships with fellow residents and program supervisors.

The institutional strategies of social support, such as the mentoring program, were considered positive elements that facilitated the communication between residents and supervisors. There is a need to evaluate with the Residency supervisors their perception of the constraints mentioned by the study participants and the possible measures to reduce their impacts.

Author Contributions

Rafael A. T. Torres: participated in the study design and research, in the structuring of the results and the text; was the lead researcher and author of the study.

Frida M. Fischer: advised and supervised the research, participated actively in the project design, discussion of the results, and conclusions. Actively participated in the writing and revision of the article.

RESUMO

INTRODUÇÃO: *A Residência Médica é uma forma reconhecida de capacitação profissional, mas há críticas em relação à sobrecarga de trabalho. Dada a extensão das jornadas de trabalho diária e semanal, os residentes desenvolvem práticas para poder conciliar a Residência com sua vida pessoal.*

OBJETIVOS: *Descrever estratégias de gestão do tempo no cotidiano de médicos residentes de Clínica Médica em hospital universitário de São Paulo, Brasil.*

MÉTODOS: *Realizadas oito entrevistas com médicos residentes do 2º ano, abordando aspectos da vida pessoal, familiar, estudo teórico, atividades práticas e vínculos de trabalho. Realizada análise de conteúdo com auxílio do programa MaxQDA.*

RESULTADOS: *Seis categorias temáticas emergiram dos relatos: organização do trabalho na Residência Médica; atividade para aprendizado ou atividade profissional?; moradia, planejamento financeiro e atividades domésticas; tempo para lazer e relacionamentos interpessoais; planejamento familiar/filhos; repouso/sono.*

DISCUSSÃO: *Diversas estratégias são adotadas para gestão do tempo: residir próximo ao hospital, auxílio das atividades domésticas por diaristas, adiamento da maternidade e apoio social centrado no convívio com outros residentes. Há realização de atividades remuneradas não vinculadas à Residência, o que leva à redução do tempo previsto para repouso, estudo e lazer, com maior prejuízo nos períodos de plantões noturnos.*

CONCLUSÕES: *Os residentes vivenciam um período de aprendizado intenso, mas que exige uma carga horária elevada e trabalho complexo. A avaliação da organização do trabalho de médicos residentes deve incluir não somente tempo para repouso, mas também estratégias de gestão do tempo para atividades cotidianas. Estas podem reduzir o prejuízo associado às longas jornadas de trabalho.*

PALAVRAS-CHAVE: *Internato e Residência. Jornada de trabalho em turnos. Gerenciamento do tempo.*

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ORIGINAL ARTICLE

Causes of misdiagnosis in assessing tubal patency by transvaginal real-time three-dimensional hysterosalpingo-contrast sonography

Na Liang [1]
Qing-Qing Wu*[1]
Jing-Hua Li [1]
Feng-Yun Gao [1]
Fu-Li Sun [1]
Cui-Xia Guo [1]

1. Department of Ultrasound, Beijing Obstetrics and Gynecology Hospital of Capital Medical University, Beijing 100026, China

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SUMMARY

OBJECTIVE: *This study aims to investigate the causes of misdiagnosis in assessing tubal patency by transvaginal real-time three-dimensional hysterosalpingo-contrast sonography (TVS RT-3D-HyCoSy), in order to improve the diagnostic efficiency of TVS RT-3D-HyCoSy.*

METHODS: *A total of 162 oviducts of 83 infertility patients were examined by TVS RT-3D-HyCoSy. These results were compared with the gold standard for laparoscopic dye studies, and the misdiagnosed cases were analyzed.*

RESULTS: *TVS RT-3D-HyCoSy revealed that 68 oviducts were unobstructed and 94 obstructed. The results for the 144 oviducts were in line with the gold standard, while those for 18 oviducts were not. The accuracy rate of the TVS RT-3D-HyCoSy was 88.9%, and the misdiagnosis rate was 11.1%. The main causes of misdiagnosis included contrast medium countercurrent and diffusion, oviduct spasm, abnormal shape or position of the oviduct, pelvic adhesion, and poor imaging operation.*

CONCLUSION: *TVS RT-3D-HyCoSy can well-evaluate tubal patency, and understand and improve the cause of misdiagnosis. Furthermore, the diagnostic efficiency of TVS RT-3D-HyCoSy can still be further improved.*

KEYWORDS: *Hysterosalpingography. Infertility. Diagnostic Techniques, Obstetrical and Gynecological. Diagnostic Errors.*

INTRODUCTION

Transvaginal real-time three-dimensional hysterosalpingo-contrast sonography (TVS RT-3D-HyCoSy) has the advantages of non-radiation and non-invasion. At present, it could be easily accepted by infertility patients, when compared to traditional lipiodol oviduct radiography, and has become a new method to check for tubal patency.[1] In recent years, several multi-center studies at home and abroad have revealed that, compared with the gold standard, TVS RT-3D-HyCoSy has better consistency in the diagnosis of tubal patency[2-4]. However, the misdiagnosis rate continues to bring some difficulties in clinic practice.[5] In the present study, misdiagnosed cases of TVS RT-3D-HyCoSy were analyzed in order to determine the causes of misdiagnosis, and improve the diagnostic efficiency of TVS RT-3D-HyCoSy in evaluating tubal patency.

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CORRESPONDING AUTHOR: Qing-Qing Wu
Department of Ultrasound – Beijing Obstetrics and Gynecology Hospital of Capital Medical University
No. 251 of Yaojiayuan Street, Chaoyang District – Beijing 100026 – China – Tel: +86 10 5227 6433 – Fax: +86 10 5227 6433
E-mail: wuqingqing1939@163.com

METHODS

Clinical data

A total of 83 infertility patients (162 oviducts), who underwent TVS RT-3D-HyCoSy from April 2015 to April 2017 in our hospital, were included in the present study. Among these patients, 31 had primary infertility, while 52 had secondary infertility. The age of these patients ranged between 23-45 years old, with an average age of 31.4 ± 5.7 years old. The course of infertility ranged from 1-9 years, with an average of 3.9 ± 2.1 years. The selected infertility patients were examined at 3-7 days after the flow of the menstrual cycle, when they provided a signed informed consent and met the indications of the oviduct ultrasound contrast examination.

TVS RT-3D-HyCoSy inspection methods

The GE Voluson E8 ultrasonic diagnostic apparatus was used, and Bracco SonoVue was the contrast agent used. Five ml of normal saline was infused and oscillated to mix. Then, a 2-ml suspension was obtained, added with normal saline, and prepared into a 20-ml diluent for future use. An intramuscular injection of 0.5 mg of atropine was given before radiography. After intrauterine catheterization, the real-time three-dimensional imaging mode was started, and the contrast medium was slowly and uniformly injected into the catheter. The distribution of the contrast medium in the uterus, oviduct, pelvic cavity, and surrounding areas of the nests was observed, and the images were preserved. Afterward, three- and two-dimensional radiography images and three-dimensional volume images of the uterus were acquired. Then, the preserved images were processed. According to the image results, the patients were classified into two types based on tubal patency: unobstructed and obstructed. All infertility patients underwent the gold standard laparoscopic dye study after TVS RT-3D-HyCoSy. Then, the accuracy and misdiagnosis rate of TVS RT-3D-HyCoSy in assessing tubal patency was drawn, and the misdiagnosed cases were analyzed.

Statistical analysis

Data were statistically analyzed using SPSS 19.0 statistical software. Measurement data were expressed as mean ± standard deviation (x ± SD), and compared between different groups using the Chi-square test. $P<0.05$ was considered statistically significant. The consistency of the test results was analyzed using the Kappa value.

RESULTS

TVS RT-3D-HyCoSy results

Among these 83 infertility patients, 15 were diagnosed with patency of bilateral oviducts by TVS RT-3D-HyCoSy, 29 were diagnosed with obstruction of bilateral oviducts, 35 had patency in one side and obstruction on the other side (Figures 1A and 1B), and four patients had only one oviduct due to a previous operation for ectopic pregnancy, among which three oviducts were unobstructed, and one was obstructed. Therefore, among these 162 oviducts, 68 oviducts were unobstructed, and 94 were obstructed.

Comparison of the TVS RT-3D-HyCoSy and laparoscopic dye study results

With the results of the laparoscopic dye studies as the gold standard, the accuracy rate of TVS RT-3D-HyCoSy in diagnosing tubal patency was 88.9% (144/162), and the misdiagnosis rate was 11.1% (18/162). Furthermore, the sensitivity of diagnosing oviduct obstruction was 89.6% (86/96), the positive predictive value was 91.5% (86/94), the specificity of diagnosing tubal patency was 87.9% (58/66), and the negative predictive value was 85.3% (58/68). The accuracy of TVS RT-3D-HyCoSy was similar to that of these laparoscopic dye studies, the difference was not statistically significant, and the consistency between these two was good (Table 1).

TABLE 1. COMPARISON OF THE TVS RT-3D-HYCOSY AND LAPAROSCOPIC DYE STUDY RESULTS

TVS RT-3D-HyCoSy	Laparoscopic		Total
	unobstructed	obstructed	
unobstructed	58	10	68
obstructed	8	86	94
Total	66	96	162

Note: P=0.815, κ=0.771

TABLE 2. CAUSES OF MISDIAGNOSIS

Results	Causes	n
False positive	contrast medium countercurrent and diffusion	2
	oviduct spasm	2
	abnormal shape or position of the oviduct	3
	poor imaging operation	1
False negative	contrast medium countercurrent and diffusion	4
	oviduct hydrops	2
	contralateral contrast medium diffused to the affected side	2
	pelvic adhesion	2
Total		18

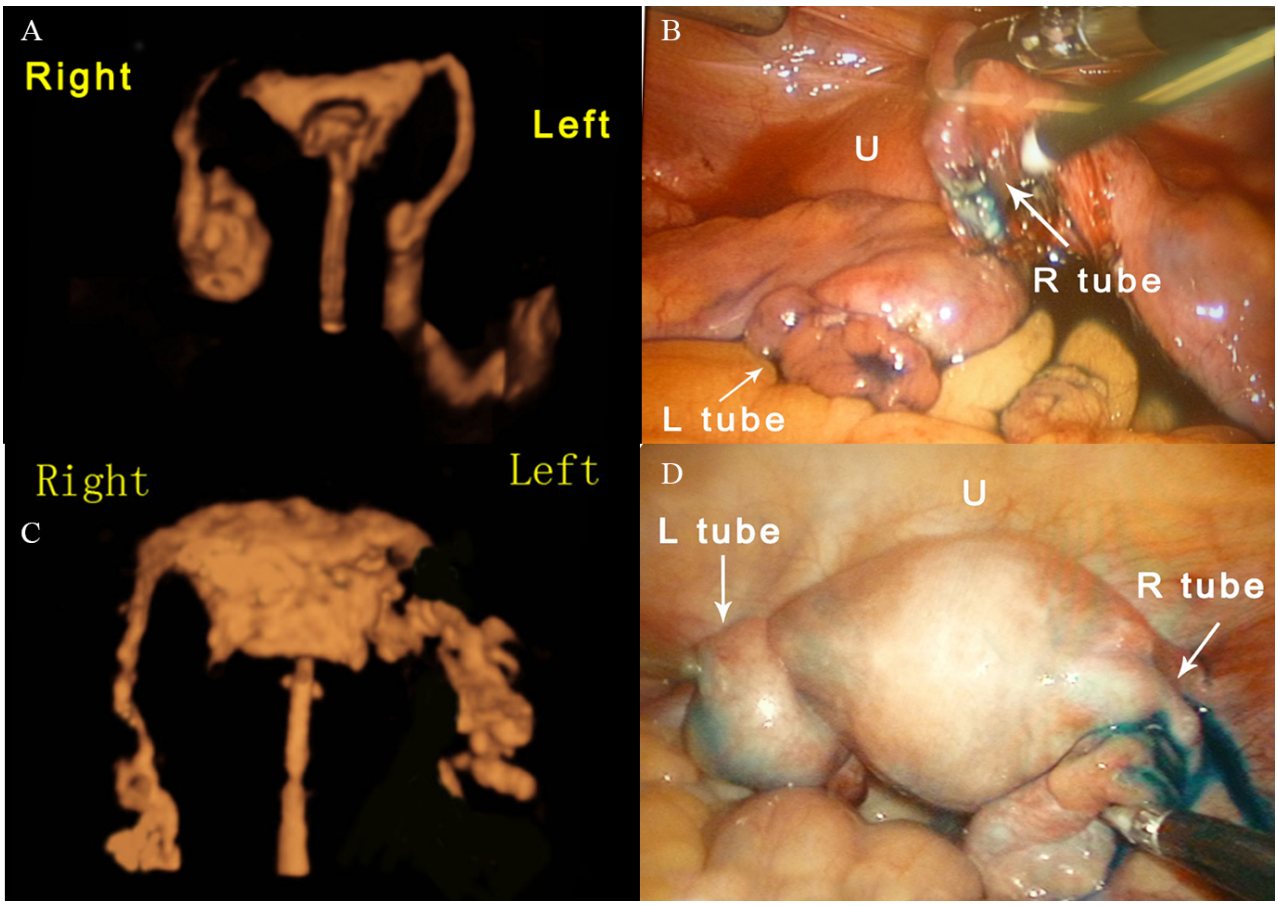

FIGURE 1A. TVS RT-3D-HyCoSy reveals that the left oviduct is unobstructed, and the right oviduct is obstructed.
FIGURE 1B. The laparoscopic surgery revealed that the left oviduct fimbria has an outflow of methylene blue, the right oviduct was conglutinated, and no outflow of methylene blue was found on the oviduct fimbria.
FIGURE 1C. The TVS RT-3D-HyCoSy revealed that that right oviduct is unobstructed, and contrast agent countercurrent was found on the left side of the uterus.
FIGURE 1D. The laparoscopic surgery revealed that the right oviduct fimbria had an outflow of methylene blue, while the left oviduct fimbria had no outflow of Meilan, but blue staining beside the uterus could be observed.

Causes of misdiagnosis

The TVS RT-3D-HyCoSy results were false negative in eight oviducts and false positive in 10 (Figures 1C and 1D). The specific reasons are presented in Table 2.

DISCUSSION

In the present study, the advantages of TVS RT-3D-HyCoSy were fully verified. This method is safe, accurate, simple, easy to operate, noninvasive, has no radiation or impact on pregnancy, is a new and ideal method to check tubal patency, and has been well accepted by doctors and patients. Furthermore, TVS RT-3D-HyCoSy is a better examination technique for infertile women in the field of assisted reproductive technology, even expected to replace traditional lipiodol radiography, and is suitable for wider clinical promotion.[6] However, the present study revealed that approximately 30% of images acquired by TVS RT-3D-HyCoSy were poor in quality, and some of these even affected the analysis of results. Furthermore, the patency of 11.1% of oviducts (18 oviducts) was not consistent with the real results, causing excessive laparoscopic surgery induced by false-positive results, or delayed surgery and pregnancy induced by false-negative results. This brings some perplexity and interference in clinic practice.

TVS RT-3D-HyCoSy can automatically display the three-dimensional shape of the oviduct in real-time. The tubal patency is mainly determined by the shape of the oviducts after radiography. Indeed, it must be combined with other factors, including the resistance of the bolus injection of contrast agent and the situations of the outflow of the contrast agent from the oviduct fimbria, especially the contrast agent

surrounding the ipsilateral ovary.[7,8] Therefore, the quality of oviduct images by TVS RT-3D-HyCoSy is the key to determine tubal patency.[9] The present study revealed that the quality of the image was closely correlated to the situation in the pelvic cavity of the patient. In patients with a history of pelvic or intrauterine surgery, patients with pelvic adhesions or pelvic endometriosis, and patients with secondary infertility, the proportion of poor image quality was relatively high. Among the causes of misdiagnosis, the proportion of countercurrent and diffusion of the contrast agent was the highest. The countercurrent and diffusion of the contrast agent would conceal the image of the oviduct. Furthermore, a small amount of concealment can be distinguished based on experience, while excessive interference of the contrast agent would make the image complex. Moreover, it would sometimes be difficult to find the true oviduct, or it would be difficult to determine whether there was any outflow of contrast agent from the oviduct. TVS RT-3D-HyCoSy can retain the whole video showing the moment when the contrast agent flows into the uterine cavity until it outward flows to the pelvic cavity. However, the countercurrent and dispersion of the contrast agents also appear in this process. Although it can be identified and excluded by playback, there is still a situation in which there is no way to distinguish the oviduct after the interference. Hence, the real oviduct could not be found, or the countercurrent of the contrast agent is regarded as an oviduct, leading to a false positive. A previous study revealed that the countercurrent is correlated to the thickness of the endometrium, the timing of radiography, and the history of pelvic operation. Choosing the right time for radiography and reducing the damage to the endometrium caused by radiography can reduce but not completely avoid the occurrence of countercurrent.[10] If image interference is obvious, the pelvic cavity can also be scanned by energy or color Doppler ultrasound, in which injection imaging is performed again after blasting the contrast agent microbubbles.[11]

Abnormal shape or position of the oviduct itself is also a common cause of misdiagnosis. Pelvic adhesions can induce the oviduct to be lifted, or cause excessive distortion and a complex image of the oviduct.[12] The oviduct is pulled by the lateral adhesion to change the original distributions, the outflow of contrast agent from the oviduct fimbria can flow to the periphery of the contralateral ovary, and adhesion can make the distal oviduct to go beyond the scope of scanning, making it difficult to distinguish whether the distal oviduct is unobstructed or obstructed. In a two-dimensional ultrasound examination, the topographical relationship of the ovary and uterus can roughly predict the distribution of the oviduct. The results of the present study revealed that when the ovary was tightly attached to the uterus, the oviduct would also be close to the uterus, making it difficult to identify. If the patient has uterine fibroids and ovarian cysts that pushes the oviduct, it can also change the normal distribution of the oviduct, making the image difficult to be recognized[13]. Furthermore, the outflow of the contrast agent from the oviduct fimbria is sometimes not distributed along the periphery of the ipsilateral ovary, affecting the judgment of the results. When the uterus is in its middle position, the proximal oviduct would be far in the three-dimensional space image, and the proximal oviduct would be thinner. Therefore, when the oviduct is in the middle position, the image of the proximal oviduct is not well-displayed; that is, the starting point is not clear. This affects the search for the distal oviduct, which can easily lead to errors. The median uterus can be turned into the anterior or posterior uterus by pressing the abdomen or shaking the probe, and an examination would be conducted again. When the oviduct is longer, the image of the outflow of the contrast agent from the distal oviduct is not collected, and it would be mistaken as an obstruction in the distal end. Therefore, in radiography, it is necessary to increase the acquisition frame appropriately and concomitantly keep both the uterine horn and bilateral ovary in the acquisition frame in order to allow the full length of the oviduct to be observed. When the oviduct presents hydrops, the accumulation of the contrast agent in the distal end would sometimes be regarded as an outflow of the contrast agent from the oviduct fimbria, causing a false negative. In patients with uterine malformation, especially those with complete double horns or mediastinum, the contrast tube can only be inserted into one side of the uterus. Furthermore, intrauterine adhesions make the uterine cavity narrow and even cause the opening of the oviduct to be blocked. This causes the contrast agent to fail to flow smoothly into the oviduct, leading to misdiagnosis. Therefore, attention should be given to the three-dimensional ultrasound examination of the uterine cavity shape.[14]

Unreasonable operation during radiography

would also cause misdiagnosis.[15] When the balloon is filled and becomes too large, or the position of the balloon is too high, the resistance of the bolus injection of contrast agent would be increased, and the contrast agent would not be able to flow outward smoothly. This situation would be mistaken as obstructed oviduct. When the contrast tube inserted into the uterine cavity is on the side of the uterine horn, there would be a difference in the time when the contrast agent flows outward from the bilateral oviducts. If the time interval is too long, it would be mistaken as an obstruction of the contralateral oviduct. The results of the present study revealed that these factors could be corrected by changing the size and location of the balloon. In radiography, attention should be given to the situation of the contrast agent reflux. If the inside of the cervix is loose, especially in the multipara, and when reflux occurs, intrauterine pressure would not be enough, and the amount of contrast agent entering into oviduct decreases. This would easily lead to a misdiagnosis. This can be corrected by tightening the contrast tube or enlarging the balloon, allowing it to be plugged to the cervical intraoral cavity. This way, it can allow the contrast agent to enter the oviduct smoothly, obtaining the correct results. The diameter of the contrast agent particles is equal to that of red blood cells, so a very fine oviduct can allow the contrast agent to enter.[16] Therefore, when the internal oviduct is narrow, but not completely occluded, there would be a small amount of contrast agent entering the oviduct, and an unobstructed result may be obtained. However, in clinic practice, such oviducts are fine, stiff, and dysfunctional. In addition, when oviduct spasm occurs, the contrast agent cannot temporarily pass through the oviduct, leading to a false-positive result. Spasms are correlated to tension or pain of the patient.[17] In operation, the incidence of spasms can be reduced by soothing the patient or delaying the bolus injection of the contrast agent for a short period of time. Slight adhesion of the oviduct or the accumulation of endometrial debris at the opening of the oviduct may hinder the contrast agent from passing through at the beginning. The pressurizing bolus injection of the contrast agent can sometimes remove interference, making the oviduct unobstructed.

In summary, through the analysis of misdiagnosed cases, the researchers consider that some causes for misdiagnosis of TVS RT-3D-HyCoSy can be avoided, while some are very difficult to avoid. Patients with poor image quality still need doctors with some experience to process the images and determine the results. Furthermore, understanding and improving the cause of misdiagnosis can improve the diagnostic performance of TVS RT-3D-HyCoSy.

Conflicts of interest

All authors have contributed significantly to the manuscript and declare that the work is original and has not been submitted or published elsewhere. None of the authors have any financial disclosure or conflict of interest.

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RESUMO

OBJETIVO: *Este estudo tem como objetivo investigar as causas do diagnóstico equivocado na avaliação da patência tubária por meio da ultrassonografia de contraste histerosalpingo em tempo real transvaginal (TVS RT-3D-HyCoSy), a fim de melhorar a eficiência diagnóstica das TVS RT-3D-HyCoSy.*

MÉTODOS: *Um total de 162 ovidutos em 83 pacientes da infertilidade foi examinado por TVS RT-3D-HyCoSy. Esses resultados foram comparados com o padrão ouro para estudos de tintura laparoscópica, e os casos diagnosticados erroneamente foram analisados.*

RESULTADOS: *TVS RT-3D-HyCoSy revelaram que 68 ovidutos foram desobstruídos e 94 ovidutos foram obstruídos. Os resultados para os 144 ovidutos estavam em consonância com o padrão ouro, enquanto que aqueles para os 18 ovidutos, não. A taxa de acurácia do TVS RT-3D-HyCoSy foi de 88,9%, e a taxa de erro de diagnóstico foi de 11,1%. As principais causas de erro de diagnóstico incluíram contraponto e difusão do meio de contraste, espasmo do oviduto, forma ou posição anormal do oviduto, adesão pélvica e má operação de imagem.*

CONCLUSÃO: *TVS RT-3D-HyCoSy pode bem avaliar a patência tubária, e compreender e melhorar a causa do erro de diagnóstico. Além disso, a eficiência diagnóstica do TVS RT-3D-HyCoSy ainda pode ser melhorada.*

PALAVRAS-CHAVE: *Histerossalpingografia. Infertilidade. Técnicas de diagnóstico obstétrico e ginecológico. Erros de diagnóstico.*

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ORIGINAL ARTICLE

Plasmatic adipocyte biomarkers and foot pain associated with flatfoot in schoolchildren with obesity

Ahmad H. Alghadir[1]
Sami A. Gabr[1]
Ashraf A. Rizk[2]

1. Rehabilitation Research Chair, College of Applied Medical Sciences, King Saud University, Riyadh, KSA.
2. Department of Health Science, College of Health Science and physical activity, King Saud University, Riyadh, KSA.

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SUMMARY

OBJECTIVE: *The aim of this study was to determine the potential association of foot pain and plasmatic adipocytes as physiological biomarkers of childhood obesity with the incidence of flatfoot in a cohort of Egyptian school children aged 6 -12 years.*

METHODS: *A total of 550 Egyptian schoolchildren (220 boys and 330 girls) aged 6-12 years were randomly invited to participate in this descriptive survey analysis. For all children, we assessed the diagnosis and severity of flatfoot as well as plasma adipocytes, as well as adiponectin, leptin, resistin, IL-6, and TNF-α, using the Dennis method and immunoassay techniques respectively. Foot pain was assessed by using a standard VAS of 100 mm and Faces Pain Scale, respectively.*

RESULTS: *Flat foot was predicted in 30.4% of school-age children, most of them showed a higher frequency of overweight (33.3%) and obesity (62.5%). Boys showed higher ranges of flat foot than girls. Foot pain significantly correlated with flat foot and obesity among the studied populations. In overweight-obese children, plasmatic adipocyte variables, as well as adiponectin, leptin, resistin, IL-6, TNF-α showed significant correlations with foot stance, especially in boys. Also, the studied adipocyte variables along with BMI, age, gender explained about~65% of the variance of flatfoot with pain among our school-age students.*

CONCLUSION: *Foot pain showed an association with flat foot and childhood obesity in 30.4% of school-age students (6-12 years). Foot pain was shown to correlate positively with the incidence of flat foot and changes in adiposity markers, as well as adiponectin, leptin, resistin, Il-6, TNF-α.*

KEYWORDS: *Flatfoot. Pediatric Obesity. Foot Diseases. Biomarkers. Obesity/complications.*

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CORRESPONDING AUTHOR: Sami A. Gabr
Rehabilitation Research Chair, College of Applied Medical Sciences, King Saud University - P.O. Box 10219 Riyadh 11433, KSA
Tel: +966562060018 – Fax: +96614698541
E-mail: dr.samigabr@gmail.com

INTRODUCTION

Childhood is frequently associated with deformities in the lower extremities.[1] In children, flexible or flat feet are the most (90%) frequent foot problems.[2] These abnormalities result in other feet complications such as gait disorders, foot depression, poor foot function, severe musculoskeletal pathologies, and a negative effect on the quality of life.[1-4] A limited ankle joint range of motion with a higher risk of foot pain, knee pain, foot injury, stress fracture, abnormal gait, and poor exercise performance were associated with flat foot, particularly in children.[4-7]

In children with obesity, higher frequency of flatfoot was reported compared to those of normal weight.[7-9] Also, weight-bearing or obesity increases pressure on the plantar area, which consequently produces an increase in foot length, width, and as a result, a change in foot size.[8-10]

In obese adolescents, higher levels of visfatin, leptin, and lower adiponectin concentrations were evaluated compared to their healthy counterparts.[8-13] Also, subjects with obesity showed an increase in the levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) as pro-inflammatory markers.[11-13] In addition, leptin and resistin as biomarkers of childhood obesity showed a correlation with bone mineral density (BMD) and bone mineral content (BMC) of the femoral neck and lumbar spine,[12-17] thus playing a significant role in mediating the foot-bone axis. After considering the aforementioned studies,[8-15] the aim of this study was to determine the potential association of foot pain and plasmatic adipocytes as physiological biomarkers of childhood obesity with the incidence of flatfoot in a cohort of Egyptian school children aged 6 -12 years.

METHODS

Subjects

This cross-sectional study included a total of 550 Egyptian schoolchildren (boys n=220 and girls n=330) aged 6-12 years who were randomly invited in September 2014 and June 2015 from different elementary and Prep public schools in Mansoura, Egypt, to participate in this descriptive survey analyses. Children with muscle weakness, paralysis, intellectual disability, endocrine and cardiovascular disorders, congenital abnormalities below the level of the ankle, cerebral palsy, history of surgery, and musculoskeletal or neurologic diseases were excluded from this study. The demographic and clinical data of the participants are in Table (1).

Anthropometric measurements

Based on the BMI cut off criteria previously devolved by Cole et al.[16] and specific to age and gender, the participants in the study were divided into four categories: underweight (BMI; > 16.2 kg/m^2), normal weight (BMI; 16.2 -17.3kg/m^2), overweight (BMI; 17.4 -21.45kg/m^2), and obese (BMI; ≥ 22 kg/m^2). In addition, waist-to-height ratio (WHtR) was also calculated according to Ashwel et al.[17] and other authors.[18-22]

Assessments of adipocytokines

ELISA kits specific for leptin and resistin were used to estimate adipocytokines in plasma samples of all the participants. Also, IL-6 and TNF-α Cytokines production in plasma samples was evaluated with an ELISA assay kit (EndogenTema, USA).

Assessment of Flat foot

Flat foot was significantly estimated by a well-trained trauma physician and orthopedist by using a classic wooden podoscope (60 x 40 x 40 cm). Finally, based upon Denis measurements, children who displayed second- and third-grade plantar footprint were well-defined as flatfooted.[20]

Foot pain was assessed by using a standard validated VAS of 100 mm.[21] Due to the conceptual complexity required to understand VAS, especially for younger ages,[22,23] VAS scores of our subjects with ages below eight years were standardized based on a pre-validated Faces Pain Scale.[19-24]

Statistical analysis

The statistical SPSS software for Windows v.20 package was used to calculate frequencies of the studied variables. Both ANOVA and Dunnett post hoc test was used to compare the data between groups. The χ^2 test was applied to determine the statistical association between the studied variables; the data were considered significant at $p < 0.05$ with 95% CI.

RESULTS

The study sample was comprised of 550 school-age students, 220 boys, and 330 girls (60%). Mean age was 10.1± 0.3 years; p= 0.11 for boys and p= 0.50 for girls. The participants were classified, based on BMI, into four groups. Abnormal body weight was reported

in 240 (43.6%) of the subjects; they were classified into low weight (4.16%), overweight (33.3%), and obese (62.5%) (Table 1).

In this study, flatfoot was estimated in 30.4% (n=167), higher in boys than in girls (boys: 32.27, girls: 28.8%; p=0.01) up to the age of 10 years (16.9%), without difference in the prevalence rates with respect to age (p=0.21), as shown in figure (1A), and figure (1B). The prevalence of flatfoot peaked at 6, 7, and eight years old for both boys and girls. The prevalence of flatfoot in schoolchildren aged seven and eight years was significantly higher (all P=0.001) than in older children, regardless of gender (Figure 1B).

In addition, compared to subjects with normal weight, higher significant rates of flatfoot were reported in subjects with obesity (52%, P=0.001), overweight (31.25%, P=0.01), and low weight (33.3 %, p=0.01), respectively, as seen in the figure (1C).

Foot pain was shown to increase in overweight and obese children with flat foot compared to children with normal weight (Figure 1A & 1B). Foot pain scores correlated positively with adiposity markers in obese children with flatfoot (P=0.001).

In figure (1D) and table (2), there was a significant increase in the plasma levels of adiponectin, leptin, resistin, Il-6, and TNF-α in overweight (P=0.01) and obese (P=0.001) subjects compared to normal-weight subjects. Underweight subjects showed lower levels of the studied parameters, but the data obtained was not statistically significant (P=0.13). Subjects with flatfoot showed higher (P=0.001) levels of adiponectin, leptin, resistin, Il-6, and TNF-α compared to subjects with normal foot stance (Figure 1D).

In this study, younger age (β = -0.256), male gender (β =-0.089), and overweight (β =0.316) were closely related to flat foot incidence among obese subjects (table 2). Along with age and gender, adiponectin (β = 0.125), leptin (β = 0.235), resistin (β = 0.136), Il-6 (β = 0.146), TNF-α (β = 0.325), and BMI were also predictors of flatfoot (β =0.258). The studied variables explained 65% of the variance among our school-aged students.

In addition, our data showed that school children with obesity had a higher risk of flat foot (6.1 times; P=0.001) and foot pain (8.5 times;P=0.001), with significant increase (p=0.005) in the level of adipocytes cytokines, as well as adiponectin, leptin, resistin, Il-6, and TNF-α, compared to subjects with normal BMI (Table 2). Also, there were detrimental effects of foot pain (1.11 times; P=0.001) and flat foot (2.69 times;

TABLE 1. ANTHROPOMETRIC AND DEMOGRAPHIC MEASUREMENTS OF THE DIFFERENT POPULATION GROUPS OF A SAMPLE OF SCHOOLCHILDREN AGED 6-12YRS (MEAN ± STANDARD DEVIATION).

Parameters	*Boys (n=220)*							
	LW(n=14)		*NW(n=126)*		*OW(n=30)*		*OB (n=50)*	
	Mean ±SD	*95% CI*	*Mean ±SD*	*95%CI*	*Mean ±SD*	*95% CI*	*Mean ±SD*	*95% CI*
Age (Years)	*9.9±0.8*	*9.4-10*	*10.1±0.8*	*9.7-10*	*9.6±0.8*	*9.2-10*	*10.3±0.8*	*9.9-10.2*
Weight (kg)	*28.3±3.6***	*25.1-29.2*	*34.2±5.3*	*32.5-36*	*48.6±5.9***	*39.2-52*	*58.9±12.4****	*51.4-65*
Height (m)	*1.46±0.2*	*1.4-1.49*	*1.5±0.2*	*1.47-1.6*	*1.7±0.2***	*1.6-1.78*	*1.79±0.2***	*1.76-1.9*
BMI (kg/m2)	*15±0.3***	*14.6-15.4*	*16.9±0.3*	*16-17.2*	*21.4±0.29***	*20.1-22*	*26.5±3.6****	*25.6-27*
WC (cm)	*54±4.8***	*52.6-55.3*	*63.1±6.4*	*62.7-64*	*75.9±8.5***	*74.8-76*	*86.5±9.1****	*85.7-87*
HP (cm)	*61±3.7***	*60.8-62.3*	*76.1±4.8*	*75.7-77*	*82.9±5.4***	*81.8-84*	*92.5±9.6****	*90.7-93*
WHR	*0.44±0.8***	*0.43-0.45*	*0.48±0.8*	*0.47-0.5*	*0.54±0.8***	*0.53-0.6*	*0.62±0.8****	*0.63-0.7*
WHtR	*0.48±0.3***	*0.47-0.49*	*0.53±0.3*	*0.5-0.54*	*0.58±0.3***	*0.57-0.6*	*0.69±0.3****	*0.68-0.8*
Parameters	*Girls (n=330)*							
	LW(n=16)		*NW(n=164)*		*OW(n=50)*		*OB (n=100)*	
	Mean±SD	*95% CI*	*Mean±SD*	*95%CI*	*Mean ±SD*	*95% CI*	*Mean ±SD*	*95% CI*
Age (Years)	*9.9±0.8*	*9.7-10*	*10.3±0.8*	*9.9-10.1*	*9.8±0.8*	*9.3-10.2*	*10.2±0.8*	*9.8-10.1*
Weight (kg)	*28.6±3.8***	*26-29.4*	*36.4±5.6*	*35.8-37*	*51.8±6.9***	*51.2-53*	*62.9±11.2****	*61.4-64*
Height (m)	*1.47±0.2*	*1.4-1.49*	*1.53±0.2*	*1.51-1.6*	*1.76±0.2***	*1.7-1.78*	*1.8±0.2****	*1.76-1.9*
BMI (kg/m2)	*14.6±0.6***	*13.8-15*	*17.2±0.7*	*16-17.3*	*21.6±0.29***	*20.1-22*	*26.9±3.4****	*26.1-28*
WC (cm)	*53.9±4.6***	*52.6-55*	*64.4±6.1*	*63.7-65*	*76.3±8.1***	*75.4-77*	*84.7±8.9****	*83.7-86*
HP (cm)	*61.6±3.9***	*60.5-63*	*77.4±4.9*	*76.8-78*	*84.8±5.8***	*83.5-86*	*93.8±9.8****	*91.5-95*
WHR	*0.44±0.8***	*0.4-0.44*	*0.48±0.8*	*0.46-0.5*	*0.55±0.8***	*0.54-0.6*	*0.60±0.8****	*0.62-0.6*
WHtR	*0.47±0.3***	*0.46-0.5*	*0.54±0.3*	*0.51-0.6*	*0.63±0.3***	*0.6-0.7*	*0.67±0.3****	*0.65-0.7*

*Statistical analyses by One-way ANOVA with Dunnett post-hoc test. **p < 0.01, ***p < 0.001. LW, low weight; OW, overweight; OB, obesity; NW: normal weight; BMI, body mass index; WC, waist circumference; HP, hip circumference; WHR, waist to height ratio; WHtR: Waist-to-height ratio.*

P=0.001) reported in subjects with low weight compared to normal subjects.

DISCUSSION

The findings of this study showed that foot pain was significantly associated with flat foot and adiposity markers in all subjects. The prevalence of flatfoot significantly (all P=0.001) peaked at six, seven, and eight years of age for both boys and girls compared to older children, regardless of gender. Other studies had similar results, most of which on younger or infants and showing ratios of flatfoot.[16-19,22-24] During childhood, age, gender, adiposity, and ligamentous laxity are considered the most predisposing factors and strongly determine the prevalence of flatfoot.[20-22]

Overall, in this study, flatfoot was found in 30.4% of the study population. Boys had higher prevalence rates for flatfoot than girls (32.27 in boys vs28.8% in girls; p=0.01). Consistent with current results, previous research found flat foot in 52% of boys and 36% of girls aged 3-6 years old.[12,21-24]

This study showed that 43.6% of the subjects had an abnormal change in BMI. The prevalence of increased BMI was 33.3% in overweight and 62.5% in obese schoolchildren, respectively. Our results are consistent with other studies, which reported a relatively high prevalence of overweight, and obesity among children aged 6–12 years.[12,14,15]

In this study, BMI correlated positively with flat foot among school-age children. The prevalence for flatfoot was significantly higher in schoolchildren with obesity (52%, P=0.001), overweight (31.25%, P=0.01), and low weight (33.3 %, p=0.01) compared to the prevalence of flat foot (17.2 %) in children with normal weight. Consistent to our results, significant variations in the prevalence of flatfoot were reported in under-weight (13.9%), normal-weight (16.1%), overweight (26.9%), and obese (30.8%) schoolchildren aged 3-18 years.[12,15] This may be explained by the fact that

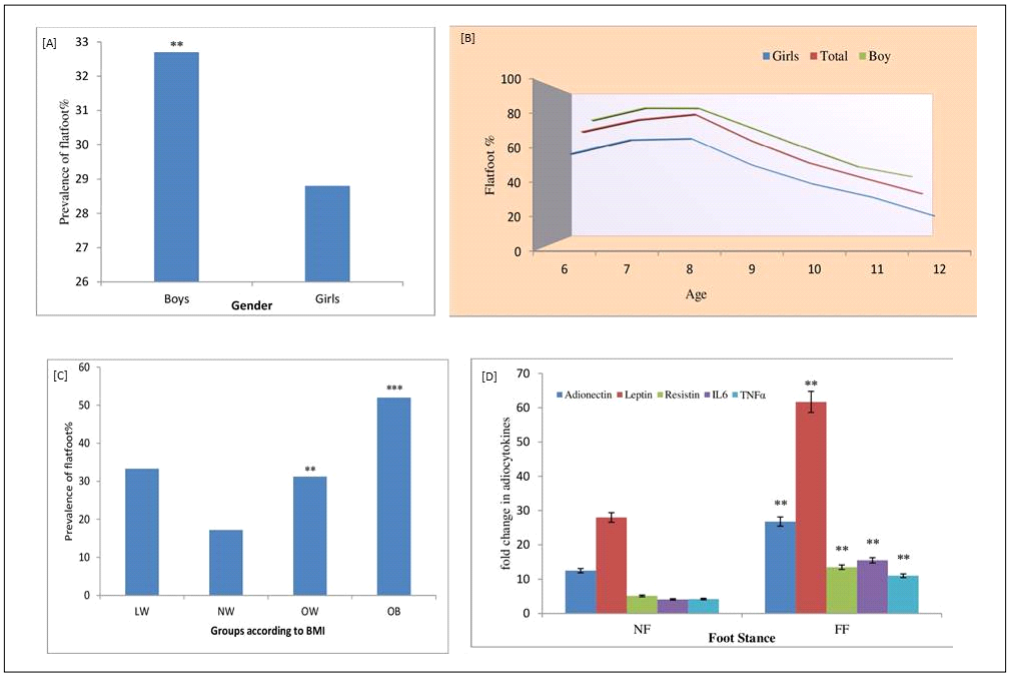

FIGURE 1. PREVALENCE OF FLATFOOT IN SCHOOL-AGE STUDENTS (6-12 YEARS) BASED ON GENDER [A], AGE [B], OBESITY [C], AND PHYSIOLOGICAL CHANGES IN ADIPOCYTOKINES AND INFLAMMATORY CYTOKINES [D]. STATISTICAL ANALYSES BY ONE-WAY ANOVA WITH DUNNETT POST-HOC TEST; TEST (¬ χ2) WERE USED TO ANALYZE CORRELATIONS. HIGHER RATES OF FLAT FOOT WAS ESTIMATED IN BOYS *** P <0.01 (BOYS VS. GIRLS) [A]. THE PREVALENCE OF FLATFOOT IN SCHOOLCHILDREN AGED 7 AND 8 YEARS WAS SIGNIFICANTLY HIGHER (ALL P=0.001) THAN IN CHILDREN WITH OLDER AGES REGARDLESS OF GENDER [B]. ALSO, STUDENTS WITH OVERWEIGHT AND OBESITY SHOWED HIGHER RATES OF FLAT FOOT COMPARED TO THOSE WITH LOW OR NORMAL WEIGHTS [C]. ** P = 0.02, *** P <0.001 (OW/OB VS LW OR NW), * P = 0.01(LW VS NW). ALSO, ADIPOCYTOKINES AND INFLAMMATORY CYTOKINES SIGNIFICANTLY CORRELATED WITH FOOT STANCE [D]. ** P <0.001(FFVSNF). NORMAL FOOT (NF); FLAT FOOT (FF); LW: LOW WEIGHT; NW: NORMAL WEIGHT; OW: OVERWEIGHT; OB: OBESITY.

TABLE 2. BETA REGRESSION COEFFICIENT (β), ORS (95% CI), AND P-VALUES FOR FACTORS EFFECT ON THE PROBABILITIES OF FOOT PAIN AND FLATFOOT IN SCHOOLCHILDREN WITH OBESITY.

Parameters	Low weight			Overweight			Obesity		
	β	OR (95% CI)	P-value	β	OR (95% CI)	P-value	β	OR (95% CI)	P-value
Age	-0.256	1.2(0.6-1.5)	0.01	-0.135	1.1(0.86-1.3)	0.01	-0.245	1.0(0.9-1.3)	0.01
Gender	-0.089	1.4(1.1-2.2)	0.05	-0.314	1.7(1.1-2.7)	0.05	-0.258	1.1(0.6-1.5)	0.01
Weight	0.316	2.1(1.1-3.4)	0.01	0.224	1.3(1.1-1.8)	0.01	0.315	1.5(1.1-1.6)	0.01
BMI	0.258	2.6(1.1-3.1)	0.01	0.124	1.6(1.1-1.9)	0.01	0.139	1.3(1.1-1.8)	0.01
Foot Pain (Yes/No)	0.125	1.11 (0. 8 - 1.7)	0.001	0.321	3.2 (1.8-7.6)	0.003	0.345	8.5 (4. 5 - 11.6)	0.001
Flat foot (Yes/No)	0.235	2.69 (3. 5 - 5.8)	0.001	0.145	4.8 (3. 1 - 8.2)	0.005	0.128	6.1 (3. 5 - 9.1)	0.001
Adiponectin, ng/mL	0.136	2.1 (1.8 – 4.3)	0.05	0.321	1.8 (1.1 - 3.2)	0.01	0.215	2.9 (1.5 - 3.2)	0.005
Leptin, ng/mL	0.146	1.5 (1.1 – 2.3)	0.01	0.324	1.2 (0.94 - 2.4)	0.05	0.124	3.7 (2.94 - 5.1)	0.005
Resistin, ng/mL	0.325	1.18 (0.8 – 2.5)	0.01	0.324	1.5 (1.1 – 2.9)	0.01	0.384	1.6 (1.1 – 3.2)	0.005
IL-6, pg/mL	0.450	2.3 (1.2 – 3.7)	0.05	0.314	3.7 (2.9 – 4.9)	0.05	0.131	3.1 (2.9 – 5.6)	0.005
TNF-α, pg/mL	-0.256	1.45 (1.2 – 2.9)	0.03	0.245	1.4 (1.1 – 2.8)	0.04	0.214	1.9 (1.3 - 3.6)	0.005
ΣR2 (%)	0.135	35 (28.6- 45.7)	0.001	0.425	62.7 (48.9 – 75.1)	0.001	0.512	65.7 (56.7 – 86.4)	0.001

Entered variables to the model (multinomial logistic regression): ΣR2 = summation of cumulative values of R relating to studied variables. Adiponectin, leptin, resistin, IL-6, TNF-α. IL-6, Interleukin-6 cytokine; TNF-α, tumor necrosis-α cytokine; ORs, odds ratios; CI, confidence interval; Beta regression coefficient (β); BMI, body mass index. Hierarchical multiple regressions were used to estimate correlations

excess weight exerts more stress and load on the ligaments and soft tissues of the foot, which induces injuries and deformities in the feet.[12-16]

In our study, a significantly higher likelihood of flatfoot (33.3 %, p=0.01) was reported in elementary school children aged 6-8 years old who were underweight, suggesting that underweight children were likely to have flatfoot, compared with those of normal weight (17.2 %). Similarly, a higher likelihood of flatfoot was reported in younger ages of underweight preschool children.[12,17] Obesity was shown to be correlated with the incidence and development of foot pain.[2-9,12-16]

The results of the current study showed that in overweight (P=0.01) and in obese (P=0.001) children with flatfoot, there is a significant increase in the levels of adiponectin, leptin, resistin, IL-6, and TNF-α, in comparison to normal subjects. Similarly, a positive higher prevalence of foot pain with obesity was reported in young and adult subjects with flatfoot.[12-24] Pain and biomechanical changes in weight-bearing joints, such as the hip, knee, or foot structures were shown to be correlated with metabolic changes in adipokine profile, particularly higher levels of adiponectin and leptin.[12,20-24]

CONCLUSION

Foot pain showed an association with flat foot and childhood obesity in 30.4% of school-age children (6-12 years). Foot pain was shown to correlate positively with the incidence of flat foot and changes in adiposity markers, such as adiponectin, leptin, resistin, Il-6, TNF-α.

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Author Contributions

Research idea, design, and practical work were proposed by GA. Review of literature was done by AR and AA. Data collection and analysis was executed by GA. Manuscript preparation and submission was done by GA.

RESUMO

OBJETIVO: *O objetivo deste estudo foi determinar a potencial associação de dor no pé e adipócitos plasmáticos como biomarcadores fisiológicos da obesidade infantil com incidência de pé plano em uma coorte de escolares egípcios de 6 a 12 anos.*

MÉTODOS: *Um total de 550 escolares egípcios (220 meninos e 330 meninas) com idades entre 6 e 12 anos foram convidados aleatoriamente para participar desta análise descritiva. Para todas as crianças, diagnóstico e gravidade do flatfoot, bem como adipócitos plasmáticos; adiponectina, leptina, resistina, IL-6 eTNF-α foram avaliados pelo método de Dennis e técnicas de imunoensaio, respectivamente. A dor no pé foi avaliada usando uma EVA padrão de 100 mm e a Faces Pain Scale, respectivamente.*

RESULTADOS: *O pé plano foi predito em 30,4% das crianças em idade escolar; a maioria apresentou maior frequência de sobrepeso (33,3%) e obesidade (62,5%). Os meninos apresentaram maiores faixas de pé plano do que as meninas. A dor no pé correlacionou-se significativamente com pé plano e obesidade entre as populações estudadas. Em crianças obesas com sobrepeso, variáveis adipocitárias plasmáticas; adiponectina, leptina, resistina, IL-6 e TNF-α apresentaram correlação significativa com a postura do pé, em meninos e meninas. Além disso, as variáveis estudadas dos adipócitos, juntamente com o IMC, idade e sexo, explicaram cerca de 65% da variância do pé plano com a dor entre os nossos alunos em idade escolar.*

CONCLUSÃO: *A dor no pé mostrou associação com pé plano e obesidade infantil em 30,4% dos estudantes em idade escolar (6-12 anos). A dor no pé se correlacionou positivamente com a incidência de pé plano e a mudança nos marcadores de adiposidade; adiponectina, leptina, resistina, IL-6, TNF-α.*

PALAVRAS-CHAVE: *Pé chato. Obesidade pediátrica. Doenças do pé. Biomarcadores. Obesidade/complicações.*

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ORIGINAL ARTICLE

TLR7 mediates increased vulnerability to ischemic acute kidney injury in diabetes

Yayi Huang[1]
Fang Zhou[1]
Yeda Xiao[1]
Cheng Shen[2]
Kang Liu[3]
Bo Zhao[1]

1. PhD, Department of Anesthesia, Renmin Hospital, Wuhan University, China.
2. Master, Department of Anesthesia, Renmin Hospital, Wuhan University, China.
3. Department of Anesthesia, Renmin Hospital, Wuhan University, China.

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SUMMARY

OBJECTIVE: *Diabetes is a risk factor for acute kidney injury (AKI). However, its mechanism of pathogenesis has not been elucidated. The aim of the study was to investigate the role of inflammation and the toll-like receptor 7 (TLR7) in ischemic AKI for diabetes.*

METHODS: *A high glucose hypoxia-reoxygenation model of human renal tubular epithelial (HK-2) cells was used to generate AKI induced by ischemia-reperfusion in diabetes. The activity of cells was measured by CCK-8 assay and LDH activity. Inflammatory cytokines were assessed by ELISA. TLR7, MyD88, and NF-κB expressions were examined by western blotting. Apoptosis was evaluated by flow cytometry.*

RESULTS: *The high glucose group and low glucose group were subjected to hypoxia-reoxygenation. The low glucose group developed only mild cell damage, apoptosis, and inflammatory response. In contrast, an equivalent hypoxia-reoxygenation injury provoked severe cell damage, apoptosis, and inflammatory response in the high glucose group. Expression of TLR7 and its related proteins were measured in the high glucose group before and after hypoxia-reoxygenation. The high glucose group exhibited more significant increases in TLR7 expression following hypoxia-reoxygenation than the low glucose group. In addition, the expression of TLR7 and its related proteins after hypoxia-reoxygenation were higher in the high glucose group than in the low glucose group. Inhibition of TLR7 provides significant protection against ischemic injury in diabetes.*

CONCLUSION: *Our results suggest that diabetes increases the vulnerability to ischemia-induced renal injury. This increased vulnerability originates from a heightened inflammatory response involving the TLR7 signal transduction pathway.*

KEYWORDS: *Health Vulnerability. Ischemia. Acute kidney injury. Diabetes mellitus. Toll-Like Receptor 7.*

INTRODUCTION

Diabetes mellitus is a chronic immune response disease that affects millions of people worldwide and leads to progressive whole-body organ damage, especially of the kidney. The discovery of toll-like receptors (TLRs) has provided great contributions in the field of diabetes research. TLRs are an important part of the innate immune system and play a crucial role in the detection of microbial infections and the antibacterial response defense of the host. In recent years, the role of TLRs in the cardiovascular and nervous system, liver, spleen, and particularly in kidney disease has attracted more attention. After activation,

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CORRESPONDING AUTHOR: Yayi Huang
Jiefang Road 238, Wuhan, Hubei, China - 430060 – Tel: 15872394498
E-mail: huangyayi@126.com

TLRs promote cell proliferation, differentiation, apoptosis, secretion of inflammatory factors through signal transduction pathways, and are involved in the occurrence and development of multiple diseases[1]. The association of TLRs with inflammatory reactions and cell apoptosis has become a topic of concern for many researchers[2,3]. Therefore, in this study, we seek to address the hypothesis that diabetes increases the vulnerability to ischemic acute kidney injury (AKI) by enhancing the production of inflammatory cytokines and expression of TLR7 within the kidney.

METHODS

Antibodies and reagents

The following antibodies and antagonists were used in this study: TLR7 (NBP2-24906, Novus Biologicals, USA), MyD88 (ab2064, Abcam, Cambridge, UK), NF-κBP65 (8242S, Cell Signaling Technology, USA) and chloroquine diphosphate (CQ) (Sigma-Aldrich Inc., St. Louis, MO, USA). TLR7 siRNA plasmid (sc-40266, Santa Cruz Biotechnology, Inc, California, USA) and control siRNA plasmid were from Santa Cruz Biotechnology. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were evaluated by commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (Elabscience Biotechnology, Wuhan, China). Chloroquine was dissolved in calcium- and magnesium-free phosphate-buffered saline at a concentration of 10 mM. Stock solutions were diluted to the desired final concentrations with growth medium just before use.

Cell culture and experimental conditions

Experiments were performed with human renal tubular epithelial (HK-2) cells (ATCC, American Type Culture Collection, Manassas, VA, USA), which are human proximal tubular epithelial cells. Cells were cultured in MEM medium (#31985, Gibco, Grand Island, NY, USA), which was supplemented with 10% fetal bovine serum (FBS, #10099-141, Gibco) and 1% Penicillin-Streptomycin solution in 5% CO_2.

Transfection of siRNA and experimental grouping

HK-2 cells were seeded in 6-well plates (2×10^5 per well) and transfected with Lipofectamine 2000 reagent (#11668-019, Invitrogen, USA) according to the manufacturer's instructions. Cells were used for further experiments 48 hours after transfection. After this time period, the HK-2 cells were randomly divided into eight groups (n = 6): 1- low-glycemic group (stimulated with LG (5.6 mM), LG group); 2 - high glucose group (stimulated with LG (30 mM), HG group); 3 - low glucose + mannitol group (24.4 mM, M group); 4 - hypoxia complex oxygen group (hypoxia for four hours and reoxygenation for two hours, LH/R group); 5 - hypoxia complex oxygenation group (HH/R group); 6 - high glucose + anoxic complex oxygen + TLR7 gene silencing group (HH/R-siRNA group); 7 - high glucose + hypoxia + reoxygenation, RNA interference in the control group (HH/R-Scrambled siRNA group); and 8 - a pre-treatment group (HH/R-CQ group) of high sugar + anoxic + hypoxic + chloroquine (50 μM)[4].

Cell in vitro simulated ischemia/reperfusion model

For the hypoxic treatment, after 72 h HG stimulation in the absence or presence of Chloroquine[5], HK-2 cells were incubated in glucose-free Krebs-Ringer bicarbonate buffer for four hours in a hypoxic chamber equilibrated with 5% CO2, 1% O2, and 94% N2. After hypoxic incubation, the cells were returned to complete medium and reoxidized for two hours with 5% CO2, 21% O2, and 74% N2. Control cells were incubated in a regular cell culture incubator with 21% O2[6].

Cell viability and lactate dehydrogenase (LDH) activity

Cell viability was determined by using the Cell Counting Kit-8 (CCK-8) assay according to the manufacturer's instructions. HK-2 cells (1×10^5 cells/well) were seeded into 96-well plates and pretreated with various conditions as described[6]. 10μL CCK-8 (Beyotime: C0037, Beijing, China) was then added and cells were incubated for four hours (37°C, 5% CO2), and the absorbance was measured at 450 nm with an ELISA assay plate reader. The LDH content was measured by an LDH Cytotoxicity Assay Kit (Jiancheng Biotech, Nanjing, China).

Measurement of inflammatory cytokines

IL-6 and TNF-α levels in HK-2 cells were assessed using a rat ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions.

Western blotting analysis

Expression of TLR7, MyD88, and NF-κB was examined using western blotting. The protein content was

determined by a Bicinchoninic Acid (BCA) protein assay. The protein samples were separated by polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. Membranes were blocked with 5% milk and incubated overnight with the appropriate primary antibodies (anti-TLR3 1:500, anti-MyD88 1:600, and anti-NF-κB 1:1000 antibody) at 4°C. The next day, membranes were incubated with HRP-labeled secondary antibodies (anti-rabbit, 1:5000) for 1 h and then washed with TBST. The membranes were developed on ECL prime solution, and the chemiluminescent signal was measured by BandScan 5.0 software. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Apoptosis assay

After reoxygenation, the cells were trypsinized, washed twice with PBS, and resuspended in binding buffer. The percent apoptosis was assessed by using the Annexin V-APC/7-AAD detection kit (Nanjing KeyGen Biotech, Nanjing, China) according to the manufacturer's instructions. Samples were determined by flow cytometry with the FACScan system (BD Biosciences). Apoptotic cells were defined as the cells located in the right two quadrants of each figure, and the percentages were determined by flow cytometry.

Statistical Analysis

The data were reported as means ± SE. All statistical analyses were performed using SPSS19.0 software. Statistical significance was analyzed by one-way or two-way ANOVA.

RESULTS

Detection of cell injury

Hypoxia reoxygenation was induced in the high glucose group and low glucose group. As seen in Figure 1A and B ($p < 0.05$), the high glucose group subjected to four hours of hypoxia developed marked cell damage while the low glucose group maintained relatively little cell damage. TLR7 expression is inhibited by TLR7 siRNA in HK-2 cells. Moreover, the HH/R group enhanced the effects described above compared to the HH/R-siRNA group, whereas transfection of TLR7-siRNA and treatment with chloroquine inhibited the injury of HK-2 under high glucose and hypoxia-reoxygenation. Flow cytometry showed that the apoptosis rate of the HG, LH/R and HH/R groups was higher than the LG group (Figure 1C, D) ($p < 0.05$). The apoptosis rate of the LH/R group was significantly lower than the HH/R group ($p < 0.05$). Our results demonstrate that TLR7-siRNA transfection or chloroquine pretreatment can significantly reduce apoptotic cells under high glucose hypoxia-reoxygenation.

Inflammatory cytokines in HK-2 cells exposed to high glucose and hypoxia-reoxygenation

Since inflammation is an important mediator of ischemic injury and since diabetes is characterized by an increase in inflammatory mediators, we examined the inflammatory factors IL-6 and TNF-α before and after hypoxia-reoxygenation (Figure 2A, B). As shown in Figure 2, the activation of inflammatory factors was significantly greater in the HH/R group than in the LH/R group ($p < 0.05$). TLR7-siRNA transfection or chloroquine pretreatment can significantly reduce the release of inflammatory factors under high glucose hypoxia-reoxygenation (HH/R group vs. HH/R-siRNA group; HH/R group vs. HH/R-CQ group) ($p < 0.05$).

Expression of TLR7 and related protein (MyD88 and NF-κB) in HK-2 cells impaired by high glucose and hypoxia-reoxygenation

First, the mannitol group (24.4 mM) was used as an osmotic control group, and there was no significant difference between the LG group and LG plus mannitol group (data not shown). However, after 72 hours of high glucose (30 mM) stimulation, TLR7 protein expression was significantly upregulated ($p < 0.05$) (Figure 3A, B) (LG group vs. HG group). Following four hours of hypoxia and two hours of reoxygenation, TLR7 protein expression was further increased (LG group vs. HH/R group; HG group vs. HH/R group). Meanwhile, MyD88 protein expression (Figure 3A, C) and NF-κB protein expression (Figure 3 A, D) also showed the same trend as TLR7 protein expression. Compared with the LH/R group, TLR7 protein, MyD88 protein, and NF-κB protein of the HH/R group were more significantly increased. Our data demonstrate that renal ischemia induces a significantly greater accumulation of TLR7 and its related proteins in the renal tubular epithelial cells in diabetic kidneys compared with nondiabetic kidneys.

After successful transfection of specific TLR7-siRNA, the cells were divided into groups according to the experimental steps. TLR7 protein expression (Figure 3A, B), MyD88 protein expression (Figure 3A, C) and NF-κB protein expression (Figure 3A, D) were significantly lower in the HH/R-siRNA group than in

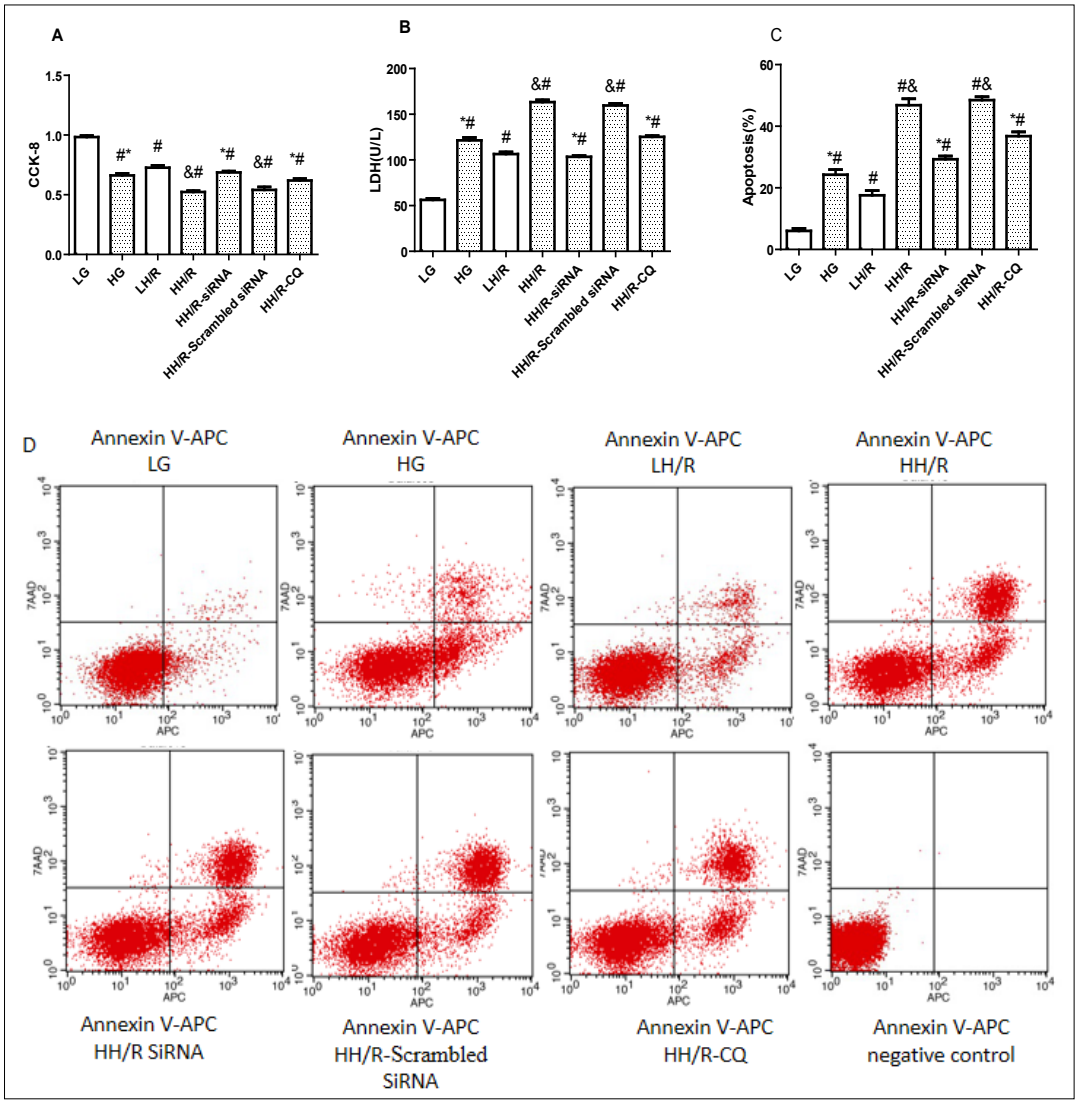

FIGURE 1. DETECTION OF CCK-8 AND LDH LEVELS IN EACH GROUP OF CELLS. EACH GROUP OF HK-2 CELLS WAS TESTED FOR (A) CCK-8 AND (B) LDH AFTER HYPOXIA FOR 4 HOURS AND REOXYGENATION FOR 2 HOURS (N=6). APOPTOSIS(C) OF HK-2 CELLS IN EACH GROUP. (D) APOPTOSIS RATE = UPPER RIGHT QUADRANT CELL (LATE APOPTOTIC CELL) LOWER RIGHT QUADRANT CELL (EARLY APOPTOTIC CELL), APOPTOSIS RATE WAS DETECTED BY FLOW CYTOMETRY AND STATISTICAL ANALYSIS (N = 6). THE HG GROUP WAS MORE VULNERABLE TO ISCHEMIC ACUTE KIDNEY INJURY (AKI) (COMPARED WITH THE LG GROUP, #P < 0.05; COMPARED WITH THE LH/R GROUP, &P < 0.05; COMPARED WITH THE HH/R GROUP, *P < 0.05).

the non-transfected group. TLR7 inhibitor chloroquine pretreatment showed the same trend as TLR7-siRNA transfection. TLR7-siRNA transfected cells and chloroquine pretreatment can inhibit the expression of TLR7, MyD88, and NF-κB proteins under high glucose hypoxia-reoxygenation conditions.

DISCUSSION

The basal expression of TLR7, under conditions of immune mechanism balance, functions to initiate protective signaling mechanisms that protect the immune mechanism during innate immunity from kidney injury. However, imbalances in immune mechanisms, whether at the receptor level or at the level of downstream effectors, can result in severe kidney damage. Our study elucidated several of the downstream effectors involved in TLR7-mediated damage in high glucose and hypoxia-reoxygenation injury of HK-2 cells. Previous animal experiments by Yayi et al.[7] showed that TLR7 protein expression is mainly concentrated in renal tubular epithelial cells. Renal ischemia-reperfusion in diabetic rats causes renal function decline, pathological renal damage, and release of inflammatory factors[7]. Our study further confirmed from HK-2 cell culture that inhibition of TLR7 can

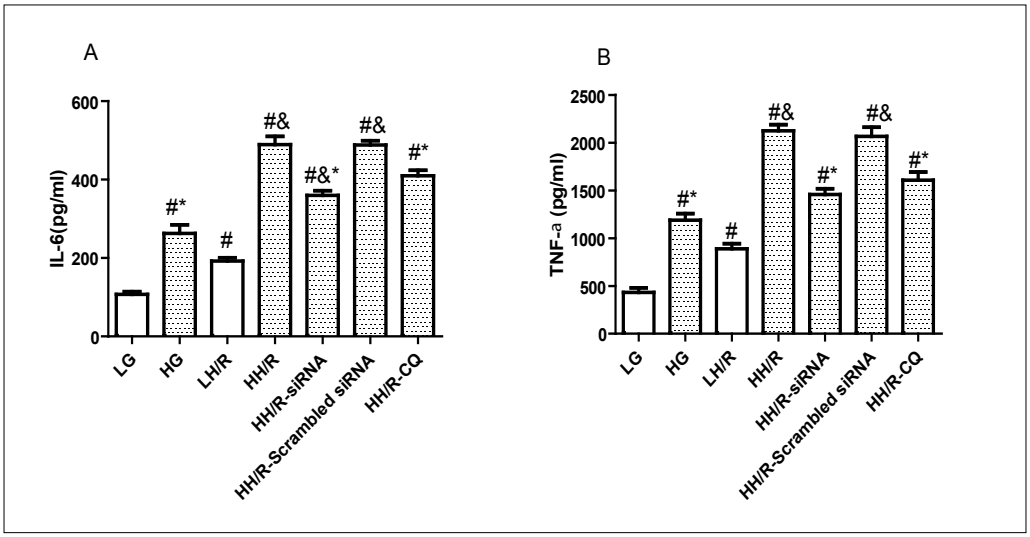

FIGURE 2. IL-6 AND TNF-α EXPRESSION LEVELS IN EACH GROUP. THE CONCENTRATION OF (A) IL-6 AND (B) TNF-α IN HK-2 CELL SUPERNATANT WAS DETERMINED BY ELISA (N = 6) (COMPARED WITH THE LG GROUP, #P < 0.05; COMPARED WITH THE LG/R GROUP, &P < 0.05; COMPARED WITH HH/R GROUP, *P < 0.05).

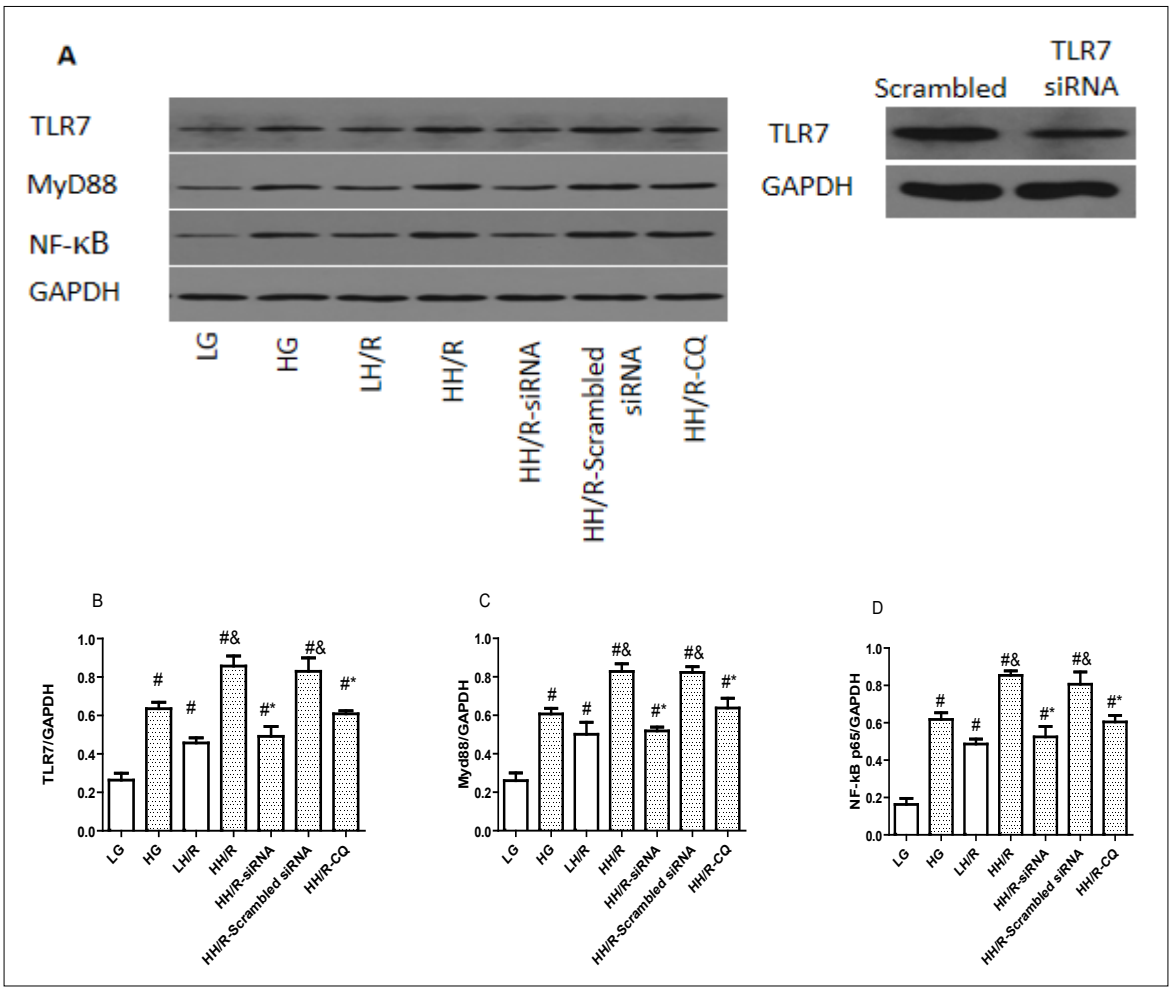

FIGURE 3. TLR7, MYD88, AND NF-κB PROTEIN EXPRESSION IN HK-2 CELLS OF EACH GROUP (N = 6). THE PROTEIN BANDS AND WESTERN BLOTTING WERE USED TO DETECT THE EXPRESSION OF TLR7, MYD88, AND NF-κB PROTEINS IN HK-2 CELLS.

improve the inflammatory response induced by high glucose and hypoxia-reoxygenation, reduce the apoptosis rate and enhance its viability, all of which alleviates the vulnerability of HK-2 cells to high glucose hypoxia-reoxygenation injury. This mechanism may occur by inhibiting TLR7/MyD88/NF-κB signaling to achieve renal protection.

Kidney vulnerability to ischemic injury has been reported in experimental models of diabetes and in clinical studies of diabetic patients, but the mechanism remains unclear[8]. This study showed that high glucose is a key factor in increasing the vulnerability of acute ischemia-reperfusion injury in the kidney. In vitro experiments showed that high glucose reduces cell activity, aggravates cell apoptosis, and increases inflammatory factor release. The results reported in an in vitro model of diabetes confirm that diabetes increases the vulnerability to ischemic AKI and implicates a serious inflammatory response as a cause of this vulnerability. Specifically, we found that upregulation of TLR7 markedly enhances renal injury in diabetes after ischemic AKI. We, therefore, hypothesize that the abnormal expression of TLR7 is associated with the vulnerability of high glucose and hypoxia-reoxygenation in human renal tubular epithelial cells. Our experiments confirmed that the upregulation of TLR7 is an important factor of vulnerability to renal injury.

Recent studies have shown increased expression of certain inflammatory cytokines such as TNF-α, IL-6, CCL2/MCP-1, CXCL1, and CXCl10/IP-10 in diabetic kidneys[9-12]. Renal ischemia in AKI animals results in upregulation of several inflammatory cytokines and chemokines, and some leukocytes infiltrate the kidneys, including dendritic cells, natural killer T cells, T and B lymphocytes, neutrophils, and macrophages[13]. Deletion or inhibition of certain cytokines or leukocyte subtypes may be one of the causes of diabetic renal insufficiency or ischemic AKI. Therefore, the highly reactive inflammatory response of diabetes is one of the causes of increased renal vulnerability to diabetes. The reason for this vulnerability requires further investigation. Our experiments showed that the levels of inflammatory factors IL-6 and TNF-α in the diabetic renal ischemia-reperfusion group were much higher than in the nondiabetic ischemia/reperfusion group. We speculate that high glucose-induced inflammatory factor release is one cause of diabetic renal injury vulnerability.

In this study, we used HK-2 cells to simulate a diabetic renal ischemia-reperfusion injury model with high glucose for 72 hours, hypoxia for four hours, and reoxygenation for two hours. The data obtained in this study indicate that downregulation of TLR7 attenuates acute renal injury induced by hypoxia-reoxygenation in HK-2 cells, which is characterized by increased cell activity, decreased apoptosis, and decreased release of inflammatory factors. Our study suggests that hyperactivation of TLR7 may exacerbate existing kidney damage. Inhibition of TLR7 expression reduces renal damage. TLR7 protein, MyD88 protein, and NF-κB protein were detected by western blotting. Studies have shown that high glucose stimulation and hypoxia-reoxygenation can significantly stimulate TLR7 MyD88 and NF-κB protein expression. TLR7-siRNA transfected cells and chloroquine pretreatment can downregulate TLR7, MyD88, and NF-κB protein expression under high glucose and hypoxia-reoxygenation conditions, reduce apoptosis, and increase cell viability. We speculate that TLR7 activates immune cells by relying on the signaling pathway of MyD88 to produce a cascade inflammatory response, releasing a large number of inflammatory factors. The release of these inflammatory factors further aggravates kidney damage.

In summary, the upregulation of TLR7 in the kidney might account for the enhanced inflammatory response and vulnerability of the diabetic kidney to ischemia. Our results may be helpful to find new targets for the treatment of diabetic ischemic AKI and provides a theoretical basis and new clinical strategies for reducing the incidence of perioperative diabetic renal ischemic AKI.

Acknowledgments

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Conflict of interests

The authors declare that they have no conflict of interests.

RESUMO

OBJETIVO: *O diabetes é um fator de risco para a lesão renal aguda (LRA). No entanto, seu mecanismo de patogênese não foi elucidado. O objetivo do estudo foi investigar o papel da inflamação e do receptor Toll-like 7 (TLR7) na LRA isquêmica no diabetes.*

MÉTODOS: *Um modelo de hipóxia-reoxigenação de células epiteliais tubulares renais humanas (HK-2) na presença de concentrações altas de glicose foi utilizado para gerar LRA induzida por isquemia-reperfusão em diabetes. A atividade das células foi medida pelo ensaio Cell Counting Kit-8 (CCK-8) e pela atividade da lactato desidrogenase (LDH). As citocinas inflamatórias foram avaliadas por ensaio imunoenzimático (Elisa). A expressão de TLR7, do fator de diferenciação mieloide 88 (MyD88) e do fator de transcrição nuclear-κB (NF-κB) foi examinada por Western blotting. A apoptose foi avaliada por citometria de fluxo.*

RESULTADOS: *Os grupos glicose alta e glicose baixa foram submetidos à hipóxia-reoxigenação. O grupo de baixa glicose desenvolveu apenas danos celulares ligeiros, apoptose e uma resposta inflamatória. Em contraste, no grupo de alta glicose, uma lesão equivalente de hipóxia-reoxigenação provocou danos celulares graves, apoptose e uma resposta inflamatória. A expressão de TLR7 e suas proteínas relacionadas foi medida no grupo de alta glicose antes e após a hipóxia-reoxigenação. O grupo de alta glicose exibiu maiores aumentos na expressão de TLR7 após hipóxia-reoxigenação do que o grupo de baixa glicose. Além disso, a expressão de TLR7 e suas proteínas relacionadas após a hipóxia-reoxigenação foi maior no grupo com alto nível de glicose do que no grupo com baixo nível de glicose. A inibição do TLR7 fornece proteção significativa contra a lesão isquêmica no diabetes.*

CONCLUSÃO: *Nossos resultados sugerem que o diabetes aumenta a vulnerabilidade à lesão renal induzida por isquemia. Essa vulnerabilidade acrescida tem por origem uma resposta inflamatória aumentada envolvendo a via de transdução de sinal do TLR7.*

PALAVRAS-CHAVE: *Vulnerabilidade em saúde. Isquemia. Lesão renal aguda. Diabetes mellitus. Receptor 7 Toll-Like.*

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ORIGINAL ARTICLE

Performance of cardiovascular risk scores in mortality prediction ten years after Acute Coronary Syndromes

Amanda Aparecida Petek[1]
Nara Aline Costa[1]
Filipe Welson Leal Pereira[1]
Ezequiel Aparecido dos Santos[1]
Katashi Okoshi[1]
Silmeia Garcia Zanati[1]
Paula Schmidt Azevedo[1]
Bertha Furlan Polegato[1]
Sergio Alberto Rupp de Paiva[1]
Leonardo Antônio Mamede Zornoff[1]
Marcos Ferreira Minicucci[1]

1. Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, Unesp – Univ Estadual Paulista, Botucatu, Brasil

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SUMMARY

BACKGROUND: *The objective of this study was to evaluate the performance of the Framingham risk score (FRS) and risk score by the American College of Cardiology/American Heart Association (SR ACC/AHA) in predicting mortality of patients ten years after acute coronary syndrome (ACS).*

METHODS: *This is a retrospective cohort study that included patients aged ≥ 18 years with ACS who were hospitalized at the Coronary Intensive Care Unit (ICU) of the Botucatu Medical School Hospital from January 2005 to December of 2006.*

RESULTS: *A total of 447 patients were evaluated. Of these, 118 were excluded because the mortality in 10 years was not obtained. Thus, 329 patients aged 62.9 ± 13.0 years were studied. Among them, 58.4% were men, and 44.4% died within ten years of hospitalization. The median FRS was 16 (14-18) %, and the ACC/AHA RS was 18.5 (9.1-31.6). Patients who died had higher values of both scores. However, when we classified patients at high cardiovascular risk, only the ACC/AHA RS was associated with mortality (p <0.001). In the logistic regression analysis, both scores were associated with mortality at ten years (p <0.001).*

CONCLUSIONS: *Both FRS and SR ACC/AHA were associated with mortality. However, for patients classified as high risk, only the ACC/AHA RS was associated with mortality within ten years.*

KEYWORDS: *Risk Assessment. Myocardial infarction. Angina, Unstable. Mortality.*

INTRODUCTION

The ischemic heart diseases are the most common causes of death worldwide.[1] Among them are the acute coronary syndromes (ACS), which can be divided into myocardial infarction with ST-seg-

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CORRESPONDING AUTHOR: Nara Aline Costa,
Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, Unesp. Rubião Júnior s/n, Botucatu, SP, Brasil CEP: 18618-970–
Phone: +55 (014) 38222969, Fax: +55 (014) 38222238
E-mail: na.costa@unesp.br

ment elevation and non-ST-segment elevation ACS. Despite the reduction tendency of mortality rates, ischemic heart diseases account for more than 1.8 million deaths every year, approximately 20% of all deaths in Europe.[1-4]

Thus, the identification of patients at risk of developing of coronary artery disease is crucial. The goal of the strategy of screening asymptomatic individuals is to have a more aggressive treatment and, consequently, a reduction of ischemic events and cardiovascular mortality. Among these scores are the Framingham risk score (FRS) and the score proposed by the American College of Cardiology and the American Heart Association (ACC/AHA RS).[5-7]

Over the last two decades, risk scores have gone through significant changes in order to concentrate on the overall absolute risk as an important key for the assessment of individual risk. In addition, more recent studies seek to improve the performance of the scores already used with the introduction of serum markers of inflammation, heart failure, and myocardial injury. Until the present time, however, risk scores have been developed and validated almost exclusively in developed countries.

In addition, the scores have been built, in their majority, in populations with apparently no cardiovascular disease, so their conclusions relate to primary prevention and do not apply to patients with diagnosed coronary disease. For patients post ACS, some instruments of risk stratification have been developed, such as the Grace, Pursuit, and Timi scores.[8-10] However, these scores assess intra-hospital mortality or, at most, the mortality after six months of an acute coronary event. Thus, instruments that assess the long-term mortality, such as the Framingham RS and the ACC/AHA RS, have still not been validated for patients with a prior coronary event.

In view of these aspects, the objective of this study was to evaluate the performance of the Framingham RS and the ACC/AHA RS in predicting mortality in patients ten years after an ACS.

METHODS

The present study was approved by the Ethics Committee of our institution, under the protocol (61090116.1.0000.5411). The Informed Consent Form (ICF) was waived in cases which death was identified in the medical or administrative records of the hospital.

The present work is an observational retrospective cohort study that included patients aged ≥18 years, with ACS, who were admitted to the Coronary Intensive Care Unit (ICU) of the Hospital das Clínicas de Botucatu - UNESP, in the period from January 2005 to December 2006, and who had the exams for the calculation of cardiovascular risk scores. We excluded patients whose final outcome after ten years was not obtained or those who refused to participate in the study.

For the calculation of the sample size, we used the Fisher and Belle formula, with the following variables: mortality ten years after AMI of 30% to 40%, confidence interval of 95%, and a sampling error of 5%. The result was a minimum of 323 patients.

The clinical and demographic data and laboratory examinations were collected upon admission to the Coronary Care Unit. The systolic blood pressure, blood glucose, lipid profile used were those of the first measurement taken at the time of admission. The FRS and the ACC/AHA RS were calculated and the overall mortality after ten years of ACS was recorded.[5-7]

In relation to acute coronary syndromes, they were classified as AMI with ST-segment elevation and non-ST-segment elevation ACS (AMI with no ST elevation and unstable angina). The diagnosis of the ACS was performed according to the guidelines by the American Heart Association.[11,12]

Systemic arterial hypertension (SAH) and the presence of diabetes mellitus (DM) were defined according to previous studies[13,14]. Smoking was regarded as the current use of tobacco.

Laboratory evaluation

For the measurement of sodium, potassium, urea, creatinine, CT, HDL, creatine phosphokinase (CPK) and creatine phosphokinase-MB (CKMB), we used the dry chemical method (Ortho-Clinical Diagnostics Vitros 950®, Johnson & Johnson). CPK and CKMB were dosed every six hours after admission and the highest value was recorded.

Framingham risk score (FRS)

We calculated the Framingham risk score. Patients were considered at high cardiovascular risk when they presented a risk of death greater than 20%. For the calculation of risk, patients younger than 20 years old or older than 80 were classified into the youngest and oldest categories of age, respectively.[5,6]

Risk score proposed by the American College of Cardiology and the American Heart Association (ACC/AHA)

We calculated the risk score proposed by the ACC/AHA. Patients were considered at high risk for atherosclerotic cardiovascular disease when they had a percentage greater than or equal to 7.5%. For the calculation of risk, patients younger than 40 years old or older than 80 were classified into the youngest and oldest categories of age, respectively.[7]

STATISTICAL ANALYSIS

The data were presented as mean and standard deviation or median and percentiles of 25% and 75%. The categorical variables were analyzed using the χ^2 test or Fisher's exact test. Continuous variables were analyzed by Student's t-test when they presented normal distribution and by the Mann-Whitney test for non-normal distribution. For the evaluation of score performance in relation to mortality, we used the ROC curve. We also used uni- and multivariate logistic regression analyses, adjusted for the variables, which were different in the univariate analysis, except for variables that were already included in the scores and those with multicollinearity. The level of significance was 5%.

RESULTS

We evaluated 447 patients with ACS admitted to the coronary ICU. Of these, 118 were excluded since their mortality ten years after admission to the ICU was not obtained. Thus, 329 patients with an average age of 62.9±13.0 years were studied. Out of that total, 58.4% were men, and 44.4% died within ten years after hospitalization. Of these, 63% presented non-ST-segment elevation ACS, while 37% had AMI with ST-segment elevation. After comparing these two groups of ACS, mortality was similar (p=0.97).

The median of the FRS was 16 (14-18%), with 17% of the patients classified at high cardiovascular risk. In relation to the ACC/AHA RS, the median was 18.5 (9.1-31.6%), with 79% of the patients classified at high cardiovascular risk, according to this score.

The demographic, clinical, and laboratory data of the patients evaluated and their relationship with ten-year mortality are presented in Table 1. The patients who died were older and had higher values of creatinine, urea, and potassium, and lower values of total cholesterol upon admission to the coronary ICU.

TABLE 1. DEMOGRAPHIC AND CLINICAL DATA AND LABORATORY CHARACTERISTICS OF 329 PATIENTS WITH ACUTE CORONARY SYNDROME.

Variables	Death within 10 years		P-value
	No (n=183)	Yes (n=146)	
Age, years	57.9±11.4	69.2±12.1	<0.001
Male, No. (%)	108 (59.0)	84 (57.5)	0.87
SAH, No. (%)	138 (75.4)	116 (79.5)	0.46
DM, No. (%)	64 (35.0)	56 (38.4)	0.60
Smoking, No. (%)	73 (39.9)	63 (43.2)	0.63
SBP, mmHg	130 (120-150)	130 (115-150)	0.99
TC, mg/dL	181 (154-218)	169 (143-208)	0.04
HDL, mg/dL	36 (30-46)	36 (30-47)	0.96
CPK, U/L	207 (93-1075)	222 (87-984)	0.72
CKMB, U/L	23 (11-135)	36 (14-115)	0.14
Creatinine, mg/dL	0.9 (0.8-1.11)	1.2 (0.9-1.5)	<0.001
Ureia, mg/dL	34.0 (26.5-41.8)	43.0 (34.0-55.0)	<0.001
Sodium, mmol/L	138 (137-141)	139 (136-141)	0.93
Potassium, mmol/L	4.1 (3.8-4.5)	4.3 (3.9-4.7)	0.02

SAH: systemic arterial hypertension; DM: diabetes mellitus; SBP: systolic blood pressure; TC: total cholesterol; HDL: HDL-cholesterol; CPK: creatine phosphokinase; CKMB: creatine phosphokinase-MB. Data in percentages and median (25% and 75% percentiles).

TABLE 2. CARDIOVASCULAR RISK SCORES OF 329 PATIENTS WITH ACUTE CORONARY SYNDROME.

Variables	Death within 10 years		P-value
	No (n=183)	Yes (146)	
FRS, %	15 (13-17)	17 (14-18)	<0.001
FRS > 20%	26 (14.2)	30 (20.5)	0.17
ACC/AHA RS, %	14.1 (6.2-26.1)	23.7 (15.0-40.1)	<0.001
ACC/AHA RS ≥ 7,5%	125 (68.3)	136 (93.2)	<0.001

FRS: Framingham risk score; AHA RS: risk score proposed by the ACC/AHA. Data in percentages and median (25% and 75% percentiles).

Furthermore, we found that the patients who progressed to death had higher scores, both on the FRS and the ACC/AHA RS. However, when we classified only patients with high cardiovascular risk, only the ACC/AHA RS was associated with mortality (Table 2). The area under the curve (AUC) of the association between the FRS and ten-year mortality in patients who had ACS was 0.6307, with 95% CI 0.5708-0.6905 and p <0.001. The cut-off point of the score that is associated with the highest mortality is over 14.3%. With the cut-off point of 14.3%, sensitivity was 74.66%, specificity 42.62%, positive predictive 50.93%, and negative predictive value 67.83% (Figure 1).

The area under the curve (AUC) of the association between the ACC/AHA RS and ten-year mortality

in patients who had ACS was 0.7015, with 95% CI 0.6455-0.7576 and p <0.001. The cut-off point of the score that is associated with the highest mortality is over 23.56%. With the cut-off point of 23.56%, sensitivity was 50.00%, specificity 71.04%, positive predictive 57.94%, and negative predictive value 64.04% (Figure 1).

There was an association between the FRS and the overall mortality after ten years in the univariate logistic regression (OR: 1.115; IC95%: 1.052-1.181; p<0.001) and in the multivariate analysis adjusted for creatinine and potassium (OR: 1.127; IC95%: 1.056-1.204, p<0.001). There was also an association between the ACC/AHA RS and the overall mortality after ten years in the univariate logistic regression (OR: 1.047; IC95%: 1.030-1.064; p<0.001) and in the multivariate analysis adjusted for creatinine and potassium (OR: 1.043; IC95%: 1.025-1.060, p<0.001).

DISCUSSION

The objective of this study was to evaluate the performance of two known risk scores for cardiovascular events in predicting mortality during the period of ten years in post-ACS patients. Our results show that both the FRS and the ACC/AHA RS were associated with mortality after ten years, with better performance by the ACC/AHA RS. In addition, for patients classified at high risk, only the ACC/AHA RS was associated with mortality after ten years.

The first aspect to be considered refers to the fact that, even though hospital mortality is relatively low in ACS, the same concept does not apply for longer periods of observation. Thus, in a period between one and ten years, patients with ACS present a mortality rate of around 10% and 45%, respectively.[1,15] In our study, the mortality after ten years of follow-up was 44%. Our data are in line with those found in the literature, but they also show that, despite the advances introduced in the treatment of these patients, the long-term prognosis remains poor.

It is also important to note that there was no difference in mortality after ten years among the patients who had ACS with and without ST-segment elevation, and the same is true n in relation to the CPK and CKMB markers of myocardial injury. This fact is in agreement with studies that show that, in the long term, there is no difference in mortality between patients who had ACS with or without ST-segment elevation.[1-3]

Another relevant aspect to be observed is that considering that approximately half of the patients with ACS will die in a period of ten years, the risk stratification of these patients becomes a priority. In this sense, the identification of patients at higher risk of unfavorable outcomes after the acute episode would

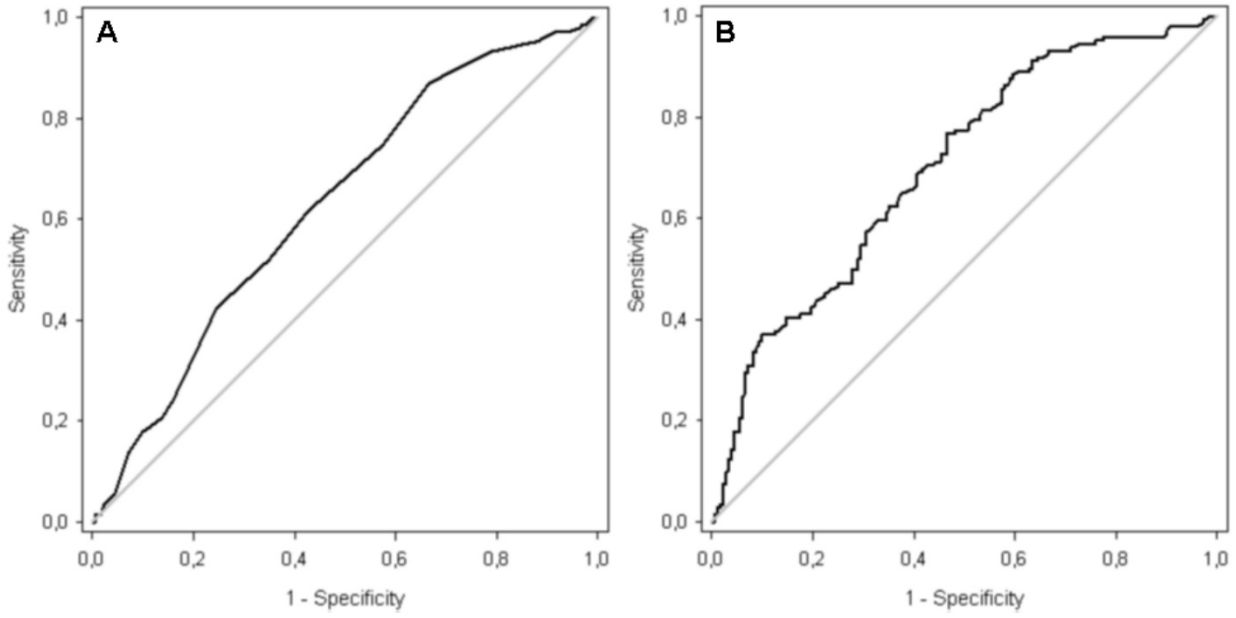

FIGURE 1 A. ROC CURVE OF THE ASSOCIATION BETWEEN THE FRAMINGHAM RISK SCORE AND TEN-YEAR MORTALITY AFTER ACUTE CORONARY SYNDROME. **B.** ROC CURVE OF THE ASSOCIATION BETWEEN THE RISK SCORE PROPOSED BY THE ACC/AHA AND TEN-YEAR MORTALITY AFTER ACUTE CORONARY SYNDROME

allow for an individualized and more aggressive approach in these cases, improving the prognosis.

Taking into account the factors associated with ten-year outcomes, in our study, in addition to advanced age, higher values of the markers of renal function and potassium were also associated with greater mortality. This result is important because creatinine and potassium are not considered in most risk scores for coronary patients.[16-18] In addition, there is an association between the decrease in renal function, associated or not to hyperkalemia, with increased risk of cardiovascular events.[19,20]

The Framingham and ACC/AHA scores are well-established and used for risk stratification of asymptomatic individuals.[21-25] It is important to emphasize that our study was the first to assess and compare these scores in a population that has already presented coronary events. Furthermore, the use of these scores, even in a population that is mostly treated from a perspective of controlling risk factors and comorbidities, allows for the identification of patients who would benefit from more aggressive treatment. Another interesting point is that these scores are quick to be calculated and use laboratory examinations that are cheap and already part of the routine evaluation of patients with ACS.

In our study, both the FRS and the ACC/AHA RS were associated with mortality in ten years. Additionally, the ACC/AHA RS had a better performance, despite discreet. Another important finding was that for patients classified at high risk, only the ACC/AHA RS was associated with mortality after ten years. Our results as a whole suggest that, specifically for patients with ACS, the ACC/AHA RS may be a better tool than the FRS for risk stratification in this scenario.

Finally, our study must be interpreted considering some limitations. This is a retrospective study, which included patients from a single center. Furthermore, the number of patients without information related to mortality after ten years was high. However, despite these restrictions, we believe that our data provide important information about the use of scores for risk stratification, in the long term, for patients with ACS.

CONCLUSION

Our data indicate that both the FRS and the ACC/AHA RS were associated with mortality after ten years, with better performance by the ACC/AHA RS. In addition, for patients classified at high risk, only the ACC/AHA RS was associated with mortality after ten years.

RESUMO

OBJETIVO: *Avaliar a performance do escore de risco de Framingham (ERF) e do escore proposto pela American College of Cardiology/American Heart Association (ER ACC/AHA) em predizer a mortalidade em pacientes dez anos após síndrome coronariana aguda (SCA).*

MÉTODOS: *Trata-se de um estudo de coorte retrospectivo que incluiu pacientes com idade ≥18 anos, com SCA, que estiveram internados na Unidade de Terapia Intensiva Coronariana (UTI) do Hospital das Clínicas de Botucatu, no período de janeiro de 2005 a dezembro de 2006.*

RESULTADOS: *Foram avaliados 447 pacientes. Destes, 118 foram excluídos, pois a mortalidade em dez anos não foi obtida. Logo, 329 pacientes com idade de 62,9±13,0 anos foram estudados. Dentre eles, 58,4% eram homens e 44,4% morreram no período de dez anos após a internação. A mediana do ERF foi de 16 (14-18)%, e do ER ACC/AHA foi 18,5 (9,1-31,6)%. Os pacientes que evoluíram a óbito apresentaram maiores valores dos escores. No entanto, quando classificamos os pacientes em alto risco cardiovascular, apenas o ER ACC/AHA foi associado com a mortalidade (p<0,001). Na análise de regressão logística, ambos os escores foram associados com a mortalidade em dez anos (p<0,001).*

CONCLUSÕES: *Tanto o ERF quanto o ER ACC/AHA foram associados com a mortalidade. No entanto, para os pacientes classificados como alto risco, apenas o ER ACC/AHA foi associado com a mortalidade em dez anos.*

PALAVRAS-CHAVE: *Medição de risco. Infarto do miocárdio. Angina instável. Mortalidade.*

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ORIGINAL ARTICLE

Radiofrequency catheter ablation increases mean platelet volume

Fatih Aksoy[1]

1. Department of Cardiology, Suleyman Demirel University, Isparta, Turkey

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SUMMARY

OBJECTIVE: *Radiofrequency ablation (RFA) may increase the risk of thromboembolic events. The objective of this study was to evaluate the effect of RFA on mean platelet volume (MPV), an indicator of platelet activity.*

METHODS: *A total of 95 patients undergoing RFA were included in the study. MPV was measured before and one month after the procedure. The control group was formed by 83 individuals of the same sex and age as those in the study group.*

RESULTS: *Beta-blockers, non-dihydropyridine calcium channel blockers, and acetylsalicylic acid use was higher in the ablation group compared with the control group. Other baseline clinical characteristics and baseline hemoglobin, white blood cell count, platelet count, and MPV values were similar between the ablation and control groups. In the ablation group, baseline and post-procedural hemoglobin, white blood cell counts were similar. However, postprocedural MPV values were higher, and platelet counts were lower compared with the preprocedural values.*

CONCLUSION: *Our results indicate that MPV values are higher after RFA compared with baseline values.*

KEYWORDS: *Mean platelet volume. Catheter Ablation. Thromboembolism.*

INTRODUCTION

Platelet activation is involved in the pathogenesis of many thromboembolic diseases and has been shown to be associated with cardiovascular risk factors such as diabetes mellitus or hypertension and increased risk of cardiovascular events. [1-6]

Several methods, including platelet counts and size, aggregation and the levels of substances released from platelets have been described for determining platelet activation. However, most of them require complicated equipment, with high costs and time-consuming analysis methods, which prevents them from being used routinely in daily clinical practice. Mean platelet volume (MPV), has been introduced as an indicator of in vivo platelet activation. It is measured in the routine complete blood count test with a very simple method that can be performed at a low cost[1]. Increased MPV indicates larger platelets, which are more likely to be more reactive, increased

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CORRESPONDING AUTHOR: Fatih Aksoy
Suleyman Demirel Unşversity – Faculty of Medicine, Cardiology Department
Isparta, Turkey – 32040
E-mail: dr.aksoy@hotmail.com

aggregability, and decreased inhibition of aggregation.[4,5] Increased MPV may also be associated with shortened bleeding time, increased thromboxane A2 and B2 concentrations, and higher expression of adhesion molecules.[6] It has been shown that MPV is increased with cardiovascular risk factors, in cardiovascular, cerebrovascular or hematological diseases and in low-grade inflammatory diseases prone to arterial and venous thrombosis. On the other hand, high-grade inflammatory conditions such as rheumatic arthritis or Familial Mediterranean Fever attacks are associated with low values of MPV, which increase with anti-inflammatory treatment. [1,3,7] Radiofrequency ablation (RFA) is a very effective method for treating a wide variety of cardiac arrhythmias. However, this procedure has the potential to cause thromboembolism.[8] To the best of our knowledge, no study has evaluated the effect of RFA on MPV. Therefore, the aim of this study was to assess the effect of RFA on MPV.

METHODS

Population

This study included 95 patients undergoing RFA and 83 apparently healthy individuals of the same age and sex as those in the study group. Patients with renal or hepatic failure, thrombocytopenia, thrombocytosis, active infection, malignancy, venous thrombosis, pulmonary embolism, congenital hemorrhagic disease, inflammatory disease, autoimmune disease, recent surgery, on oral contraceptives were excluded. Between May 2009 and May 2012, 105 patients undergoing RFA were screened, and 10 patients were excluded based on the exclusion criteria (diabetes mellitus, n = 3; heart failure, n = 2; hypertension, n = 4 and increased creatinine levels, n = 1). Therefore, 95 patients were included in the RFA group (atrioventricular nodal reentrant tachycardia, n = 44; orthodromic reentrant tachycardia, n = 14; antidromic reentrant tachycardia, n = 20; idiopathic ventricular tachycardia, n = 12; atrial tachycardia, n = 4; and atrial flutter, n = 1). There were 21 left-sided procedures (antidromic reentrant tachycardia, n = 8 and orthodromic reentrant tachycardia, n = 13) and the remaining 75 cases were right-sided procedures. The control group was chosen from the subjects who attended our cardiology clinics between 2009 and 2012 and were found to be healthy.

Electrophysiological study and RF ablation procedures

The procedures were performed in the fasting state without sedation. All the antiarrhythmics were discontinued for at least 5 half-lives before the procedure. Three sheaths were positioned into the femoral veins, and one sheath was positioned into the femoral artery that required arterial approach. Diagnostic 6 Fr catheters were positioned into the high right atrial, and its bundle locations (Medtronic, USA) and 6 Fr coronary sinus catheter were introduced into the coronary sinus (Bard, USA). An electrophysiological study with standard stimulation techniques was performed in each patient, and RFA was applied with the use of 4-mm tip 7 Fr deflectable catheter (Medtronic, USA). The pacing was performed with a stimulator at twice the diastolic threshold and with a pulse width of 2 ms (EP Tracer, Cardiotek, Netherlands). Intravenous heparin was used in the left-sided procedures with a target ACT level between 250-300 sec. Antiplatelet therapies with 100 mg acetylsalicylic acid or clopidogrel in patients unable to receive acetylsalicylic acid were given for three months after the procedure. All the patients underwent transthoracic echocardiography before and one day after the procedure. The local Research Ethics Committee approved the study, and all the patients gave written informed consent.

Hematologic measurements

Hematologic measurements were performed before and one month after the procedure. Blood samples were drawn from the antecubital vein by careful venipuncture using a 21 G sterile syringe without stasis in the morning. MPV was measured in a blood sample collected in dipotassium EDTA-tubes. An automatic blood counter (Beckman Coulter LH 780) was used for the analyses. MPV was measured within 30 min after sampling to prevent EDTA-induced platelet swelling because it has been shown that platelets exhibit time-dependent swelling when blood samples are anticoagulated with EDTA and the measurement is not performed within 1 hour of sampling.[9]

Authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content. The procedures were in accordance with the ethical standards of the institution's committee on human experimentation and the Helsinki Declaration of 1975, revised in 1983.

Statistical analysis

Continuous variables were expressed as mean ± SD, and categorical variables were expressed as frequency (%). The changes in the laboratory findings before and after RFA were compared using a paired Student t-test. Continuous variables were compared by independent Student t-test. Categorical variables were compared with the chi-square test. It was assumed that baseline MVP values would be as a mean of 6.0 fl and would increase to 9.0 fl after the procedure. With this assumption, 97 RFA patients were required to detect a significance with an alpha level of 0.05, and a power of 0.80. A two-sided p-value < 0.05 was considered significant. SPSS 11.0 version (Chicago, IL, USA) was used for analysis.

RESULTS

Patients

The rates of the baseline use of beta-blockers, non-dihydropyridine calcium channel blockers, and acetylsalicylic acid were higher in the ablation group compared with the control group (All p values < 0.05). Other baseline clinical characteristics were similar between the ablation and control groups (All p values > 0.05; Table 1).

Laboratory parameters: Ablation group vs. control group

Baseline hemoglobin, white blood cell count, platelet count, and MPV values were similar between the ablation and control groups (All p values > 0.05). In the ablation group, pre-procedural and post-procedural hemoglobin and white blood cell count values were similar (Both p values > 0.05). However, post-procedural MPV values were significantly higher, and platelet counts were lower compared with pre-procedural values (Both p < 0.05 values; Table 2). No thromboembolic events occurred in any group.

Laboratory parameters: Right-sided vs. left-sided ablation groups

Pre-procedural and post-procedural hemoglobin and white blood cell count values were similar in both right-sided and left-sided ablation groups (All p values > 0.05). However, post-procedural MPV values were significantly higher, and platelet counts were

TABLE 1. BASELINE CHARACTERISTICS

Variables	Ablation group (n=95)	Control group (n=83)	P value
Age (years)	47 ± 16	48 ± 8	0.54
Male gender n, %	46 (48.4)	36(43.4)	0.42
Body mass index (kg/m2)	27 ± 3.8	26 ± 4	0.42
Ejection fraction (%)	63 ± 8	63 ± 2	0.92
Heart rate (beats/min)	79 ± 20	77 ± 8	0.34
Systolic blood pressure (mmhg)	129 ± 10	132 ± 6	0.15
Diastolic blood pressure (mmhg)	81 ± 6	79 ± 3	0.19
Glucose (mg/dl)	102 ± 24	97 ± 10	0.12
Creatinine (mg/dl)	0.9 ± 0.19	0.86 ± 0.2	0.07
LDL cholesterol (mg/dl)	103 ± 30	105 ± 28	0.72
HDL cholesterol (mg/dl)	47 ± 13	47 ±11	0.90
Smoking n, %	13 (13.7)	12 (14.5)	1
Pre-procedural treatment			
Amiodarone n, %	6 (6.3)	-	-
Propafenone n, %	3 (3.2)	-	-
Beta-blockers n, %	25 (26.3)	4 (4.8)	<0.0001
Non-dihidropiridine calcium channel blocker n, %	14 (14.7)	4 (4.8)	0.04
Acetylsalicylic acid n, %	43 (45.3)	12 (14.5)	<0.0001
Clopidogrel n, %	2 (2.1)	-	-
Statin n, %	8 (8.4)	-	-
ACEI/ARB n, %	13 (13.7)	6 (7.2)	0.6

Abbreviations: LDL= low density cholesterol; HDL= high density cholesterol; ACEI= angiotensin-converting enzyme inhibitor; ARB,=angiotensin receptor antagonist. Data were given as mean ± SD or n (%).

TABLE 2. COMPARISON OF HEMOGRAM PARAMETERS BETWEEN THE ABLATION AND CONTROL GROUPS.

Laboratory findings	Ablation group (n= 95)		Control group (N=83)	P values	
	Pre-RFA	Post-RFA		Pre-RFA vs. Control	Pre-RFA vs. Post-RFA
White blood cell counts	8280 ± 2298	7922 ± 2110	7933 ± 2148	0.31	0.2
Hemoglobin (g/dl)	14 ± 1.8	14 ± 1.9	14 ± 1.4	0.25	0.9
Platelet count (x 10^3)	305 ± 82	262 ± 67	288 ± 67	0.15	<0.0001
MPV (fl)	7.98 ± 0.75	8.88 ± 1.06	7.87 ± 0.81	0.35	<0.0001

Abbreviations: RFA, radiofrequency ablation; MPV, mean platelet volume. Data presented as mean ± SD.

lower compared with the pre-procedural values in both right-sided and left-sided ablation groups ($p < 0.05$; Table 3).

Relationship between MPV and ablation parameters

There was no correlation between MPV and temperature ($r = -0.04$, $p = 0.69$), energy ($r = -08$, $p = 0.46$), or ablation application duration ($r = 0.19$, $p = 0.1$).

DISCUSSION

The main finding of the present study is that MPV, an indicator of platelet activation, is higher after RFA when compared with the baseline. This was found after both the right-sided and leftsided ablation procedures. In the present study, no thromboembolic events occurred; however, we speculate that increased MPV might indicate increased platelet activity, which has been shown to be associated with thromboembolic events.

In the literature, it has been indicated that RFA may be associated with thromboembolism. Zhou et al.[8] have reported in their review that the overall incidence of thromboembolic complications of RFA is 0.6% and that it is increased to 1.8% to 2.0% in left heart procedures and to 2.8% when the procedure is performed for ventricular tachycardia. Haman et al.[10] have analyzed data from 400 patients who underwent supraventricular tachycardia ablations and have found thromboembolic events at a rate of 1.75%. Thakur et al.[11] and Epstein et al.[12] have also reported the incidence of thromboembolic events at a rate of ~2%. In a dog model, Khairy et al.[13] showed that radiofrequency energy caused a higher incidence of thrombus formation and larger thrombus volumes than cryoablation. In 232 patients undergoing atrial fibrillation ablation, periprocedural symptomatic cerebrovascular events occurred at a rate of 0.4%, and diffusion magnetic resonance imaging showed embolic events in 14% of the patients. [14]In a large study, transthoracic echocardiography showed ~0.1% of thrombotic complications. [15]

Thromboembolism most commonly occurs after left-sided RFA procedures (particularly when performed for the treatment of atrial fibrillation). In the present study, no atrial fibrillation ablation procedures were included. However, our results indicate that MPV increased after both the left-sided and right-sided ablation procedures, probably indicating that platelet activation is increased after both procedures. Hyperthermia induced by RFA energy may cause endothelial injury, protein denaturation, tissue necrosis, platelet adhesion and activation, and thus, thrombus formation.[8,10,13] In the literature, neurological[11,12,16-18], pulmonary[18,19], and arterial[18-20] thromboembolic events have been reported after this procedure. Radiofrequency ablation probably causes thromboembolism in patients who have a prothrombotic status, such as factor V Leiden mutation, protein C and S deficiencies, among others. However, we have not searched for those conditions in the present study.

No correlations were found between thromboembolic complications and duration of the procedure and number of the RF applications in a previous study.[21] Similarly, although we did not detect any thromboembolic events, we did not find any correlation between RF parameters and MPV. In the daily clinical practice, heparin is used in left-sided RFA procedures, and acetylsalicylic acid is given for at least one month after all the RFA procedures.

It has been shown that D-dimer levels are lower with the combination of acetylsalicylic acid and ticlopidine after RFA.[22] Also, catheter coagulum was found to be lower with temperature-controlled procedures compared with power-controlled procedures.[23] We used heparin in the left-sided procedures

TABLE 3. COMPARISON OF HEMOGRAM PARAMETERS BETWEEN THE RIGHT-SIDED AND LEFT-SIDED- ABLATION GROUPS.

Laboratory findings	Right-sided ablation group (n = 74)		P-values	Left-sided ablation group (n = 21)		P values
	Pre-RFA	Post-RFA		Pre-RFA	Post-RFA	
White blood cell counts	8169 ± 2141	7886 ± 1976	0.7	8600 ± 2905	7994 ± 2583	0.25
Hemoglobin (g/dl)	13 ± 1.8	13 ± 1.9	0.63	14 ± 1.7	14 ± 2	0.77
Platelet count (x 103)	298 ± 81	261± 68	<0.0001	332 ± 86	267 ± 69	0.004
MPV (fl)	8.05 ± 0.79	8.98± 1.04	<0.0001	7.76 ± 0.61	8.75 ± 1.0	0.002

Abbreviations: RFA, radiofrequency ablation; MPV, mean platelet volume. Data presented as mean ± SD.

and acetylsalicylic acid at 100 mg/d for three months after all the procedures. On the other hand, it has also been reported that the use of heparin and temperature control do not completely prevent thromboembolic events.[8,13] In addition, recent evidence shows that acetylsalicylic acid does not affect MPV.[7]

Our results may indicate that anticoagulant or more aggressive antiplatelet therapy may be required during RFA since MPV, an indicator of platelet activity, is increased after this procedure.

Limitations

The central problem was that we did not investigate the risk factors for thrombosis, such as factor V Leiden mutation, and protein C and S deficiencies. Although the present study indicates increased MPV values after RFA, no thromboembolic events have been detected. This might be due to the small sample size of the study. MPV alone may not correctly reflect platelet functions. The use of other assessment methods for platelet function would be more reliable; however, they are time- consuming, costly, and require complex equipment. In addition, there are many studies in the literature that indicate the reliability of MPV; therefore, we used just MPV. No screening methods were used to detect thromboembolism, and the patients were just followed clinically. We did not include patients with diabetes mellitus, hypertension, heart failure, left ventricular systolic dysfunction, acute coronary syndrome, and moderate to severe heart valve disease. The duration of the recruitment was too long (Between 2009 and 2012). The expanded exclusion criteria and long duration of the study might represent a selection bias and influence the validity of the results. We did not measure inflammatory markers. Finally, control-group subjects were not tested for MPV at the post-ablation period.

CONCLUSIONS

Our results indicate that MPV is increased after RFA compared with the baseline. This increase occurred after both right-sided and left-sided ablation procedures. Larger studies are required to show the reliability of MPV as an indicator of increased platelet activity after RFA.

RESUMO

OBJETIVOS: *A ablação por radiofrequência (ARF) pode aumentar o risco de eventos tromboembólicos. O objetivo foi avaliar o efeito da ARF no volume plaquetário médio (VPM), um indicador de atividade plaquetária.*

MÉTODO: *No total de 95 pacientes submetidos à ARF, o VPM foi medido antes e um mês após o procedimento. Oitenta e três pessoas do mesmo sexo e faixa etária constituíram o grupo controle.*

RESULTADOS: *Betabloqueadores, bloqueadores dos canais de cálcio não diidropiridínicos e uso de ácido acetilsalicílico foram maiores no grupo ablação quando comparados ao grupo controle. Outras características clínicas basais e hemoglobina basal, contagem de leucócitos, contagem de plaquetas e valores de VPM foram semelhantes entre os grupos de ablação e controle. No grupo de ablação, linha de base e hemoglobina pós-procedimento, as contagens de glóbulos brancos foram semelhantes. No entanto, os valores de VPM pós-procedimento foram maiores e as contagens de plaquetas foram menores em comparação com os valores pré-procedimento.*

CONCLUSÕES: *Nossos resultados indicam que os valores de VPM são maiores após a ARF em comparação com os valores basais.*

PALAVRAS-CHAVE: *Volume plaquetário médio. Ablação por cateter. Tromboembolia.*

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ORIGINAL ARTICLE

Causes for hospitalization of elderly individuals due to primary care sensitive conditions and its associated contextual factors

Angela Maria de Medeiros Soares[1]
Tamires Carneiro de Oliveira Mendes[2]
Kenio Costa Lima[3]
Márcio Moreira de Menezes[4]

1. Doutora em saúde coletiva pelo programa de pós-graduação em saúde coletiva da Universidade Federal do Rio Grande do Norte (PPGSCol-UFRN) do departamento de odontologia, professora substituta do departamento de Saúde Coletiva da UFRN, Natal, RN, Brasil.
2. Doutora em saúde coletiva pelo programa de pós-graduação em saúde coletiva da Universidade Federal do Rio Grande do Norte (PPGSCol-UFRN) do departamento de odontologia, Natal, RN, Brasil.
3. Pós-doutor pela Agência de Saúde Pública de Barcelona, professor associado IV da Universidade Federal do Rio Grande do Norte (UFRN) e do programa de pós-graduação em saúde coletiva da UFRN (PPGSCol) do departamento de odontologia, Natal, RN, Brasil .
4. Médico Clínico-Geral pela Universidade Potiguar. Médico da Estratégia de Saúde da Família da Unidade Básica de Saúde de Cajazeiras-RN, Brasil.

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SUMMARY

OBJECTIVE: *The objective of this study was to analyze the hospitalizations of the elderly for conditions sensitive to primary care (ICSAP) and associated contextual factors, referring to elderly people aged 60 and over, living in municipalities in the Northeast region.*

METHODS: *Characterized as being an ecological study using data from the Hospital Information System (SIH) and the Basic Attention Information System (SIAB) referring to elderly people aged 60 and over.*

RESULTS: *The total hospitalization rate was 527,524, with the highest number due to heart failure, followed by cerebrovascular diseases, and infectious gastroenteritis. Analyzing the ICSAP rates with the contextual factors, all were significant. Regarding the coverage of basic care, a similarity occurred between them, and for the rate of the number of consultations among the elderly, despite the greater number of these in the municipalities with higher hospitalization rates, there was no significant difference between them.*

CONCLUSION: *We conclude that the contextual factors interfere in the conditions of this hospitalization, necessitating, besides the improvement of primary care, an improvement in the living conditions of the elderly population.*

KEYWORDS: *Aged. Hospitalization. Epidemiologic factors.*

INTRODUCTION

The increase in the elderly population worldwide is currently the subject of many discussions. In Brasil, this has also become relevant, since the country is going through an intense process of population aging.

This process brings many changes - psychological, physical, and emotional -, which increases the need for

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CORRESPONDING AUTHOR: Angela Maria de Medeiros Soares
Rua Dom José Tomaz, 1126, apartamento 1202. Tirol, Natal, RN 59022-250, Brasil
E-mail: angelammedeiros@gmail.com

attention to the health of elderly individuals, mainly due to the increase in the number of chronic-degenerative diseases. In this context, the health system needs to adapt to this inversion of the population pyramid, including changes in primary care, which is characterized by being the first line of patient treatment. This type of care is paramount in the process of health promotion, prevention of injuries, hospitalizations, and early deaths. Effective primary care is associated with a reduction of costs, user satisfaction, and improved health indicators.[1-4]

To evaluate and monitor the effectiveness of primary care, a list of Hospitalizations due to Primary Care Sensitive Conditions (PCSC), published in Decree No 221 of the Ministry of Health (MS), on 17 April 2008, was drawn up and validated in Brasil.[2] It comprises a number of diseases and injuries that can be prevented with timely and effective outpatient care, control of acute episodes, or management of chronic conditions and diseases. It lists a set of events that would hardly evolve to the point of requiring hospitalization if approached appropriately in outpatient care promotion, prevention, early treatment, and follow-up.[5,6] By studying ICSAP, it is possible to analyze the impact of primary care based on hospitalizations database records.[7]

Along with these factors, it is important to evaluate the social factors that affect the population health, since studies[5,7] have reported that, in addition to the improvement of basic care, it is necessary to assess the socio-economic conditions of the population so that along with the improved access to health there is also improved quality of life as a whole.

Thus, this study aims to study the degree of hospitalizations of elderly individuals in the Northeast region of Brasil, in the period from 2010 to 2015, evaluating the frequency of Hospitalizations due to Primary Care Sensitive Conditions (PCSC) and its possible relationships with contextual factors.

METHODS

This is a descriptive ecological study that included elderly individuals aged 60 years or older, residing in the northeast region of Brasil, in the years 2010 to 2015. The sample units are composed of municipalities in the region, which includes nine states and 1,794 municipalities.

We selected data of Hospitalizations due to Primary Care Sensitive Conditions (PCSC) in the municipalities, per location of residence, obtained from the Hospital Information System (SIH), which is based on Hospital Admission Authorizations (HAA type 1).

As a dependent variable, we evaluated the rate of ICSAP in the elderly. This was calculated by the ratio between the number of ICSAP in elderly individuals residents of the municipalities of the Northeast Region and the entire elderly population in these places in the year 2010, multiplied by 6, because it represents the period analyzed (2010-2015), for a cluster of 10 thousand inhabitants.[7]

In relation to the independent variables, the contextual socioeconomic variables, regarding the last census (2010), were collected from secondary sources: The Institute for Applied Economic Research (IPEA, 2015) and the Brasilian Institute of Geography and Statistics (IBGE, 2015), both in its raw form, as well as transformed into indicators by the United Nations Development Programme (UNDP, 2015). In addition to these variables, we collected data regarding the coverage of basic care in the municipalities and the number of consultations of elderly patients in primary care from the Siab, which used the rate of the number of consultations, consisting of the ratio between the total number of consultations in the years 2010-2015 and the total number of elderly people in the year 2010, multiplied by 6, for a conglomerate of 10,000.

Based on a theoretical and statistical analysis, we selected 11 contextual variables: mean per capita income (income); illiteracy rate in the population aged 18 years old or older (illit.); percentage of the population aged 6 to 14 years old in primary education with no delay (primary-e); degree of formalization of employment (form); rate of activity of individuals aged 25 to 29 years old (actv25-29); unemployment rate of the population 18 years old or older (unemp); percentage of people employed in the agricultural sector (agro); appropriate households (house); dependency ratio (dep_ratio); urbanization rate (urb_rate), and social welfare programs (soc-well).

Since this study is based on official secondary data in public domain, there was no need for approval by the Research Ethics Committee, which followed the guidelines of the Resolution of the National Health Council (CNS) No. 466, December 12, 2012.

Statistical analysis

Of the 1,794 municipalities in the Northeast, we carried out a K-means non-hierarchical clustering analysis, which produced clusters of the northeastern

municipalities, based on the chapters of the causes for Hospitalizations due to Primary Care Sensitive Conditions. As a result, three clusters were formed: 1, 2, and 3, with intermediate, low, and high rates of ICSAP, consecutively.

In relation to more than 800 contextual socioeconomic variables, the selection for the multivariate analysis was based on a theoretical analysis of the dimensions of the models of Social Determinants of Health, followed by descriptive analysis, in order to identify those that best differentiate the municipalities and with a distribution closer to normality. Then, we calculated the Spearman's correlation coefficient between all the variables to select those with the greatest potential to represent the others.

We chose to summarize the set of variables selected by R-type factorial analysis, in which these variables are grouped according to the latent dimensions in its group and interpreted based on what they represent collectively. We used a correlation matrix, the measurement of the adequacy of the sample, the Bartlett test, and anti-image matrix to evaluate the correlation between the variables and the applicability of the factor analysis. The extraction of the factors was done using Principal Component Analysis (PCA), whose number of components extracted was determined by the Kaiser criterion. To facilitate the interpretation of factors, the Varimax rotation method was used.

For the PCA, we included the contextual variables that, from their correlations, were reduced to three components representing the different dimensions related to the contextual level of the Social Determinants of Health. Once we confirmed the applicability of the factor analysis by observing the correlations matrix, with the determinant different from zero (0.006), the anti-image matrix, the Bartlett test ($p<0.001$) and the Kaiser-Meyer-Olkin index (KMO=0.86), we proceeded to the extraction of components using the 11 variables adequate to it. Based on the Kaiser criterion, we selected three factors that, together, explained 65.3% of the total variance of the variables included in the model.

Therefore, the contextual variables were reduced to three components of the different contextual dimensions of the Social Determinants of Health, component 1, called "Urbanization and Its Reflexes"; component 2, "Favorable Socioeconomic Context"; and 3, "Low Formal Education and Dependence on the State".

In addition to these contextual variables, we also analyzed the Social Vulnerability Index (SVI), the Human Development Index (HDI) and the Firjan Index of Municipal Development (IFDM) as a way to complement the analysis with the latent variables produced by the factors.

In the analysis of the hospitalization rate of the clusters, along with the contextual variables, we used the analysis of variance with the Bonferroni test for a significance level of 5%.

RESULTS

Evaluating the hospitalization of the elderly individuals due to ICSAP in the given period, the total number of ICSAP in the Northeast region was 1,727,043, with a higher number of cases due to cardiac insufficiency, followed by cerebrovascular diseases and, in third place, infectious gastroenteritis. In the analysis of ICSAP rates using a population of 32,455,176 elderly individuals in a period of six years, we found a total rate of 527,524 hospitalizations for every 10,000 elderly inhabitants - Table 1.

After forming the clusters, according to Figure 1, we observed that in the cluster with the lowest and highest rates of hospitalization, hospitalizations due to heart failure were the most frequent, followed by gastrointestinal diseases and, in third place, by cerebrovascular diseases, which differs from the cluster with intermediate rates, in which infectious gastroenteritis were more frequent, followed by hypertension and, in third place, diabetes mellitus.

After testing the difference between the rates per ICSAP group in elderly patients with the contextual factors, according to Table 2, a significant difference was found in both clusters and the contextual variables. Cluster 3, which has the highest rates of hospitalization, was the one with the best values for the Favorable Socioeconomic Context and higher values for Low Formal Education and Dependence on the State. Cluster 2, which has lower rates of hospitalization, had the best means for the "Urbanization and Its Reflexes". In relation to the coverage of basic care, there was a similarity between the clusters, and regarding the rate of the number of consultations of elderly individuals, we found that, despite the greater number of consultations in clusters with higher rates of hospital admission, there was no significant difference between them. The VSI was higher for the clusters with intermediate rates. Whereas the IFDM was greater in the clusters with lower rates of

TABLE 1. CAUSES OF HOSPITALIZATION OF ELDERLY INDIVIDUALS DUE TO PRIMARY CARE SENSITIVE CONDITIONS AND ITS RATES IN THE NORTHEAST REGION, 2010-2015.

CID-10	Total number of ICSAP (2010-2015)	ICSAP rate (2010-2015)
1 - Diseases preventable by immunization and sensitive conditions	10,680	3.26
2 - Infectious gastroenteritis and complications	241,289	73.705
3 - Anemia	12,189	3.723
4- Nutritional deficiencies	55,872	17.067
5 - Infections of the ear, nose and throat	5,525	1.688
6- Bacterial pneumonia	69,199	21.138
7- Asthma	67,589	20.646
8- Pulmonary diseases	105,072	32.096
9 - Hypertension	143,963	43.976
10 - Angina	71,618	21.877
11 – Heart failure	291,675	89.096
12 – Cerebrovascular diseases	245,564	75.011
13 – Diabetes mellitus	193,189	59.012
14 - Epilepsy	9,585	2.928
15 - Kidney and urinary tract infections	106,097	32.409
16 - Infection of the skin and subcutaneous tissue	65,521	20.014
17 - Inflammatory disease of female pelvic organs	1,895	0.579
18 - Gastrointestinal ulcers	30,435	9.297

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

TABLE 2. DIFFERENCE IN THE RATES OF HOSPITALIZATION OF ELDERLY INDIVIDUALS DUE TO PRIMARY CARE SENSITIVE CONDITIONS IN RELATION TO CONTEXTUAL VARIABLES, 2010-2015.

Variables	Groups per rates of causes of hospitalization of elderly individuals			p
	Intermediate rates of ICSAP (Cluster 1) Mean (standard deviation)	Lower rates of ICSAP (Cluster 2) Mean (standard deviation)	Higher rates of ICSAP (Cluster 3) Mean (standard deviation)	
Urbanization and Its Reflexes	–022 (0.77)a	0.06 (1.05)b	–0.04 (0.90)a b	<0.001
Favorable Socioeconomic Context	0.05 (0.95)a	0.00 (1.00)b	0.32 (0.90)c	<0.001
Low Formal Education and Dependence on the State	0.05 (0.88)a	-0.04 (1.00)a	0.29 (1.09)b	<0.001
Social Vulnerability Index (SVI)	0.49 (0.10)a	0.46 (0.04)b c	0.45 (0.04)a c	<0.001
Human Development Index (HDI)	0.58 (0.03)a	0.59 (0.10)b	0.52 (0.11)a b	0.009
Firjan Municipal Human Development Index (IFDM)	0.47 (0.10)a	0.52 (13.1)b	0.46 (13.1)a	<0.001
BC Coverage	93.9 (10.5)a	91.6 (13.1)b	91.2 (13.1)b c	0.012
Rate of elderly consultations BC	25850.0(98704.3)	28629.8(66962.5)	31504.7(146739.0)	0.774

*Different letters demonstrate the existence of significant differences between the clusters for a confidence level of 95%.

hospitalization, as was the HDI, but the means were not as significantly different for these indexes.

DISCUSSION

The results obtained showed that the rate of ICSAP was 527.524 per 10 thousand inhabitants, and the three main causes of ICSAP were congestive heart failure, cerebrovascular diseases, and infectious gastroenteritis. These causes together, considered sensitive to primary care, demonstrate that good attention at this level of care could prevent such events, and these findings may represent a warning to the services of primary health care, so they can identify flaws in the care system of the entire Northeast Region.

On this same premise, studies have reported that the rates of hospitalization due to PCSC serve as indicators that are being increasingly used in the assessment of this type of care, showing that primary care

services of better quality are associated with lower rates of hospitalization due to CSAP.[8,9]

The two greatest causes of ICSAP identified in this study corroborate other studies found in the literature and performed in Brasil; however, they evaluate not only the population of elderly individuals but also the population in general. In the evaluation of age groups over 60 years old, illnesses related to the circulatory system are among the main causes of ICSAP.[1-4,9-13]

The highest rate of hospitalization due to heart failure in the elderly population is confirmed by data published in Brasil, in which this represents the second most frequent cause of hospitalization due to CSAP.[1,2] Among the risk factors for heart failure are hypertension, smoking, obesity, sedentary lifestyle, and family history, factors that can be addressed in basic care through health promotion and disease prevention.

Regarding infectious gastroenteritis, the third greatest cause of hospital admissions, they are related to social factors, such as living conditions and adequate sanitation. Studies have reported that these are more often related to the children population, with diseases linked to public health problems in Brasil, with a strong social and economic impact.[10,12,13]

The clusters with the lowest rates of hospitalization were also those who had the highest mean for the "Urbanization and Its Reflexes" factor, followed by the cluster with the highest rates of hospitalization. In connection with the data found, findings have reported that the process of urbanization and the consequent demographic transition contribute to an increase in chronic non-communicable diseases,[14] because this process generates industrial development, unhealthy life habits, and environmental pollution, factors that contribute to a greater increase of CNCD,[10] which explains the greater number of diseases caused by heart failure in the clusters mentioned.

Favorable Socioeconomic Context was also predominant in the cluster with the highest rates of hospitalization. This larger number may be related to greater use of hospital services by elderly individuals, which is related to the higher number of cases of chronic diseases in this stage of life, often with greater intensity and severity, which increases the number of hospitalizations, regardless of social class. In addition, elderly individuals with favorable socioeconomic context also use hospital services often.[14]

The Low Formal Education and Dependence on the State factor also had the highest mean in clusters with higher rates of hospital admission. This finding is related to the precariousness of services in primary care, since this factor includes people with greater dependence of state health services, and these higher rates may reflect a deficit in primary care, which would entail a greater number of hospitalizations, assuming CSAP was not solved in basic care. In addition, the population with this profile has, consequently, greater exposure to risk factors such as tobacco use, poor diet, among other risk factors related to chronic diseases.[10,12]

A study carried out in the districts of the municipality of Goiânia also had similar findings, districts that had better socioeconomic contexts had higher rates of hospitalization. The study concluded that this result was motivated by a lack of access to primary health care.[12]

When analyzing the number of consultations of elderly individuals in primary care, there was no significant difference. The factor does not portray the effectiveness of basic care and its relationship with a greater or lower frequency of ICSAPS in the clusters. It is also important that, in addition to evaluating the primary care, we evaluate the other networks of health services, because their appropriate structuring can provide improvements in the health care of elderly individuals, reducing hospitalizations due to CSAP.

Another study related to the creation of the Family Health Strategy (ESF) with the ICSAP. The results showed no correlation between its expansion and the decrease of ICSAP. Although the population coverage by the FHS has increased in all regional health services of the cities studied, this did not happen evenly.[15] Studies with 1,622 Brasilian municipalities have also identified a negative correlation between FHS coverage and ICSAP.[16]

It is stressed that the implementation of the FHS has expanded the coverage of basic care and contributed to the organization of a care model that used the health of the family as a first-line health service. However, this was likely not accompanied by a corresponding improvement in the level of organization and practices of these services, which did not reach the expected levels of effectiveness, and did not follow the improvement in the quality of life of the elderly population, since we observed significant differences between contextual factors and the number of ICSAP in the municipalities.

This is also due to the lack of efforts to improve the integrality of care by many health professionals,

since this principle takes into account not only the disease but the individual as a whole, including the social determinants of health. A study carried out in Porto Alegre[17] reported that, when assessing the effectiveness of the integrality of care, significant problems were found. Some explanations were related; evidence shows that the basic health units still operate under the biomedical logic that focuses on the diseases, and not on the individual, and have lower scores of integrality when compared to the ESFs and to the educational units who have a Multiprofessional Residency. This finding is possibly related to the challenge of breaking away from the traditional care model of such establishments.[18,19]

The shift towards integrality requires a rupture from the biomedical model through policies of family health prioritization, changes in the building of human resources, and the logic of care that disregards the use of protocols and evidence in the APS care. In addition, the Family Health Strategy also has limitations that may hinder the process for integral care, such as the precarization of work contracts and the absence of Family Health Support Centers.[17-19]

Authors have reported that, when assessing the primary care coverage or the number of consultations, it is necessary to check beyond the quality of primary care that it can reflect since there are other factors associated with the results, such as the adequacy of the professionals, mainly the permanence of physicians; the association to social, economic, political, and environmental determinants; and other aspects of the structural dimension.[10,16]

The findings of the present study reflect the importance of studying the ICSAP in elderly individuals and monitoring social determinants of health, which will reflect on further assistance to the activity of health promotion among elderly individuals and, consequently, will reduce diseases related to the CSAP and improve the quality of life.

Regarding the limitations of the present study, it is worth noting that this paper is limited to initial hospitalizations, in which there may be diagnostic errors at the time of admission, which could mask some of the causes. However, it should be emphasized that this study corroborates several others in the literature, which confirms the most common findings.

CONCLUSION

We found a predominance of diseases due to heart failure in Hospitalizations due to Primary Care Sensitive Conditions and that contextual factors interfere in the conditions of admission. This makes us conclude that, besides the improvement of primary care, it is necessary to improve the living conditions of the elderly population, because this has great potential for reducing injuries and complications caused by chronic diseases in this population. Therefore, it is essential that public policies work in interventions that ensure care for chronic diseases and strengthen the promotion of healthy aging.

Author Contributions

A. M. M. Soares was responsible for a good part of the writing of this document and data collection; T. C. O. Mendes performed the statistical analyses; M. M. Menezes contributed in data collection and in writing the article; K. C. Lima advised the entire study.

RESUMO

OBJETIVO: *Objetivou-se realizar uma análise das internações dos idosos por condições sensíveis à atenção primária (ICSAP) e fatores contextuais associados em idosos com 60 anos ou mais, residentes em municípios da Região Nordeste.*

MÉTODOS: *Caracterizou-se por ser um estudo ecológico utilizando dados do Sistema de Informação Hospitalar (SIH) e do Sistema de Informação da Atenção Básica (Siab) referentes a idosos com 60 anos ou mais.*

RESULTADOS: *A taxa total de internação foi de 527,524, sendo em maior quantidade aquelas por insuficiência cardíaca, seguidas das doenças cerebrovasculares e, em terceiro, as gastroenterites infecciosas. Analisando as taxas de ICSAP com os fatores contextuais, todas foram significativas. Em relação à cobertura da atenção básica, ocorreu uma similaridade entre eles, e para a taxa do número de consultas entre idosos, apesar do maior número destas nos municípios com maiores taxas de internação, não existiu diferença significativa entre eles.*

CONCLUSÃO: *Concluímos que os fatores contextuais interferem nas condições dessa internação, necessitando, além da melhoria da atenção primária, uma melhoria nas condições de vida da população idosa.*

PALAVRAS-CHAVE: *Idoso. Hospitalização. Fatores epidemiológicos.*

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COMMENTARY

Comments: "Causes for hospitalization of elderly individuals due to primary care sensitive conditions and its associated contextual factors"

Yan Nogueira Leite de Freitas[1]

1. Professor Adjunto da Universidade Federal do Amazonas (UFAM) - Manaus, AM, Brasil.
Email: nlfyan@hotmail.com

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An evaluation of the epidemiological profile of the leading causes of hospitalization in the elderly population in Brasil shows us, first and foremost, how much health policies contribute to the quality of life of these individuals. Based on such an evaluation, it is possible to understand, too, how we are aging, i.e., the price we pay for longer survival in a context somewhat unfavorable.

By studying the morbidities that lead to the hospitalization of elderly individuals, we notice that, in their majority, they are represented by diseases or injuries that should be prevented and treated in Primary Health Care (PHC). Therefore, studies of this nature show that primary care is often not able to fulfill its role in the face of the complexity of elderly individuals[1,4].

Santos et al.[2] strengthen these findings by arguing that the hospitalization rates among the elderly population are much higher than that of other age groups, precisely on account of the high prevalence of chronic-degenerative diseases and of multi-morbidities that are frequent in these individuals. Thus, we can see that health policies are still unable to meet, in full, the demands generated by the Brazilian demographic/epidemiological transition.

Regarding the costs, Góis, and Veras[3] found that the average cost of hospitalizations in the Unified Health System (SUS) is higher in the age range of 60 to 69 years, and it decreases with the increase of age. These findings, curiously, show that the cost of medical/hospital care by elderly Brazilians is not directly related to increased costs of procedures, but with their use rate.

Therefore, to meet the demands from the increasing elderly Brazilian population, it is not necessary to raise expenditure on health, but improve the quality of procedures already offered and invest in the PHC, so that elderly individuals would need to use these services less frequently.

In this context, we can understand how important it is to investigate and discuss the causes of hospitalizations of the elderly individuals due to primary care sensitive conditions, especially in the Northeast of Brasil, where the population is extremely vulnerable and aged.

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An epidemiologic overview of acute kidney injury in intensive care units

Reginaldo Passoni dos Santos[1*]
Ariana Rodrigues Silva Carvalho[1,2]
Luis Alberto Batista Peres[1,3]
Claudio Ronco[4]
Etienne Macedo[5]

1. Programa de Pós-Graduação em Biociências e Saúde, Universidade Estadual do Oeste do Paraná – Cascavel, PR, Brasil
2. Departamento de Enfermagem, Universidade Estadual do Oeste do Paraná – Cascavel, PR, Brasil
3. Departamento de Medicina, Divisão de Nefrologia, Universidade Estadual do Oeste do Paraná – Cascavel, PR, Brasil
4. San Bortolo of Vicenza; Department of Nephorology, Dialysis & Transplantation; International Renal Research Institute - Vicenza, Italy.
5.University of California San Diego, Department of Medicine, Division of Nephrology - California, United States of America

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SUMMARY

INTRODUCTION: *Acute kidney injury (AKI) is a frequent event among critically ill patients hospitalized in intensive care units (ICU) and represents a global public health problem, being imperative an interdisciplinary approach.*

OBJECTIVE: *To investigate, through literature review, the AKI epidemiology in ICUs.*

METHODS: *Online research in Medline, Scientific Electronic Library Online, and Latin American and Caribbean Literature in Health Sciences databases, with analysis of the most relevant 47 studies published between 2010 and 2017.*

RESULTS: *Data of the 67,033 patients from more than 300 ICUs from different regions of the world were analyzed. The overall incidence of AKI ranged from 2.5% to 92.2%, and the mortality from 5% to 80%. The length of ICU stay ranged from five to twenty-one days, and the need for renal replacement therapy from 0.8% to 59.2%. AKI patients had substantially higher mortality rates and longer hospital stays than patients without AKI.*

CONCLUSION: *AKI incidence presented high variability among the studies. One of the reasons for that were the different criteria used to define the cases. Availability of local resources, renal replacement therapy needs, serum creatinine at ICU admission, volume overload, and sepsis, among others, influence mortality rates in AKI patients.*

KEYWORDS: *Acute kidney injury. Intensive care units. Epidemiology. Risk factors.*

INTRODUCTION

Acute kidney injury (AKI) occurs most frequently in critically ill patients admitted to an intensive care unit (ICU) and represents a global public health problem.[1,2] The implications of AKI go beyond the care context and involve considerable expenditure for health care institutions and systems, as well as contributing to a reduction in patients' quality of life.[3,4] In 2014, the International Society of Nephrology launched the "0by25" Project, aiming to reduce avoidable death due to AKI worldwide by promoting universal actions and encouraging scientific dissemination on the subject.[5] Thus, this study aimed to investigate the AKI epidemiology in ICUs.

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CORRESPONDING AUTHOR: Reginaldo Passoni dos Santos
Rua Cabo Clodoaldo Ursulano, 426 - Santa Felicidade - Cascavel/PR. Brasil. CEP: 85.803-450
E-mail: regi-pas@hotmail.com

METHODS

We searched the MEDLINE, Scientific Electronic Library Online, and Latin American and Caribbean Literature in Health Sciences databases for original articles published in English, Portuguese, and Spanish, with information available about the incidence or mortality of critically ill AKI patients admitted to ICUs. The keywords used in the search were: "acute kidney injury", "intensive care unit", and, "epidemiology" with the application of the Boolean operator "AND." The search occurred between January to February 2018.

RESULTS

Forty-seven studies analyzing data from 67,033 patients from more than 300 ICUs located in different regions of the world were included in this review. The overall incidence of AKI ranged from 2.5%[6] to 92.2%[7] and mortality from 5%[8,9] to 80%, reaching 100% in patients who undergo renal replacement therapy (RRT).[10-12]

Among the papers included in this review, 28 reflect data from developing countries, 17 from developed nations, and 2 are multinational, including countries with different income categories. The mean AKI incidence in developed and developing countries was 33.4% and 37.7%, while the mean mortality rate was 40.6% and 43.2%, respectively.

The need for RRT was between 0.8%[13] and 59.2%[14] and the ICU, and hospital stay length varied from [52,15] to 21 days[16] and from 102 to 36 days,[17] respectively. Overall, AKI patients showed a risk of mortality almost 50% higher than patients without AKI, and their hospital stay was up to ten days longer (see Table 1).

DISCUSSION

The use of different diagnostic criteria is one of the factors that contributed to the discrepancies observed in the studies in AKI incidence rate.[18,19] About the geographical location, we verified that AKI-associated mortality rate was higher in developing countries. As demonstrated by another international multicenter study,[2] the risk of death in cases of AKI in ICU is four-fold higher in emerging countries, mainly due to the limited resources available for the adequate/early management of the event.

The AKI occurrence in ICU increases length of stay, need for more vasopressors drugs and specialized human resources, increasing the cost of services and health care systems. The limitation of all these resources and the observed reality in emerging countries contribute to the increase in patients' morbidity and mortality in regions with lower resources.

Furthermore, the population and the total number of participants included in a study should also be considered. A study reported AKI incidence of 2.5% based on data from approximately 8,000 patients enrolled from the database Multiparameter Intelligent Monitoring in Intensive Care-II.[6] Whereas, in a study analyzing data from a single-center survey with 51 patients in the postoperative period of cardiac surgery had an incidence higher than 90%.[7]

In two recent multinational, multicenter studies[1,2] included in this review, the mean incidence of the event was 40.5%, and the mean ICU mortality was 23%, reaching more than 30% among cases that required RRT. In another multinational study published in 2005, AKI incidence in critically ill patients was close to 6%, the need for RRT was higher than 70%, and the mortality predicted in the ICU was 45.6%.[20]

These data show that the burden of AKI worldwide is alarming and has been increasing over the past ten years, requiring that the biomedical model of care be replaced by an interdisciplinary health care model, in which actions are planned by a multi-professional health team. In addition, physicians of different specialties (intensivists, nephrologists, and others) should work with a specialized nursing team, pharmacists, physiotherapists, and nutritionists, each bringing their specific professional expertise to integrate quality intensive care.[1-4]

The demographic transition that has taken place in developed nations and, more recently in developing countries, has also brought clinical-epidemiological changes in the population profile, expressed by the increase in the life expectancy of individuals. Old age and chronic diseases are among the main AKI risk factors, as they result in systemic and permanent metabolic-physiological alterations that lead to decreased basal organic-functional kidney activity.[21] However, these changes have also incurred an increase in the burden brought about by chronic-degenerative pathological conditions.[22]

Zhou et al.[23] reported a significant risk for incidence or death from AKI in ICU among individuals over 50 years of age. However, other studies have shown that morbidity and mortality are especially high among individuals older than 60, and the risk

TABLE 1. SUMMARY OF INFORMATION ABOUT THE EPIDEMIOLOGY OF AKI PRESENTED IN THE REVISED ARTICLES*.

Ref	Patients		Criteria for AKI	Epidemiological data of AKI								
	n	Setting		Incidence (%)	RRT (%)	Mortality endpoint	Mortality (%)			Length of stay (days)		
							With AKI	Without AKI	Diff.	With AKI	Without AKI	Diff.
[1]	1,802	GP	KDIGO	57.3	23.5	ICU Hospital	24 26.9	4.7 7.2	+19.3 +19.7	ICU: 6 Hospital: 15	4 12	+2 +3
[2]	6,647	GP	AKIN-m	DC: 19.1 EC: 19.9 Both: 19.2	15.5 30.2 23.7	Hospital	DC: 27.6 EC: 17.6 Both: 22 RRT: 32.3	NS	-	UTI: DC: 5 EC: 6 Hospital: DC: 11 EC: 10	NS	-
[6]	8,085	GP	KDIGO	2.5	45	NS	NS	NS	-	NS	NS	-
[7]	51	Post-surgery cardiac	KDIGO	92.2	NS	NS	NS	NS	-	NS	NS	-
[8]	1,087	Post-surgery myocardial injury	KDIGO	5.2	NS	30 days	5.3	NS	-	NS	NS	-
[9]	7,696	Volume overload	KDIGO sCr-a	24.2 25.3	NS	60 days	5.4	NS	-	NS	NS	-
[10]	73	HIV	RIFLE	All	23.3	ICU	75.3	-	-	1	-	-
[11]	269	GP versus Sepsis	RIFLE	GP: 8.9 Sepsis: 32.3	AKI: 1.9 AKI+Sepsis 15	ICU	AKI: 66.7 AKI+ Sepsis: 80	GP: 21.2 Sepsis: 45.2	+45.5 +34.8	AKI: 8 AKI+Sepsis 11	GP: 9 Sepsis: 13	-1 -2
[12]	152	GP	RIFLE	65.8	12.4	ICU	AKI: 52 RRT: 84.2	5.8	+46.2	8.5	7	+1.5
[13]	476	GP	AKIN	52.7	0.8	ICU	58	27.5	+30.5	NS	NS	-
[14]	414	Severe acute pancreatitis	AKIN	69.3	59.2	ICU	44.9	20.5	+24.4	24	22	+2
[15]	389	Cancer	KDIGO	Cr: 49.4 UO: 56.3 Both: 69.4	5.9	ICU 180 days	30 51.5	5 15.1	+25 +36.4	5	2	+3
[16]	122	V+P versus V+C	AKIN	V+P: 32.7 V+C: 28.8	V+P: 18.8 V+C: 38.1	NS	NS	-	-	V+P: 17.8 V+C: 21.4	-	-
[17]	74	RRT	NS	27	All	NS	NS	-	-	ICU: 18 Hospital: 36	-	-
[23]	1,036	GP	RIFLE AKIN KDIGO Cys-C	26.4 34.1 37.8 36.1	NS	28 days	57.9 54.4 51.8 52.1	NS	-	13.5 12.9 13.9 13.2	(±) 5	+8.5 +7.9 +8.9 +8.2
[24]	200	Elderly (>60 years)	KDIGO	27	NS	ICU	48.1	15.7	+32.4	11.4	5.2	+6.2
[25]	137	Sepsis	AKIN	77	23.4	ICU Hospital 28 days	39 45 38	NS	-	NS	NS	-
[26]	573	Volume overload	SOFA	23	50.8	28 days	50	13.4	+36.6	15.3	NS	-
[27]	1,234	RRT	RIFLE/ AKIN	All	All	ICU	69.4	-	-	9.8	-	-
[28]	14,986	Obesity	KDIGO	21.1	NS	Hospital 1 year	10 12	NS	-	NS	NS	-
[29]	832	GP	CrCl/ AKIN	CrCl: 58.3 AKIN: 27	CrCl:9.5 AKINNS	ICU	CrCl: 26.2 AKIN: NS	CrCl: 10.7 AKIN: NS	+15.5 -	CrCl: 13.5 AKIN: NS	CrCl: 10 AKIN: NS	+3.5 -
[30]	190	GP	RIFLE AKIN KDIGO	62.6 63.2 63.2	NS	ICU	42.9 42.5 42.5	17.7 17.7 17.74	+25.2 +24.8 +24.8	NS	NS	-
[31]	335	CIN	RIFLE	15.5	34.6	ICU Hospital	40.4 53.8	21.3 35.7	+19.1 +18.1	ICU: 14	ICU: 15	-1
[32]	41	RRT	RIFLE	All	All	ICU Hospital	48.8 53.7	-	-	9	-	-
[33]	548	GP	RIFLE	17.2	4.3	28 days	49	NS		NS	NS	-
[34]	254	Infections diseases	RIFLE	All	27.6	ICU	62.8	-	-	NS	-	-
[35]	3.107	GP	RIFLE/ AKIN/ KDIGO	46.9 38.4 51	(±) 20	Hospital	27.8 32.2 27.4	7 7.1 5.6	+20.8 +25.1 +21.8	5	3	+2
[36]	2,526	GP	KDIGO	46.4	18.9	28 days	25.7	10.1	+15.1	7	5	+2

CONTINUATION

Ref	Patients		Criteria for AKI	Epidemiological data of AKI								
	n	Setting		Incidence (%)	RRT (%)	Mortality endpoint	Mortality (%)			Length of stay (days)		
							With AKI	Without AKI	Diff.	With AKI	Without AKI	Diff.
[37]	65	GP	RIFLE/ AKIN	All	48	ICU	69	NS	-	NS	NS	-
[38]	901	Trauma	AKIN	6	19	30 days	29.6 RRT: 50	7.9	+21.7	NS	NS	-
[39]	114	CIAI	AKIN	58.8	28.9	ICU 28 days	17.9 23.9	NS	-	NS	NS	-
[40]	360	GP	AKIN	59.7	(±) 2.3	ICU	(±) 60.2	18.6	+41.6	ICU (±): 11.9 Hospital: (±) 22.7	ICU: 7.2 Hospital: 27.2	+4.7 -4.5
[41]	715	GP	RIFLE	16.1	39.1	ICU 28 days	7.8 49.5	NS	-	ICU: 11	NS	-
[42]	40	RRT	NS	All	All	30 days	52.5	-	-	NS	-	-
[43]	44	GP	AKIN	All	43.2	ICU	43.2	NS	-	NS	NS	-
[44]	627	GP	RILE/ AKIN	RIFLE: 69.4 AKIN: 51.8 Both: 74.4	5.4	90 days	40.9 44.6	19.8 23.5	+21.1	14	14	-
[45]	149	CIN	RIFLE	15.4	13	ICU	52	19	+33	13	12	+1
[46]	2,901	GP	AKIN/ KDIGO	39.3	10.2	Hospital 90 days	25.6 33.7	NS	-	3.7	NS	-
[47]	624	Surgical	RIFLE	58	8	Hospital 1 year	19 35	4 14	+15 +21	ICU: 6 Hospital: 19	ICU: 3 Hospital: 9	+3 +10
[48]	274	RRT	RIFLE	All	All	ICU Hospital	58.4 62	-	-	ICU: 14 Hospital: 22	-	-
[49]	182	Post-partum	Cr≥ 89µmol/ RIFLE	37.3	28	NS	NS	NS	-	4	2	+2
[50]	1,769	GP	AKIN	28.9	NS	30 days 1 year	13.3 28.8	6.0 16.5	+7.3 +12.3	NS	NS	-
[51]	40	GP	RIFLE	75	NS	ICU	30	10	+20	NS	NS	-
[52]	1,070	GP	RIFLE	Men: 35.8 Women: 38.7 Both: 37.3	NS	NS	NS	NS	-	NS	NS	-
[53]	445	GP	KDIGO	48.8	33.2	90 days	15.3	NS	-	(±) 4.3	2.1	2.2
[54]	27	MV	RIFLE	All	NS	ICU	44.4	NS	-	NS	NS	-
[55]	3,350	GP	RIFLE/ AKIN	21 21.1	NS	ICU	46.5 47	NS	-	NS	NS	-

*Note: Only articles published between January 2013 and February 2017. Abbreviations: AKIN - Acute Kidney Injury Network; AKIN-m - AKIN modified; CIAI - complicated intra-abdominal infection; CIN – Contrast-induced nephropathy; ClCr – Clearence of creatinine; Cr - Creatinine; Cys-C - Cystatin C; DC - Developed countries; Diff. - Simple difference between patients with and without AKI; EC - Emerging countries; GP - General population; HIV - Human Immunodeficiency Virus; KDIGO - Kidney Disease Improving: Global Outcomes; MV – Mechanical ventilation; NS - Not specified; Ref - Reference; RIFLE - Risk, Injury, Failure, Loss, End-stage kidney disease; RRT – Renal replacement therapy; sCr-a – Serum creatinine adjusted to the water balance; SOFA – Sequential Organ Failure Assessment; UO – Urine Output; V+P – Vancomycin with piperacillin-tazobactam; V+C – Vancomycin with cefepime.

increases linearly with age, as well as the chance of needing RRT, which further increases the mortality rate of patients.[1,2,14,17,24]

Almost all studies listed in Table 1 present diabetes mellitus and systemic arterial hypertension as the chronic diseases that are the most prevalent risk factors in critically ill adults with AKI. Moreover, these are also the chronic conditions that frequently increase morbidity and mortality for AKI in critically ill patients who are septic,[11,16,25] with fluid overload,[2,9,26] making use of nephrotoxic drugs[16] or who have undergone major surgeries.[7,8] Other chronic pathological conditions that are also noted as risk factors for AKI incidence and mortality in critically ill patients include cardiovascular,[9,17,27-30] respiratory,[10,31] cerebrovascular,[16] cirrhosis,[32,33] cancer[15,25], and human immunodeficiency virus infection.[10,34]

Several studies have shown baseline renal function as one of the most important risk factors for AKI. Luo et al.[35] performed prospective analysis of a database from Beijing (China), with more than three

thousand adult patients, and observed that AKI patients identified by KDIGO criteria already had worse baseline renal function in comparison with those without AKI. In this same sense, Yokota et al.[24] in a prospective study with critically ill elderly patients verified that AKI patients presented worse baseline serum creatinine. Worse baseline renal function also was an AKI risk factor in the study performed by Wang et al.[36]

In others studies,[12,37-39] patients with AKI during ICU stay are the ones with higher serum creatinine at ICU admission, and in the studies performed by Podoll et al.[38] and Peres et al.[12] it was also associated with a higher risk for mortality.

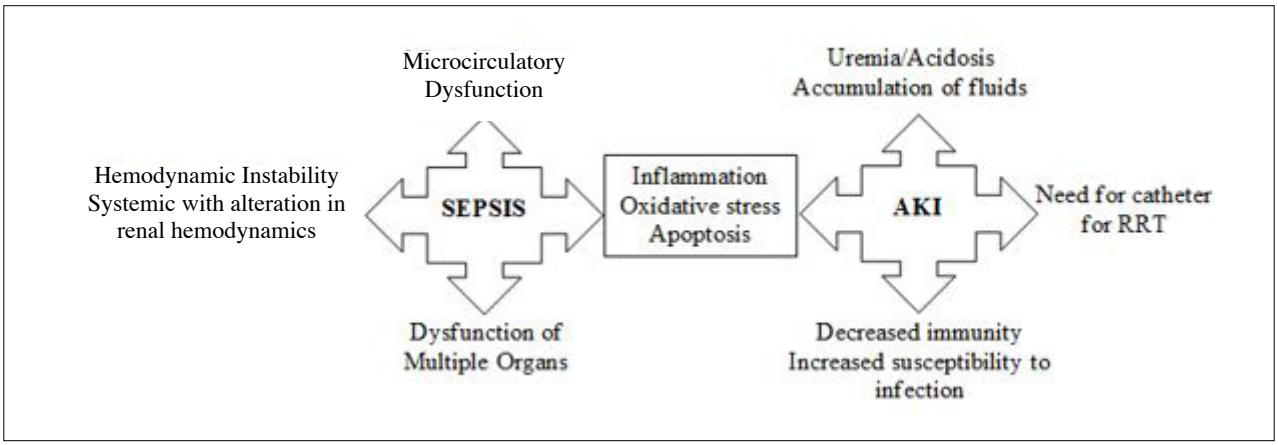
In the presence of sepsis, the reported incidence of AKI was greater than 70%, and mortality rate reached 80%, higher in patients undergoing RRT,[1,10,11,14,17,24,34,36,37,39-43] Sepsis was also an independent factor that increased the length of stay of individuals in ICUs.[11] The pathophysiological dynamics of the interaction between sepsis and AKI are not fully understood, and the association between sepsis and AKI in ICU patients can cause vascular, glomerular, tubular, and interstitial damage in the kidneys. Nevertheless, it is believed that the inflammatory process, oxidative stress, and apoptosis act as the link of the interaction between the events (see Figure 1). Vasodilation, hypoperfusion, and ischemic injury are probably the primary deleterious effects of this interaction.[3,5,11,20]

Nephrotoxic agents were an important factor contributing to AKI among critically ill patients[44]. Two recent studies have shown distinct results on the effect of contrast-induced nephropathy (CIN).[31,45] Hocine et al.[45] assessed 149 patients in a single-center study in Belgium and Kim et al.[31] 335 patients in the Republic of Korea. Although these studies showed a similar incidence of CIN, approximately 15%, CIN patients had a mortality rate 11% higher than those without CIN.[45] In another study, the ICU length of stay of patients with and without CIN was similar, nearly 15 days.[31] It should be noted that the pathophysiological genesis of CIN is not yet fully determined; however, the mechanisms may involve renal medullary ischemia and renal tubular damage by toxicity.[31,45]

Additionally, we verified that fluid overload might represent an independent risk factor for AKI development, need for RRT, and mortality.[9,26,32,36,44] Fluid overload can promote renal interstitial edema and consequent water and saline retention, increased interstitial pressure, reduction of renal blood flow and glomerular filtration rate.[26,32] Moreover, fluid overload is also a risk factor for increased intra-abdominal pressure, central venous pressure, and renal venous pressure, which contribute to worsening renal function.[26,32,36]

The use of diuretics in critically ill patients is frequent and one of the first strategies to minimize the consequences of fluid overload, but the administration may increase the risk of death in AKI patients.[37,46] In South America, the administration of diuretics was associated with higher mortality of critically ill AKI patients.[37] A multicenter Finnish study was identified in which 78.1% of patients who acquired AKI in the ICU were treated with vasoactive drugs.[46] In addition, the authors reported that over one-third of the AKI patients that used diuretics had hemodynamic stability or severe sepsis, and the use of diuretics was identified as a risk factor for the increase of AKI incidence.

FIGURE 1. PHYSIOPATHOLOGICAL INTERACTION BETWEEN SEPSIS AND AKI



Note: AKI - Acute kidney injury; RRT - Renal replacement therapy

In the United States, the combined therapy of vancomycin with piperacillin-tazobactam resulted in a higher AKI rate in ICU patients. However, no statistically significant differences were found regarding the need for RRT among patients taking vancomycin combined with cefepime.[16]

In a small prospective cohort, Nascimento et al.[7] evaluated 51 patients after bypass surgery and reported AKI incidence of 92.2%. In another study, the AKI occurrence was 5.2%, and the syndrome was an independent risk factor for myocardial injury.[8]

In addition to cardiac procedures, neurosurgeries, and transplants,[1] gastrointestinal, orthopedics, gynecological and urological surgeries[47] have also been associated with the occurrence of AKI in critically ill patients and are responsible for increasing the risk of death.

The RRT need can significantly increase both the length of hospital stay and patient mortality. In studies performed in Brasil, the mortality in patients undergoing RRT reached 58.4%[48] in the Southeast region and 84.2%[12] in the South region, and the mean length of hospital stay reached 36 days.[17] In two studies, the patients' mortality on continuous RRT modality was around 50%,[43,32] and in one study, the mortality in RRT by continuous and intermittent modalities reached 74.4%.[27]

There are studies in which the need for mechanical ventilation,[2,28,33] as well as elevated central venous pressure[30] and metabolic acidosis[28,42,45] have proved to be risk factors for AKI and death of patients who already presented the injury. Other risk factors are present in special populations such as patients with infectious diseases,[28] severe acute pancreatitis,[15] overweight/obesity,[32] obstetric complications,[49] malaria[42], and trauma victims.[38]

CONCLUSION

AKI incidence presented high variability among the studies, and the different criteria to define the cases is among the reasons. Availability of local resources, renal replacement therapy needs, serum creatinine at ICU admission, volume overload, and sepsis, among others, influence the mortality rates in AKI patients. This literature review demonstrated that a varied combination of risk factors are linked to the increasing incidence and persistence of high mortality rates in the ICU. We believe that knowing the epidemiological aspects of AKI and identifying its main risk factors to achieve early diagnosis are the first steps toward enhancing patient outcomes.

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Highlights

The incidence of AKI in critically ill patients is worrisome, regardless of geographical location;

The value of serum creatinine when the patient is admitted to the ICU can help the early diagnosis of AKI;

Sepsis persists with the cause-effect role of AKI, showing a strong association both to the incidence and to the increased mortality of critically ill patients;

Requiring RRT continues to have a relationship with worse outcomes;

Reducing preventable cases and deaths is a challenge to be faced by all health staffs.

Author Contributions

Passoni dos Santos R contributed with (1) the conception and design of the work, the acquisition, analysis, and interpretation of the data, (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be submitted; (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Carvalho ARS contributed with (1) drafting the article and revising it critically for important intellectual content; (2) final approval of the version to be submitted.

Peres LAB contributed with (1) the conception and design of the work, the acquisition, analysis, and interpretation of the data, (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be submitted.

Ronco C and Macedo E contributed with (1) drafting the article and revising it critically for important intellectual content; (2) final approval of the version to be submitted.

RESUMO

INTRODUÇÃO: *Injúria renal aguda (IRA) é um evento frequente entre pacientes criticamente enfermos internados em unidade de terapia intensiva (UTI) e representa um problema de saúde pública global, sendo imperativa uma abordagem interdisciplinar.*

OBJETIVO: *Investigar, por meio de revisão de literatura, a epidemiologia da IRA em UTIs.*

MÉTODOS: *Pesquisa on-line nas bases de dados Medline, Scientific Electronic Library Online e Literatura Latino-americana e do Caribe em Ciências da Saúde, com análise dos 47 estudos de maior relevância publicados entre 2010 e 2017.*

RESULTADOS: *Foram analisados dados de 67.033 pacientes, internados em mais de 300 UTIs de diferentes regiões do mundo. A incidência global de IRA variou de 2,5% a 92,2% e a mortalidade, entre 5% e 80%. O tempo de internação em UTI variou de cinco a 21 dias, enquanto que a necessidade de terapia renal substitutiva, de 0,8% a 59,2%. Pacientes com IRA apresentam índice de mortalidade substancialmente maior e tempo de internação mais elevado, em comparação com pacientes sem IRA.*

CONCLUSÃO: *A incidência de IRA apresentou alta variabilidade entre os estudos e, dentre os motivos, estão os diferentes critérios utilizados para definição dos casos. Disponibilidade de recursos locais, necessidade de terapia renal substitutiva, creatinina na admissão na UTI, sobrecarga volêmica e sepse, dentre outros, influenciam as taxas de mortalidade entre os pacientes com IRA.*

PALAVRAS-CHAVE: *Lesão renal aguda. Unidades de terapia intensiva. Epidemiologia. Fatores de risco.*

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REVIEW ARTICLE

Influence of physical training on bone mineral density in healthy young adults: a systematic review

Josiel Almeida de Avila[1]
Rafael Almeida de Avila[2]
Ezequiel Moreira Gonçalves[1]
Gil Guerra-Junior[1]

1. Laboratório de Crescimento e Desenvolvimento (LabCreD), Centro de Investigação em Pediatria (CIPED), Faculdade de Ciências Médicas (FCM), Universidade Estadual de Campinas (UNICAMP), Campinhas, SP, Brasil
2. Faculdade de Ciências Médicas, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil

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INTRODUCTION

Bone density is related to genetic, hormonal, nutritional, and environmental factors. Among the environmental factors, physical activity is identified as a major contributor to bone density gain during different periods of life[1,2], since the formation of bone is associated with the elastic compressive force of muscle contractions and weight support. Thus, activities that impose heavier loads on the bone structure cause more significant gains in bone density[3,4].

Bone Mineral Density (BMD) can be analyzed using x-rays, neutron activation, absorptiometry dual-energy x-ray absorptiometry (DXA), and high-resolution magnetic resonance imaging. The first two techniques present a disadvantage because they expose the patient to a large amount of radiation. Currently, the most commonly used method for evaluating bone health is DXA, which estimates the content of the bone area, and is considered the gold standard to evaluate bone density. Furthermore, this technique has low cost and little exposure to ionizing radiation[5-7].

According to the World Health Organization, cases of osteoporosis are expected to double by the year 2050[8]. Currently, osteoporosis affects about 50% of women and 20% of men over the age of 50 years-old[9]. The illnesses linked to bone health are dependent on inherent bone loss due to age, but they are also influenced by bone acquisitions that occur during adolescence and adult life[10,11]. Studies have shown that resistance exercises, impact activities, and sports preserve bone health[12,13].

Although many cross-sectional studies show that physical activity is related to BMD, longitudinal studies are still scarce. Thus, this systematic review aimed to determine how the variables of physical training (duration, volume, intensity, type of activity, and frequency of training) influence BMD evaluated by DXA in young adults.

METHODS

This is a systematic review of literature about the influence of physical activity on BMD of healthy young adults. The method utilized as reference was PRISMA (Preferred Reporting Items for Systematic reviews

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CORRESPONDING AUTHOR: Josiel Avila
126 Tessália Vieira de Camargo Rue, Cidade Universitária Zeferino Vaz, Campinas – 13083-872 – São Paulo, Brasil
E-mail: josielavila@me.com

and Meta-Analyses)[14]. The PRISMA recommendations include a checklist of 27 items that guide the authors of systematic reviews regarding information that should be clearly described in the manuscript, including specific instructions for title, abstract, methods, results, and financial support.

This systematic review conducted searches in the electronic databases PubMed and Bireme in July 2018. Only works published between 2000 and 2018 were included in this study. The search was conducted by two authors (JAA and RAA), during different moments, in English and Portuguese. Our searches had the following English language descriptors and the respective Portuguese translations: absorptiometry, Dual X-Ray, young adult or adolescent, bone density or bone mineral density or bone mineral content, motor activity or physical activity or sport or exercise.

The criteria for article selection was: studies involving healthy young adults with no history of illness or use of medications that could influence bone health original human research; the use of DXA to identify BMD, and articles published in Portuguese and English, from 2000 to 2018. In addition, the articles should use physical activity as a modifying factor for BMD. Review articles, thesis, and dissertations were not included.

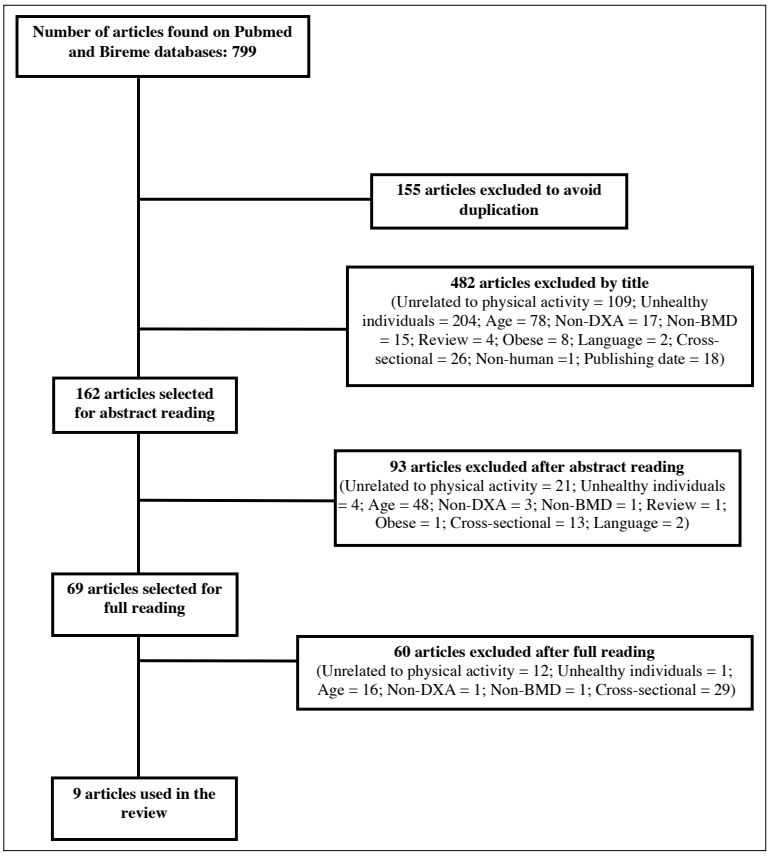
The internal quality of the selected studies was evaluated with the Downs and Black scale. This scale aims to evaluate studies that do not have a randomized clinical trial design, including five sub-items related to the form of reporting results (if the information presented in the study allows the reader to interpret the data and results without bias), external validity, bias, confounding factors, and the power of the study. The maximum score achieved, throughout the 27 gathered items, was 31 points[15].

RESULTS

A total of 799 articles were identified (PubMed=520 and Bireme=279) with the use of the previously mentioned descriptors. Of these, 155 articles were excluded due to duplicity; 482 articles were excluded after title reading; 93 were excluded after abstract reading; and 60 were excluded after full article reading. Only nine articles (PubMed=8 and Bireme=1) were finally included in this review, as illustrated in Figure 1.

According to Downs & Black[15] these nine studies had between 18 and 23 points (Table 1). Their data is presented in chronological order in Table 1.

FIGURE 1. PRISMA FLOWCHART



DISCUSSION

Longitudinal studies that evaluate the influence of physical activity on BMD in young adults are rare. However, following this systematic review, it was possible to verify that some aspects appear to exert a positive effect on BMD.

As for the type of exercise performed, it seems that resistance[21], concentric and eccentric[17] exercises, as well as impact[16] exercises, have a positive influence on BMD. In a study that evaluated impact exercises and weight training, it was found that the impact exercises caused a higher BMD. However, the difference in BMD among the groups was more substantial after 6 months than after 12 months of training. This finding shows that impact activities result in an effect on BMD that is more immediate and of greater magnitude. Therefore, resistance exercises cause more delayed effects. However, it should be noted that both activities bring beneficial changes in BMD[22].

Furthermore, a combination of resistance and aerobic exercises tend to produce better results[20]. This finding was observed in a study comparing aerobic and combined (resistance and aerobic) training. In this study, only the combined training group presented a significant increase in BMD of the tibia[20].

Duration of training appears to be efficient when it is performed during a period equal to or greater than 5 months[16,17,21,22]. The results obtained in studies with interventions of 8 and 12 weeks[20,23] appeared not to be significant. However, significant changes in biomarkers of bone formation were observed after 8 weeks. However, the same significant changes were

TABLE 1. CHARACTERISTICS OF STUDIES ANALYZING THE INFLUENCE OF PHYSICAL ACTIVITY ON BMD OF YOUNG ADULTS, 2005/2018.

Study	Downs & Black	N	Protocol	Analyzed part of the body	Intensity	Weekly training Volume	Outcome
Kato et al.16, 2006	22 Points	36 W (20-23 years old)	6 months of high jumps	Spine and proximal femur	High	30 jumps	Jump Training increased femur and spine BMD after 6 months
Nickols-Richardson et al.17, 2007	20 Points	70 W (18-26 years old)	5 months of eccentric and concentric training	Totality of body, proximal femur, distal tibia and forearm	6 RM	18 - 90 repetitions	Eccentric and concentric exercises increased total proximal femur and forearm BMD
Ryan et al.18, 2004	20 Points	13 M and 21 W (20-29 years old)	6 months of resistance exercises	Totality of body, spine, greater trochanter, Ward's triangle and femoral neck	12-15 RM	3 weekly sessions of 3 11-exercise series	No significant changes
Maimoun et al.19, 2004	19 Points	7 W (18-20 years old)	Before and after 32-week season for triathlon athletes	Totality of body, proximal femur, intertrochanteric region, spine, radio, distal tibia and forearm	High, moderate and low	Varied	No significant increases were noted between pre- and post-season
Lester et al.20, 2009	21 Points	56 W (20,3±1,8 years old)	8 weeks of resistance, combined and aerobic training	Totality of body, hips, lower body, pelvis and tibia	High, moderate and low	90 to 270 minutes	Combined training showed increased in tibia BMD
Almstedt et al.21, 2011	20 Points	14 M e 15 W (18-23 years old)	24 weeks of resistance training	Hips and spine	67 to 90% 1 RM	90 minutes	Men in the resistance training group increased spine BMD
Liang et al.22, 2011	23 Points	51 W (20-35 years old)	12 months of HIS and weight training	Totality of body, spine, hips, legs, arms, heels and wrists	IS (High) and weight training (65-70% and 80% of 1 RM)	IS (180 minutes) weight training (40 minutes)	IS increased heel BMD in women after 6 and 12 months
Ramírez-Campillo et al.23, 2013	18 Points	7 M e 4 W (23±1 years old)	12 weeks of non-dominant leg resistance training	Totality of body, upper body, arms and legs	10-30% of 1 RM	240 minutes	No significant changes in BMD
Stanforth et al.24, 2016	21 Points	212 W (18-23 years old)	Before and after 3 years of university season (Soccer, Volleyball, Running, Swimming and runners)	Totality of body, arm, leg, pelvis, and spine	High, moderate and low	Varied	IS cause larger variation in BMD

Abbreviations: M= Men; W= Women; IS= Impact Sport; DXA= Dual-energy X-ray Absorptiometry; RM = Repetition Maximum; BMD= Bone Mineral Density.

not observed in biomarkers related to bone reabsorption. This finding suggests that the results of BMD tend to appear after a greater period of intervention[20].

As for training intensity, it seems that intense[16,17,22] and moderate[21] training cause a greater effect on the variation of BMD. Low-intensity training[23], even with large volumes, does not show significant differences in BMD.

Despite the different locations of evaluation of BMD utilized in the reviewed articles, the locations where more significant changes occur are the femur and the spine[16,17,21]. However, other sites showed a significant increase in BMD. In a study that evaluated the effect of concentric and eccentric exercises on BMD, it was observed that the upper limbs are more sensitive to changes when compared with the femoral neck[17]. Therefore, it can be concluded that physical training affects both the axial skeleton as well as the appendicular skeleton.

As for the frequency of training during the week, it was not possible to draw further conclusions since all studies used 3 practice sessions a week as the training protocol. However, this variation does not appear to be essential in producing effects on BMD since even when using the same frequency of training some studies showed an increase in BMD[16,17,21,22] and others did not present significant differences[20,23].

LIMITATIONS

As a limitation of this systematic review, it was possible to determine that the analyzed studies differ on training protocols, duration, and intensity of workouts. Furthermore, some studies differ about the location of evaluations of BMD, which may have caused a bias in the analysis of these articles.

CONCLUSIONS

Regardless of the limitations described above, it can be concluded that the increase in BMD occurs on the axial skeleton as well as the appendicular skeleton. Impact, resistance, and combined exercises cause an increase in BMD. Frequency and the weekly volume of training do not necessarily produce effects on BMD. On the other hand, more intense training causes a more significant effect on BMD, and the results are obtained when training is performed with duration equal to or greater than 5 months.

The availability of longitudinal studies that evaluate the effects of physical activity on BMD is limited. Thus, further studies are necessary for better analysis of the effects of training variables on BMD in young adults.

PALAVRAS-CHAVE: *Revisão. Densidade Óssea. Exercício. Adulto Jovem.*

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COMMENTARY

Comments: "Influence of physical training on bone mineral density in healthy young adults: a systematic review"

Sami A. Gabr [1,2]

1. Professor of biochemistry and molecular biology, Rehabilitation research chair, College of Applied Medical Sciences, King Saud University, Riyadh, KSA.
2. Department of Anatomy, Faculty of Medicine, Mansoura University, Egypt.
Correspondence: P.O. Box 10219 Riyadh 11433, KSA, email: nadalab2009@hotmail.com, dr.samigabr@gmail.com, sgabr@ksu.edu.sa

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Aspects of human lifestyle such as diet, physical activity, and daytime life have positive effects on bone health, especially on bone loss or osteoporosis among older people.[1-4]

Many parameters such as genetic factors, peak bone mass (PBM), balanced nutrition, physical activity, and lifestyle risk factors, including the intake of caffeine, tea, and carbonated beverage, smoking, and alcohol consumption were collectively influencing changes in bone contents and mass among younger and older individuals.[1-6] These parameters represent the main factors that affect the accumulation and maintenance of bone mass.[5,6] Moreover, anthropometric data (body weight and body mass index [BMI]) are also considered as related factors that contribute to changes in total bone mass.[5-7] Two studies have reported that high BMD is closely associated with elevated BMI in women,[7] and that obesity significantly decreases the risk for osteoporosis, but does not decrease the risk for osteopenia.[5,6]

The strength of healthy bones can be assessed by continuous measurements of bone quality, bone mineral density (BMD), and bone structure.[8,9] Currently, these parameters are considered the ideal controlled measures for bone strength in cases of healthy and diseased bones and are particularly affected by the scores of physical activity.[10-12]

As explained from the physiology of bone, its formation is predominant during the first ten years of human growth. A homeostatic balance between the naturally occurring processes of bone formation and resorption among was observed among healthy humans aged 20–45 yrs. In older ages, a disorder in the balance state occurs via a slight increase in the resorption process, which in turn results in bone loss and a lower bone density.[13] It has been reported that individuals with low physical activity were susceptible to bone disorders, including bone loss or osteoporotic fracture.[14] Conversely, physically active people, even those who are older, resist the decrease in BMD, which reduces their risk of fracture. Furthermore, increased physical activity results in an increase in BMD and a concomitant decrease in BMI.[15,16]

Finally, supporting comments on the influence of physical training on bone mineral density among young adults, many research works have reported that physical activity provides positive effects on BMD via mechanical loading mechanisms.[17-19] Also, in previous studies, body mass index, physical activity, low calcium consumption, and abnormal lifestyle have a role in bone mineral density and prognosis of osteoporosis in young adults.[20] In addition, in younger ages (children and adolescents), low back pain (LBP) was shown to be linked with limited sun exposure, inadequate vitamin D diets, adiposity, lower PA, sedentary lifestyles, vitamin 25 (OH) D deficiency, and lower levels of Ca, CK, and LDH.[21] In the same way, training programs of different intensities,

particularly aerobic exercises of moderate intensity, were found to protect bone and cartilage by regulating body trace elements that are involved in the biosynthesis of bone matrix structures and inhibition of bone resorption process via a proposed anti-free radical mechanism.[22, 23]

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REVIEW ARTICLE

Fever of unknown origin – a literature review

Leonardo Fernandes e Santana[1]
Mateus de Sousa Rodrigues[1]
Marylice Pâmela de Araújo Silva[2]
Rodrigo José Videres Cordeiro de Brito[3]
Jandir Mendonça Nicacio[4]
Rita Marina Soares de Castro Duarte[5]
Orlando Vieira Gomes[6]

1. Discente de Medicina na Universidade Federal do Vale do São Francisco (UNIVASF), Petrolina, PE, Brasil
2. Médica formada pela UNIVASF, Petrolina, PE, Brasil
3. Médico Infectologista. Professor MSC efetivo da cadeira de Infectologia da UNIVASF, Petrolina, PE, Brasil
4. Médico Hematologista. Preceptor da Residência de Clínica Médica do Hospital Universitário da UNIVASF. Professor efetivo das cadeiras de Doenças hematológicas e de Oncologia da UNIVASF, Petrolina, PE, Brasil
5. Médica Reumatologista. Preceptora da Residência de Clínica Médica do Hospital Universitário da UNIVASF. Professora efetiva da cadeira de Reumatologia da UNIVASF, Petrolina, PE, Brasil
6. Médico Nefrologista. Preceptor da Residência de Clínica Médica do Hospital Universitário da UNIVASF. Professor efetivo da cadeira de Medicina de Urgência da UNIVASF, Petrolina, PE, Brasil

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SUMMARY

Fever of undetermined origin (FUO) is a challenging entity with a striking presence in hospitals around the world and can be associated with a myriad of differential diagnoses. It is defined as fever ≥ 37.8 ° C on several occasions, lasting ≥ three weeks, in the absence of diagnosis after three days of hospital investigation or three outpatient visits. The main etiologies are: infectious, neoplastic, and rheumatic. The diagnosis is based on the detailed clinical history and physical examination of these patients, in order to direct the specific complementary tests to be performed in each case. Empirical therapy is not recommended (with few exceptions) in patients with prolonged fever, as it may disguise and delay the diagnosis and conduct to treat the specific etiology. The prognosis encompasses mortality of 12% - 35%, varying according to the underlying etiology. In this sense, the objective of this study is to review the main topics about fever of undetermined origin, bringing historical and scientific aspects, national and international.

KEYWORDS: *Fever of Undetermined Origin; Fever of Unknown Origin; Prolonged fever.*

INTRODUCTION

Fever of undetermined origin (FUO), of obscure origin, or fever without an apparent cause is a challenging clinical entity with high worldwide prevalence, and a myriad of differential diagnoses 1-5. It affects all age groups, with some peculiarities at age extremes[6,7]. FUO presents an etiologic pattern represented by four general categories: infectious, neoplastic, non-infectious inflammatory diseases, and miscellaneous. A fifth category is considered by some authors as the idiopathic presentation of FUO, or true FUO [1,8-10]. Despite a large number of diagnostic resources, meticulous clinical examination is still the fundamental approach to patients with prolonged fever. Empirical therapy is performed only in selected cases, since some medications may mask symptoms and interfere with the early etiological diagnosis,

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CORRESPONDING AUTHOR: Leonardo Fernandes e Santana
Av. José de Sá Maniçoba, s/n. Centro, Petrolina, PE. Brasil CEP: 56304-917
E-mail: lleonardofernandes94@gmail.com

which is necessary to specify the proper therapy in most cases[2,11]. The objective of this study is to review the main historical and scientific aspects of the fever of undetermined origin.

METHODS

This is a literature review carried out on the more recent publications in the PubMed Central® database. The research used the descriptor "Fever of Unknown Origin" and the secondary descriptors: "Analysis", "Complications", "Diagnosis", "Enzymology", "Epidemiology", "Epidemiology", "Etiology", "Immunology", "Metabolism", "Microbiology", "Mortality", "Parasitology", "Pathology", "Physiology", "Physiopathology", "Statistics and numerical data", "Therapy", "Virology". All secondary descriptors were associated with the main descriptor using the tool "OR". A total of 3,736 results were found. Then, the following filters were applied: "Review", "Clinical Trial", "Meta-Analysis", "Systematic Reviews", "Full text", "published in the last five years", and "Humans". After applying the filters, the results were narrowed down to 75 items. Of these, 23 articles were selected after an analysis of their summary and abstract. The Scielo database was used as a complement to include more Brazilian studies. The descriptor "Febre de origem indeterminada" was used, retrieving a total of 15 matches, which were reduced to 13 after the filters "Brasil" and "Ciências da Saúde" were applied We did not use any filter for year of publication while selecting Brazilian articles due to the scarce scientific production/update on the subject. After analyzing the papers, five were selected based on the scope of the present study, totaling 28 references. This review was conducted in December 2017.

DISCUSSION

Concept

Fever of undetermined origin was initially characterized in 1961 by Petersdorf e Beeson as a record of oral temperature >38.3°C on at least three different occasions, for a minimum of three weeks, in the absence of diagnostic hypotheses that could explain the fever after a week of investigation [1,2,12-14]. The purpose of this concept was to eliminate autolimited infections and habitual hyperthermia, in addition to, at the same time, provide sufficient time for a robust diagnostic investigation[1,8]. One of the most accepted concepts of classical FUO encompasses axillary temperature ≥37.8 °C on several occasions, for a period ≥ three weeks, with no diagnosis after three days of hospital investigation or three outpatient consultations[8,15].

Etiology and epidemiology

Some authors defend the existence of more than 200 possible causes for FUO [1,2,16,17]. The main causes are of infectious, neoplastic, and rheumatic nature. Table 1 addresses the proportion of each FUO group and identifies the infectious causes as the main causes of fever, in most of the studies[1,8,12,18]. Despite all clinical, laboratory, and imaging research, a portion of 7-50% can still remain with no diagnosis (idiopathic)[1,9,10]. In developed countries, neoplastic and rheumatic causes are usually predominant, while in developing countries infections often prevail[1]. A Brazilian study[8] provides the prevalence of the main etiologies of FUO in the country, in 1989 (Table 2). Infectious etiologies were the main cause of FUO in most of the studies[17]. Among the infectious causes, abscesses (in the pancreas, liver), endocarditis, tuberculosis, and complicated urinary tract infections (UTIs) stand out in all age groups[1,7]. Fever is not the most common symptom in rheumatic patients. However, some rheumatic diseases can evolve with varying degrees and patterns of fever, accounting for up to 30% of FUO cases, according to some authors[16]. Most rheumatic cases that include FUO consists of arthritis (e.g., juvenile idiopathic arthritis), vasculitis (ex. Kawasaki disease), systemic lupus erythematosus (SLE), and Still's disease.[6,19-21]

A study conducted in 1961 by Browder and collaborators reported the incidence of fever in patients with neoplasms for one year[12]. They found that fever was not so common in these patients (of 343 patients, 5% had a fever). Fever in cancer patients can happen due to several factors, the main ones being secondary infections (etiology in ⅔ of oncology patients with FUO) and obstructions caused by the tumoral mass[12]. Many neoplasms can cause FUO, but the most frequent are hematological (lymphoma is the main neoplasm associated with FUO), hepatic, pancreatic colonic, and pancreatic[12,17,22]. There are other specific potential causes for FUO that do not fit the categories of infectious, neoplastic, rheumatic and hence form the miscellaneous group. Its main representatives are factitious fever, subacute thyroiditis, and drug fever[17].

Clinical history and physical examination

Brazilian studies[8,23] recommend essential precepts for the approach of patients with FUO, among which the following are particularly noteworthy: making sure that the patient actually has a fever; systematization of the clinical examination; exclusion of serious and treatable etiologies; considering the hypothesis of fever caused by medications; exclude underlying immunodepression; consider an association of diseases; good doctor-patient relationship.

Clinical history

A detailed anamnesis is the main tool for the approach of a patient with a history of prolonged fever, and it must include travel history, professional matters, home environment, housing, use of medication and illicit drugs, contact with animals and sick people[18]. It is always worth inquiring about symptoms that may be considered unimportant, such as jaundice, weight loss, chronic cough, and change in bowel movements, associated with the fever. The personal and family history of previous diseases can also provide valuable indications of specific etiologies (mainly autoimmune diseases)[8]. Up to 28% of travelers who return to their place of origin report fever as the main complaint in medical consultations[13]. The main etiologies in these patients, especially those who traveled to tropical countries, are: malaria (most common etiology), dengue, leptospirosis, rickettsiosis, salmonellosis, amoebiases, and schistosomiasis[13]. Endemic diseases should always be remembered at the time of the clinical investigation. Each FUO category presents specific details that stand out in the clinical history:

Infections: a history of invasive procedures and surgeries; search for a history of previous infections. Contact with animals must raise the suspicion of toxoplasmosis, brucellosis, cat-scratch disease, malaria, piroplasmosis, leptospirosis. Patients usually present fever associated with chills. Immunosuppressive drugs increase the risk of tuberculosis and viral infections[2].

Neoplasms: Neoplasms tend to be associated with early hyporexia or anorexia and progressive weight loss[2].

Rheumatic: Myalgias and arthralgias suggest rheumatic etiologies. A history of oral ulcers may suggest SLE or Behcet's disease. Family history usually reveals important data[2].

Miscellaneous: A history of lymphadenopathy may suggest Rosai-Dorfman or Kikuchi's disease. Neck pain can be a valuable clue for the diagnosis of thyroidites[2,8].

Physical examination

Elementary lesions on the skin, lesions in the oropharynx, teeth[24] (dental abscesses), visceromegalies, lymph node enlargement, pelvic and abdominal

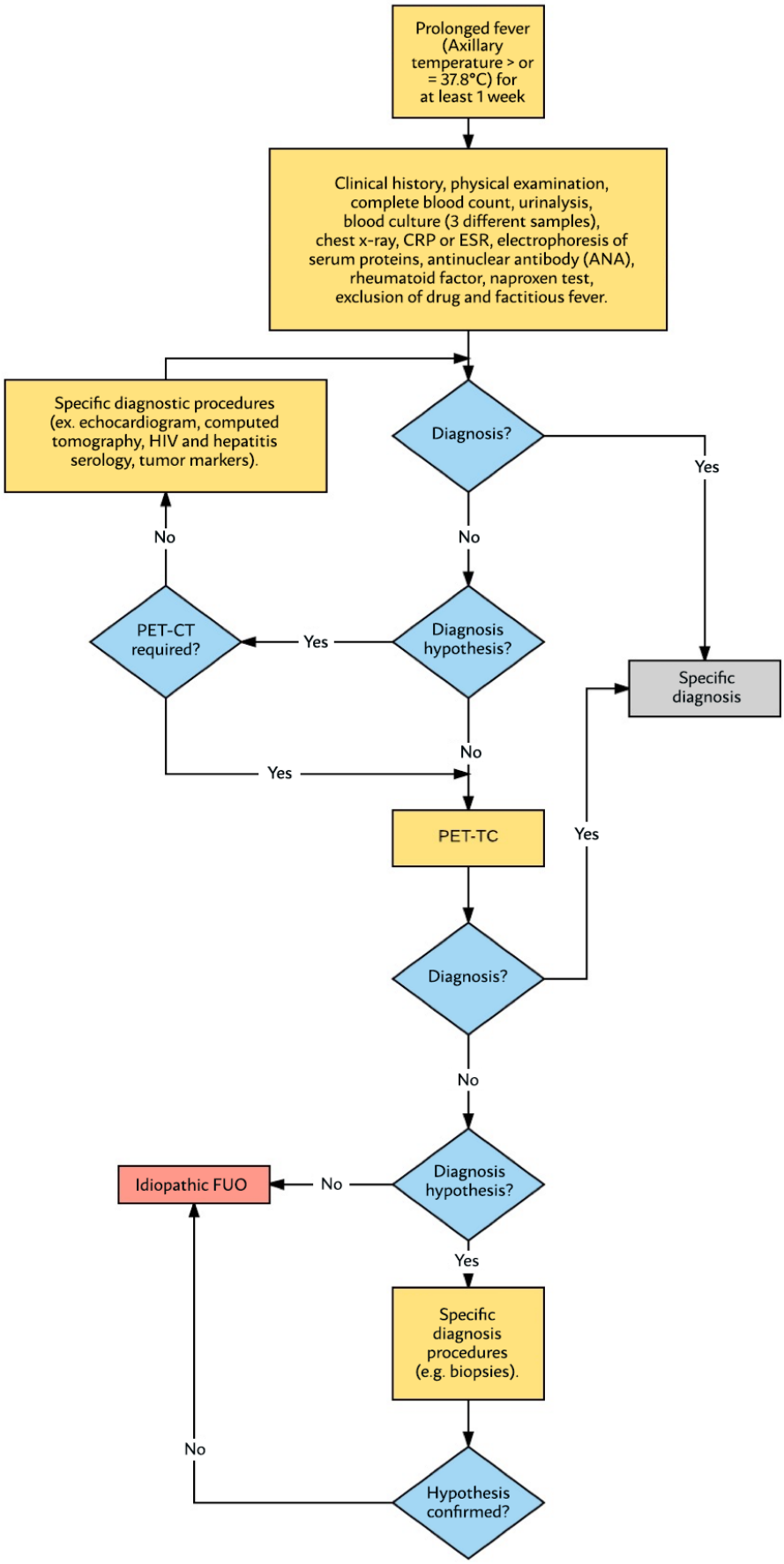
TABLE 1. CAUSES OF CLASSICAL FUO[1]. ADAPTED FROM UNGER M, KARANIKAS G, KERSCHBAUMER A, WINKLER S, ALETAHA D. FEVER OF UNKNOWN ORIGIN (FUO) REVISED. WIEN KLIN WOCHENSCHR. 2016;128(21-22):796-801.

Author (Year)	Infectious (%)	Neoplastic (%)	Rheumatic (%)	Miscellaneous (%)	Idiopathic (%)
Petersdorf (1961)	36	19	19	19	7
Larson (1982)	30	31	16	11	12
Knockaert (1992)	22.5	7	23	21.5	26
De Kleijin (1997)	26	12	25	8	30
Bleeker-Rovers (2007)	16	7	22	4	51

TABLE 2. MAIN CAUSES OF CLASSICAL FUO IN A STUDY CONDUCTED IN BRASIL (1989), ON 54 PATIENTS[8]. ADAPTED FROM LAMBERTUCCI JR, ÁVILA RED, VOIETA I. FEBRE DE ORIGEM INDETERMINADA EM ADULTOS

Infectious (43%)	Neoplastic (17%)	Rheumatic (17%)	Miscellaneous (19%)	Idiopathic (8%)
Tuberculosis Endocarditis Abscess Malaria Toxoplasmosis Gonococcal perihepatitis Salmonellosis Schistosomiasis Katayama fever	Hodgkin's disease Adenocarcinoma Metastases Leukemias	Systemic Lupus Erythematosus (SLE) Still's disease Giant cell arteritis Polymyalgia rheumatica	Subacute thyroiditis Granulomatous hepatitis	

FIGURE 1. ALGORITHM PROPOSED FOR THE DIAGNOSTIC APPROACH TO PATIENTS WITH FUO. ADAPTATION AUTHORIZED BY UNGER M, KARANIKAS G, KERSCHBAUMER A, WINKLER S, ALETAHA D. FEVER OF UNKNOWN ORIGIN (FUO) REVISED. WIEN KLIN WOCHENSCHR. 2016;128(21-22):796-801.



masses or heart murmurs[2]. Fundoscopy and anoscopy must be performed whenever possible. Ideally, the thermal curve must be obtained so that it can be associated or not with the classic patterns of some etiologies (malaria, tuberculosis, for example)[8].

Infections: FUO with morning peaks speaks can be an indication of typhoid fever and Whipple's disease. The Faget sign suggests entities such as yellow fever or typhoid fever. Two peaks of fever per day can be indicative of malaria, miliary tuberculosis, or visceral leishmaniasis (the two latter may also present a single peak in the afternoon)[2]. Roth spots observed during fundoscopy should raise a suspicion of infectious endocarditis. Q fever is an important differential diagnosis in cases of isolated hepatomegaly[2].

Neoplasms: malignancies of the central nervous system or lymphomas can cause bradycardia. Retinal hemorrhages can suggest pre-leukemia. Isolated hepatomegaly in cases of FUO can be suggestive of hepatic or kidney neoplasias, or metastases[2].

Rheumatic diseases: fever with morning peaks reinforce the hypothesis of polyarteritis nodosa. Two daily peaks raise suspicion of Still's disease. Differences between the pulses and the arterial pressure of the limbs are important findings in the diagnosis of Takayasu arteritis. The presence of oral ulcers may suggest SLE or Behcet's disease. Lymphadenopathy is a finding that may be present in SLE, sarcoidosis, or rheumatoid arthritis. Libman-Sacks endocarditis should be remembered in patients with SLE and new heart murmurs[2].

Miscellaneous: the patient has few clinical signs in addition to the fever. The causes of this group depend much more on clinical history than the physical examination[2].

DIAGNOSIS

The diagnosis must be based on the clinical history and thorough physical examination of these patients, with the purpose of establishing specific complementary examinations for each case[2,15,18]. There is no consensus on the optimal diagnostic approach for a patient with FUO[2]. However, there are several protocols and algorithms that can be used to facilitate the diagnostic investigation of prolonged fever. The most current include more sophisticated tests, such as Positron Emission Computed Tomography (PET-CT). Figure 1 presents an adaptation of a current algorithm for the diagnostic approach of prolonged fever. It should be emphasized that all approaches should be be based on the patient's clinical context, since it may not be feasible to perform a large number of exams. The physician must select the exams that can confirm or exclude common nosological entities, always taking into account the local epidemiology. The initial approach must include nonspecific tests[2]. If no diagnostic hypothesis is raised from the nonspecific tests, a series of more specific tests should be performed (serology for hepatitis, biopsies, cultures for mycobacteria, echocardiogram, CT of the abdomen and chest)[11].

Naproxen test

The naproxen test can be useful in the differential diagnosis of FUO, although there is controversy among some authors[2,8]. The test is based on the answer to the empirical therapy with naproxen in patients with prolonged fever (for at least seven days). An etiology of infectious nature should be suspected in patients with no response to the empirical treatment, while in case of a response to the test in 24 hours a neoplasm should be suspected as the cause for the fever[1].

Laboratory exams

Nonspecific laboratory investigation in the initial approach to a FUO patient can make it easier to identify the underlying etiology, based on the most likely diagnostic hypotheses, suspected from the clinical examination data[11]. The first line exams are: complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum creatinine, electrolytes, markers of liver function, rheumatoid factor, antinuclear antibodies, electrophoresis of serum proteins, and blood cultures (three samples of different punctures)[11]. The second line exams should be based on the suspected diagnosis provided by clinical examination and the first line exams, they include: serology for HIV, serum ferritin, thyroid function, lactic dehydrogenase, serology for specific agents, bacilloscopy, echocardiogram, among others[11]. More specific and/or invasive tests are the next steps in the investigation of the etiology of FUO when the preceding steps are not sufficient (ex. biopsies). There is a recommendation to perform bone marrow biopsies, even in the absence of changes in peripheral blood[8].

Blood cultures are useful in cases of bacteremia as the etiology of FUO, for example, in brucellosis, typhoid fever, and in some abscesses. It can

be dismissed in cases of low clinical probability of infectious etiologies for the FUO, such as in neoplasms and rheumatic diseases[2]. The complete blood count is very important and should be requested in virtually all cases. Very often, it shows data that should be valued, such as leukopenia, eosinophilia, lymphocytosis, thrombocytosis, anemias, and thrombocytopenias [2]. In FUO patients, isolated and elevated alkaline phosphatase can be an indication of lymphoma[2]. CRP and ESR are important evidence of inflammatory activity in the follow-up of FUO patients. Serum protein electrophoresis can provide valuable data: elevation of the alpha-1/alpha-2 globulin ratios may occur in lymphomas and in SLE; monoclonal gammopathy can suggest multiple myeloma, hyper-IgD syndrome, Castleman disease; polyclonal gammopathy may be indicative of infection by the human immunodeficiency virus, cytomegalovirus, also occurring in the presence of cirrhosis, sarcoidosis, and malaria[2]. The urinalysis is key in the initial approach to FUO patients, especially those with vague clinical examination. Microhematuria may be the first or sole indication of subacute endocarditis, renal tuberculosis, polyarteritis nodosa, brucellosis, or renal neoplasms[2]. The antinuclear antibody (ANA) test is valuable in suspected rheumatic etiologies of FUO and may assist the diagnosis in 2-12% of cases[15].

Imaging exams

It is essential that the radiologist is familiar with the case of each patient. In general, the first exams requested are cheaper and less specific, such as radiographs and ultrasound. When the first tests are inconclusive, more specific tests such as computed tomography (CT) and nuclear magnetic resonance (NMR) can be requested[17]. Masses, cavitary effusion, consolidations, abscesses, adenopathies, and visceromegalies are some of the main findings of imaging examinations[17]. CT is of extreme value in the diagnosis of tuberculosis and lymphoproliferative diseases, as well as in the staging of neoplasms. NMR may be vital in cases of central nervous system, pelvic, and vertebral diseases.[17]

A number of studies have been conducted in recent years to evaluate the role of PET-CT, which remains controversial for some authors when taking into account the cost in its applicability[5,9,17,25-27]. Some authors defend the PET-CT as a first line exam in the approach for FUO[28]. A recent study involving 52 FUO patients recorded that PET-CT was performed in 22 patients, successfully identifying inflammatory, infectious, and neoplastic causes in 10 of the 22 patients. The positive predictive value (PPV) was 83%, and the negative predictive value (NPV) was 50%[12]. This imaging method makes it easier to precisely identify the location of neoplastic and inflammatory lesions, but its cost is still high, so it should not be used routinely[11,18]. The sensitivity of the PET-CT for the diagnosis of non-infectious inflammatory diseases is 65%[15].

Figure 1. Algorithm proposed for the diagnostic approach to patients with FUO. Adaptation authorized by Unger M, Karanikas G, Kerschbaumer A, Winkler S, Aletaha D. Fever of unknown origin (FUO) revised. Wien Klin Wochenschr. 2016;128(21-22):796-801.

TREATMENT

In general, empirical therapy is not recommended for patients with prolonged fever, since it can mask and delay the diagnosis and the conduct to treat the specific etiology[11]. Some exceptions to this rule are suspected diagnosis of: infective endocarditis with a negative culture (empirical antibiotic therapy is indicated), active tuberculosis (the empirical use of tuberculostatics is indicated), as well as temporal arteritis with a risk of loss of vision (empirical corticosteroid therapy is indicated). Glucocorticoids should be used with caution and in selected cases[1]. The use of antipyretics for symptomatic relief may be necessary in cases in which the patient does not tolerate the fever or febrile peaks. It is important to emphasize that the fever pattern is important for the diagnosis of the underlying etiology and that the antipyretics may distort that finding [1,2,11].

PROGNOSIS

Despite all the current diagnostic and therapeutic tools, the mortality rate for FUO remains around 12-35%[1,8]. This variation is justified by the different etiologies associated with FUO. However, the group that remains without a definite diagnosis (idiopathic FUO) presents a good prognosis, usually with the cessation of the fever after four weeks or more, with mortality of 3.2% in five years. Records show that 51-100% of these patients can recover spontaneously[1]. The greater mortality in patients with FUO is represented by neoplastic etiologies[16].

RESUMO

Febre de origem indeterminada (FOI) é uma entidade desafiadora com presença marcante nos hospitais de todo o mundo, à qual uma miríade de diagnósticos diferenciais podem estar associados. É definida como febre ≥37,8 °C em várias ocasiões, com duração ≥ três semanas, na ausência de diagnóstico após três dias de investigação hospitalar ou três consultas ambulatoriais. As principais etiologias são de ordem infecciosa, neoplásica e reumatológica. O diagnóstico é baseado na história clínica e no exame físico minuciosos desses pacientes, com a finalidade de direcionar os exames complementares específicos a serem realizados em cada caso. A terapia empírica não é recomendada (com poucas exceções) em pacientes com febre prolongada, uma vez que ela pode camuflar e retardar o diagnóstico e a conduta para tratar a etiologia específica. O prognóstico engloba uma mortalidade de 12–35%, variando de acordo com a etiologia de base. O objetivo deste estudo é revisar os principais tópicos acerca da febre de origem indeterminada, trazendo aspectos históricos e científicos, nacionais e internacionais.

PALAVRAS-CHAVE: *Febre de Causa Desconhecida. Febre. Revisão.*

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REVIEW ARTICLE

Selective head cooling and whole body cooling as neuroprotective agents in severe perinatal asphyxia

Mahara Nonato[1*]
Larissa Gheler[2]
João Vitor Balestrieri[3]
Marise Audi[4]
Mirto Prandini[5]

1. Estudante de Medicina, Faculdade de Medicina do ABC - FMABC, Santo André, SP, Brasil
2. Estudante de Medicina, Unifai, Adamantina, SP, Brasil
3. Estudante de Medicina, Universidade dos Grandes Lagos-Unilago, São José do Rio Preto, SP, Brasil
4. Neurocirurgia Pediátrica, Hospital Beneficência Portuguesa - São Paulo, SP, Brasil
5. Médico - PhD - Professor Associado Livre Docente - Universidade Federal de São Paulo - Unifesp, São Paulo, SP, Brasil

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SUMMARY

INTRODUCTION: *The possibility that hypothermia has a therapeutic role during or after resuscitation from severe perinatal asphyxia has been a longstanding focus of research. Studies designed around this fact have shown that moderate cerebral hypothermia, initiated as early as possible, has been associated with potent, long-lasting neuroprotection in perinatal patients.*

OBJECTIVES: *To review the benefits of hypothermia in improving cellular function, based on the cellular characteristics of hypoxic-ischemic cerebral injury and compare the results of two different methods of cooling the brain parenchyma.*

METHODS: *Medline, Lilacs, Scielo, and PubMed were searched for articles registered between 1990 and 2019 in Portuguese and English, focused on trials comparing the safety and effectiveness of total body cooling with selective head cooling with HIE.*

RESULTS: *We found that full-body cooling provides homogenous cooling to all brain structures, including the peripheral and central regions of the brain. Selective head cooling provides a more extensive cooling to the cortical region of the brain than to the central structures.*

CONCLUSIONS: *Both methods demonstrated to have neuroprotective properties, although full-body cooling provides a broader area of protection. Recently, head cooling combined with some body cooling has been applied, which is the most promising approach. The challenge for the future is to find ways of improving the effectiveness of the treatment.*

KEYWORDS: *Brain Diseases. Hypothermia, Induced. Asphyxia Neonatorum. Hypoxia-Ischemia, Brain. Neuroprotection.*

INTRODUCTION

In the presence of neonatal asphyxia, the fetus uses a variety of adaptive mechanisms, which include increased blood flow to the brain, heart, and adrenal glands, and diversion of the pulmonary, intestine, liver, kidneys, and skeletal muscle flow, culminating in a slight blood pressure increase and a small change in

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CORRESPONDING AUTHOR: Mahara Nonato
Av. Lauro Gomes, 2000 - Vila Sacadura Cabral - Santo André / SP, Brasil, Tel: +55067998711224
E-mail: mbn01@hotmail.com

cardiac output. Severe hypoxia and acidosis lead to a decrease in cardiac frequency and cardiac output and a drop in blood pressure, with consequent reduction of the cerebral flow, ischemia, and cellular necrosis.[1,2]

Hypoxic-ischemic injuries of asphyxiated neonates cause Hypoxic-Ischemic Encephalopathy (HIE). This injury is due to hemodynamic changes associated with the prenatal period, during childbirth, and even neonatal events, which can selectively affect vulnerable areas of the central nervous system. The severity of the insult varies with gestational age, and the most serious are those with a high degree of brain-vascular immaturity, in addition to the time of the aggression.[3,4]

Hypoxic-Ischemic Encephalopathy is one of the most significant worldwide problems regarding newborns, leading to death in 10-60 percent of the children affected, and at least 25 percent of the survivors will present long-term neurological sequelae. In Brasil, this is the second cause of neonatal mortality when the first day or the first week of life are considered. HIE is responsible for 18.3 percent and 16.7 percent of deaths in these periods, respectively. HIE is also the most frequent cause in the etiopathogenesis of cerebral palsy, a chronic childhood encephalopathy characterized by non-progressive motor disorders, leading to motor, tonus, and posture disorders, which may or may not be associated with cognitive and sensory deficits.[5,6]

Reports of experiments with hypothermia began to be described around 1964, in a study that suggested a better neurological outcome in newborns (NB) victims of hypoxic insult who were cooled in cold-water baths for 10 minutes after birth.[7]

In order to minimize the high prevalence of HIE, the therapeutic hypothermia procedure is performed worldwide, but the great challenge lies in obtaining conclusions about the effect of hypothermia since there are multiple ways to administer the treatment. Interventions are mainly used in two methods: selective cooling of the head or cooling of the whole body and with further complexities, such as varying temperature variation and cooling time.

OBJECTIVES

The main objective is to verify the hypothermic therapy potentiality of HIE in neonatal asphyxia, based on literature data, to find the evidence level available comparing the benefits between whole-body cooling or selective head cooling

Methodology and selection criteria

Medline, Lilacs, Scielo and PubMed were searched for articles registered between 1990 and 2019 in Portuguese and English. We focused on trials comparing the safety and effectiveness of total body cooling with selective head cooling with HIE. The inclusion criteria were clinical trials comparing whole-body cooling with selective head cooling. The exclusion criteria were studies that compared temperatures or standard treatment methods since the goal was to determine which cooling method is best.

Action mechanisms of therapeutic hypothermia

Therapeutic hypothermia aims to reduce brain metabolism by approximately 5 percent for each 1 °C of body temperature decreased.[8,9]

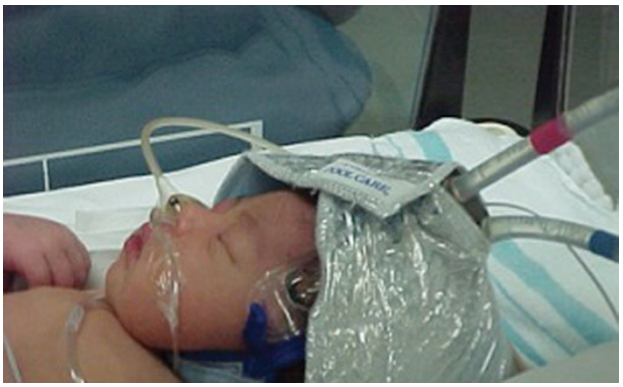

FIGURE 1. INFANT ON SELECTIVE HEAD COOLING USING THE COOL-CAP(R).[26]

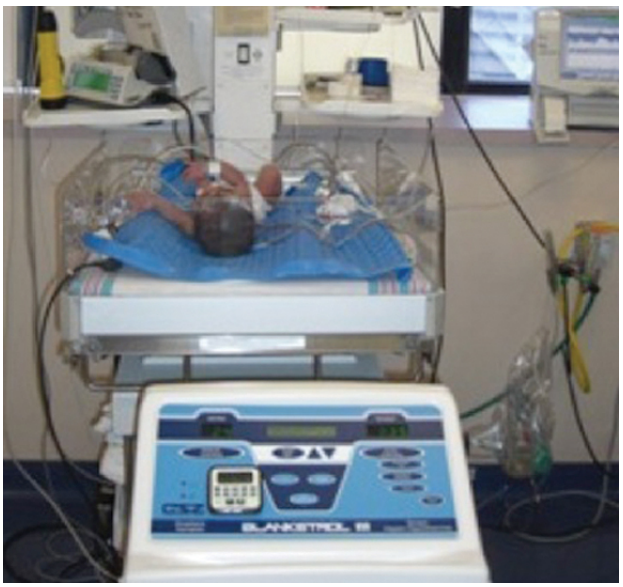

FIGURE 2. INFANT ON WHOLE BODY COLLING USING THE BLANKETROL III BY CINCINNATI SUB-ZERO [26]

Basically, such procedure has proved to have neuroprotective properties by modifying cells programmed for apoptosis, leading to survival by reducing the metabolic rate of the brain, attenuating the release of excitatory amino acids (glutamate, dopamine), improving ischemic damage by the absorption of glutamate, and decreasing the production of nitric oxide and free radicals, thus reducing neuronal death.[5,10]

Other strategies involved are the reduction of the production of reactive oxygen species, reduction of the metabolic rate with reduction of oxygen consumption, and production of carbon dioxide, and some endogenous neuroprotective effect.[5,11-14] The inhibition of the inflammatory reaction, which always accompanies the ischemic process, was demonstrated by Prandini et cols. with the use of hypothermia in brains of rats that were submitted to an inflammatory process induced by the application of a potent inflammatory substance. The reduction of brain temperature to 30ºC during 120 minutes was demonstrated to be effective in reducing the inflammatory reaction.[15,16]

Criteria for the application of cooling

Meet both criteria:

1. Evidence of perinatal asphyxia:

- Arterial blood gas analysis of cord blood or in the first hour of life with pH <7.0

or BE <-16

- Or a history of acute perinatal event (abrupt placental abruption, prolapse

of cord)

- Or Apgar score of 5 or less at the 10th minute of life
- Or a need for ventilation beyond the tenth minute of life

2. Evidence of moderate to severe encephalopathy within 6 hours of life: convulsion, level of consciousness, spontaneous activity, posture, tonus, reflexes, and autonomic system. (Table 1)

Contraindications

Gestational age less than 35 weeks and 0/7 days
Birth weight less than 1800 grams.[8,17]

RESULTS

Based on the inclusion criteria, five comparative clinical trials were selected, totaling 323 hypoxemic infants.

In the prospective randomized study conducted at the Intensive Care Unit of Newborns of the Mersin University of Medicine, the selective head cooling (SHC) and whole body cooling (WBC) methods were compared. Thirty patients with hypoxic-ischemic encephalopathy born after 35 weeks of gestation were selected. The groups were randomized, with 17 patients assigned to the selective head cooling group and 13 to the whole-body cooling group.

In both groups, the temperature was measured every thirty minutes. The WBC the goal was to keep the actual temperature at 33ºC for 72 hours, and the SHC group goal was to maintain the actual temperature at 34-35ºC for 72 hours. After cooling for 72 hours, the temperature in both groups was increased to 36.5ºC at less or equal to 0.5ºC/h.

The time of the study was twelve months, and at this time, it was possible to observe that there was no significant difference in adverse effects when comparing the two groups. During the period, seven patients in the SHC group and four in the WBC group died; a nonsignificant difference. Adverse effects related to therapy were hypotension, bradycardia, abnormalities in coagulation tests, renal malfunction, hyponatremia, hypokalemia, thrombocytopenia, hypocalcemia, hypoglycemia, high blood concentration, sepsis, and elevated liver enzymes. The complications among patients who survived after twelve months of treatment were severe deficiencies (six in the SHC group and four in the WBC group). The final results of the number of survivors at the end of the study were three survivors in the SHC group and

TABLE 1. CRITERIA FOR DEFINING MODERATE AND SEVERE ENCEPHALOPATHY

Category	Moderate encephalopathy	Severe Encephalopathy
Level of consciousness	lethargic	Stupor or coma
Spontaneous activity	Decreased activity	No activity
Posture	Distal flexion, complete extension	Decerebrate
Tone	Hypotonia (focal or general)	Flaccid
Primitive reflexes: Suck Moro	 Weak Incomplete	 Absent Absent
Autonomic System: Pupils Heart Rate Respiration	 Constricted Bradycardia Periodic Breathing	 Deviated, dilated or nonreactive to light Variable Apnea

Source: Shankaran et al.17

four in the WBC group. Therefore, we can conclude that there were no significant differences between the two treatments, both related to complications and related to death. [18]

Sakar et al.[19] at the University of Michigan conducted a randomized study on therapeutic hypothermia as a neuroprotective factor in neonates with hypoxic-ischemic encephalopathy. Both selective cranial cooling (CSR) and total body cooling (WCT) were used in infants of gestational age greater than or equal to 36 weeks and who had significant hypoxic-ischemic encephalopathy. A total of 59 infants were enrolled, 28 children who received total body cooling and 31 children who received selective head cooling. Hypotension, urinary output <0.5 ml/kg/h for> 24 hours after birth, and electrolyte abnormalities (hyponatremia, hypokalemia, hypocalcemia) were also observed in the groups. Coagulopathy, thrombocytopenia, hypotension. This study suggests that none of the cooling methods offers a lower risk of organic dysfunction[19].

In the non-randomized retrospective study conducted by the University of Michigan, the authors hypothesized that hypoxic-ischemic lesions observed on brain magnetic resonance imaging after cooling were able to differentiate both cooling modalities. The objective was to compare the frequency, distribution, and severity of hypoxic-ischemic lesions between SHC and whole-body cooling WBC. Ninety-eight newborns were selected, but it was possible to use magnetic resonance imaging in 83, of which 34 were subjected to SHC and 49 to WBC. All of them underwent magnetic resonance imaging between 7 and 10 days of life. The classification of brain lesions included two patterns of images in infants with asphyxia: primary lesion of the nucleus of the gray matter, and primary lesion in the areas of vascular borders. Hypoxic-ischemic lesions were observed on MRI after cooling in 47 of 83 infants. The distribution of the lesions was: BGT in 7, cortical not extending beyond the areas of hydrocephalus in 16, cortical not extending beyond the areas of hydrocephalus and basal nuclei in 5, and cortical extending beyond hydrocephalus and basal nuclei in 19. Magnetic resonance imaging was more frequent in patients with CRS, and they still presented more severe lesions.

When comparing the two methods, they observed that during SHC, a relatively better protective effect could be achieved on the cortex. However, no differences were observed between isolated lesions of the cortex. The cortical lesions may not be seen immediately after the examination so they may have been missed because they were examined very early. Another limitation they found was that, because of the retrospective design, it was impossible to rule out the possibility that babies in the SHC group may have had a worse prognosis because of the more severe initial brain lesions.[20]

In another perspective on the differences in hypothermia application, Sakar et al.[21] assumed that SHC allows effective cerebral cooling with less systemic hypothermia and potentially fewer systemic adverse effects. Based on this, they proposed to evaluate pulmonary dysfunction and the potential adverse systemic effects of neuroprotection compared to WBC. Sixty-three infants with gestational period greater than or equal to 36 weeks were selected to receive neuroprotective hypothermia in cases of moderate or severe hypoxic-ischemic encephalopathy. Among these, 33 NB met the clinical, laboratory, and amplitude integrated electroencephalography (aEEG) criteria for the Cool Cap protocol, and received SHC. Another 28 newborns with clinical conditions and similar laboratory criteria received WBC. They observed that the incidence of persistent pulmonary hypertension of the newborn (HPPN) was similar in the WBC and SHC groups and that pulmonary mechanics and gas exchange did not differ with the method of obtaining hypothermia. [21]

Hoque et al.[22] conducted an observational study with four groups who received hypothermia in different ways to determine the differences in temperature and hemodynamic stability between the groups. A total of 73 newborns with HIE were subdivided into four groups: selective head cooling (n = 20), whole-body cooling with manually controlled mattress (n = 23), whole-body cooling with control-wrapped body wrap (n = 28). Hemodynamic changes in mean arterial pressure (MAP) and central temperature in maintenance and rewarming were higher in the whole group, compared to the whole-body servo-controlled group. This means that the difficulty in minimizing maximum variation manually can lead to possible systemic changes, which could compromise the long-term goal of hypothermia.[22]

In a study that assessed the difference between temperatures and cerebral blood flow during selective head cooling and whole-body cooling in 17 pigs, it was observed that initial cooling of the head with constant rectal temperature resulted in an increase

in the temperature gradient in the brain from the warmest central structures to the periphery of the chiller (brain 2cm – dura temperature: 1.3 +/- 1.1 °C on the control to 7.5 +/- 3.5 °C during cooling). Hypoxia overlaid on head cooling decreased the temperature gradient by at least 50 percent. In contrast, body cooling was associated with an unchanged temperature gradient in the brain (brain 2 cm – dura temperature: 1.5 +/- 1.2 °C in the control up to 1.1 +/- 0.9 ° C during cooling).

Hypoxia overlaid on body cooling did not alter brain temperature. Both modes of brain cooling resulted in similar reductions in the overall uptake of cerebral blood flow (approximately 40 percent). This means that whole body cooling provides homogenous cooling to all brain structures, including the peripheral and central regions of the brain. Selective head cooling provides greater cooling to the periphery of the brain than to the central structures of the brain. [23]

DISCUSSION AND CONCLUSION

Based on the evidence, full-body cooling provides homogenous cooling to all brain structures, including the peripheral and central regions of the brain. Selective head cooling provides a wider cooling to the cortical region of the brain than to the central structures. Adverse effects in the five studies analyzed were not significantly different regarding vital organ dysfunction.

Another important observation is that, in order to systematize the application, regardless of the methodology chosen for the cooling, the maintenance of the temperature must be done through servo-controlled monitoring, thus minimizing variations in temperature, blood flow, mean arterial pressure, and others parameters that should be evaluated with caution since the results of hypothermia may be affected.

For improved benefit, head cooling combined with some body cooling has been applied [24,25] and showed that it minimizes temperature gradients throughout the brain and facilitates cooling of the central regions. The association of both methods may be the most promising approach.

The main remaining problems are finding better ways to identify the babies most likely to be benefited, defining the ideal mode and conditions of hypothermia, and finding ways to improve treatment efficacy further and decrease sequelae and deaths.

The limitation of the present review was that most clinical trials are based on one hypothermia manner, SHC or WBC, compared to the standard treatment (normothermia). Clinical trials comparing the modalities of hypothermia between head and the whole-body are being conducted worldwide, so we should get its true benefits, better results, and the shortest negative impact on one of the modalities.

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Author Contributions

[1] Mahara Nonato: Dissertation, interpretation and data analysis;

[2] Larissa Gheler: Dissertation, interpretation and data analysis;

[3] João Vitor Balestrieri: Dissertation, interpretation and data analysis;

[4] Marise Audi: Guidance and finalization;

[5] Mirto Prandini: Guidance and finalization

RESUMO

INTRODUÇÃO: *A possibilidade de a hipotermia ter um papel terapêutico durante ou após a reanimação da asfixia perinatal grave tem sido um foco de pesquisa de longa data. Estudos desenhados em torno desse fato mostraram que a hipotermia cerebral moderada, iniciada o mais cedo possível, tem sido associada à neuroproteção potente e duradoura em espécies perinatais.*

OBJETIVOS: *Resumidamente, analisar os benefícios da hipotermia na melhoria da função celular, com base nas características celulares da lesão cerebral hipóxico-isquêmica e comparar os resultados de dois métodos diferentes de resfriamento do parênquima cerebral.*

MATERIAL E MÉTODOS: *Medline, Lilacs, SciELO e PubMed foram pesquisados para artigos registrados entre 1990 e 2019 nos idiomas português e inglês, com foco em estudos comparando segurança e eficácia do resfriamento corporal total com o resfriamento seletivo da cabeça com EHI.*

RESULTADOS: *Descobrimos que o resfriamento de corpo inteiro fornece resfriamento homogêneo para todas as estruturas cerebrais, incluindo as regiões periférica e central do cérebro. O resfriamento seletivo da cabeça fornece um resfriamento mais amplo para a região cortical do cérebro do que para as estruturas centrais.*

CONCLUSÕES: *Ambos os métodos demonstraram ter propriedades neuroprotetoras, embora o resfriamento de corpo inteiro forneça uma área mais ampla de proteção. Recentemente, o resfriamento da cabeça combinado com algum resfriamento corporal foi aplicado e essa é a maneira mais promissora. O desafio para o futuro é encontrar formas de melhorar a eficácia do tratamento.*

PALAVRAS-CHAVE: *Encefalopatias. Hipotermia induzida. Asfixia neonatal. Hipóxia-isquemia encefálica. Neuroproteção.*

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REVIEW ARTICLE

Melatonin effects on ovarian follicular cells: a systematic review

Isabela Pasqualini Minguini[1]
Camila Maganhin Luquetti[1]
Maria Cândida Pinheiro Baracat[1]
Carla Cristina Maganhin[1]
Camila de Oliveira Nunes[1]
Ricardo Santos Simões[1]
Eduardo Carvalho de Arruda Veiga[1]
José Cipolla-Neto[2]
Edmund Chada Baracat[1]
Jose Maria Soares Junior[1]

1 Disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil
2 Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil

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SUMMARY

Melatonin is known for its effects on both the sleep and reproductive system of mammals. The latter has melatonin receptors type 1 and 2, which act to regulate, among other things, cyclic AMP. Notwithstanding all the literature data, there is still no sound knowledge or a clear understanding of the hormone's action on the physiology of ovarian follicular cells.

OBJECTIVE: *To review and evaluate studies about melatonin action on the ovarian granulosa/theca interna cells from the literature.*

METHODS: *The systematic review was carried out according to the PRISMA recommendations. The MEDLINE and Cochrane primary databases were consulted with the use of specific terms. There was no limitation on language or publication year.*

RESULTS: *Seven papers about melatonin action on granulosa cells were selected. The following can be attributed to the hormone's effects: a) progesterone increase in culture medium; b) increased estrogen production; c) antagonistic action on estrogen; d) improvement in cell quality resulting in improved embryo and higher pregnancy rates; e) improved cell proliferation via MAPK; f) reduction of free radicals. Nevertheless, there are contrarian papers reporting a reduction in progesterone production.*

CONCLUSION: *Melatonin interferes in sex steroid production, boosting progesterone output. Such action may help improve oocyte quality.*

KEYWORDS: *Melatonin. Ovary. Granulosa cells.*

INTRODUCTION

Melatonin is the main hormone produced by the pineal gland. Of all its functions, the most studied and with the best-structured data is the regulation of the circadian cycle and seasonal rhythms of the body[1,2]. Only recently have other functions been studied more intensively. Some of these are control of glucose metabolism, modulation of humoral immune activity and vascular tone, and regulation of human reproductive function[3-6].

Melatonin is an indolamine resulting from sero-

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CORRESPONDING AUTHOR: Eduardo Carvalho de Arruda Veiga
Rua Dr. Enéas de Carvalho Aguiar, 255, 10º Andar – sala 10.166 - Cerqueira César - São Paulo, SP, Brasil - CEP 05403-900 - Tel: 3069-6647
Email: eduveiga56@gmail.com

tonin acetylation and methylation[7]. As a powerful antioxidant[6,8,9], more so than glutathione[7], it has an important role in controlling free radicals. Using endocrine, paracrine, and autocrine signaling, mediated or not by membrane receptors like MT1 and MT2[1,10,11], melatonin plays a still unclear role in human ovarian physiology[7,10]. The human follicular fluid contains high melatonin concentrations – higher than those in serum concentrations – mainly in preovulatory follicles[2]. There are studies correlating melatonin concentrations in follicular fluid with those of progesterone, estradiol, and even oxytocin[12-14]. Thus, the scientific investigation into melatonin's role and action mechanisms in the ovary may open up new horizons for the treatment of several diseases of the female reproductive system, including endometriosis, ovarian neoplasms, and polycystic ovary syndrome[6,15]. In short, the key question to address, which has been the source of much discussion, is melatonin's influence on ovarian follicle cells[7].

This systematic review aimed at gathering and analyzing research work about melatonin effects on human ovarian follicle cells published in the literature up to the present. The ultimate objective was to consolidate knowledge in this field.

METHODS

The systematic review followed the procedures established by PRISMA[16]. The search strategy and the databases that were consulted are shown in Figure 1. An option was made not to exclude papers with respect to publication time to allow an analysis of the relevance of the topic throughout the decades. The search, thereby, yielded articles ranging from 1986 to 2017. The PICO was defined as follows: P (Patients) patients with infertility; I (intervention) melatonin in the granulosa cells; C (control) women with normal menstrual cycle and a male factor; and O (outcome) melatonin's effect.

The initial selection was carried out based on the title; studies unavailable in English, Portuguese, Spanish, Italian, or French or those not addressing the central issue of this review were excluded. Only original papers reporting on research conducted with humans and reviews on the subject were included. Work using animal models was excluded. This phase was followed by the reading of abstracts and the screening out of articles unrelated to the topic.

Study selection was carried out by two researchers (I.P.M. and R.S.S.) who worked independently, following the eligibility criteria. When there was disagreement, a third reviewer was consulted (J.M.S.J.).

The phase above yielded 15 studies to be read entirely (Fig. 2). In addition, reviews, as well as references, were examined to enhance our research (evaluation of the literature in the "gray area").

A table was used to organize the following data from the articles: title, authors, year, study design, number of participants (N), general objectives, methods, results, and study limitations.

TABLE 1. CHARACTERISTICS OF THE STUDIES INCLUDED IN THE SYSTEMATIC REVIEW

Authors and year of publication	Country	Sample	Kind of study	Outcome
Kim et al.3, 2013	South Korea	Human and oocyte granulosa cells	Cell culture	Improvement in embryo implantation
Taketani et al.9, 2011	Japan	Granulosa cells	Cell culture	Decrease of free radicals
Nakamura et al.7, 2003	Japan	Human granulocyte cells and follicles	Cellular and follicular culture	Increased melatonin is accompanied by increased progesterone
Woo et al.10, 2001	Canada	Human granulosa cells	Cell culture	Activation of MAPK (mitosis) and discrete progesterone increase
Bódis et al.17, 2001	Hungary	Human granulosa cells	Cell culture	Estradiol stimulation and progesterone reduction
Niles et al.11, 1999	Canada	Human granulosa cells	Without cell culture	Presence of the melatonin receptor
Yie et al.18, 1995	Canada	Human granulosa cells	Cell culture	Presence of the melatonin receptor Increased production of progesterone

RESULTS

The database search produced a total of 116 articles. Selection narrowed this number down to 7 studies about melatonin action on the granulosa cells (Fig. 2). The data are summarized in Table 1. Most papers were from Canada. Only one study did not use cells from a cell culture; measurements were made directly on the cells after collection. The patients were in the 22 to 35 age range. The studies had a transverse design.

What follows can be attributed to melatonin action on the granulosa cells: a) progesterone increase in culture medium; b) stepped up estrogen production; c) antagonistic action on estrogen; d) improvement in cell quality resulting in improved embryo and higher pregnancy rates; e) improved cell proliferation via MAPK; and f) reduction in free radicals. Nevertheless, there are contrarian papers reporting a reduction in progesterone production[8].

The studies imposed limitations on this review, given the diverse stimulation protocols for the women in the assisted fertility programs and different methods for evaluating melatonin. The varied intracellular signaling pathways further compounded the complexities of the review.

DISCUSSION

Ovulation is a process involving adequate interaction between follicular cells and substances participating in the inflammatory process, such as prostaglandins and cytokines, as well as the action of proteolytic enzymes and vasoactive substances[9]. Regulating this process is crucial for successful egg release and oocyte quality. It is also known that the macrophages, neutrophils, and vascular endothelium itself in the follicles produce reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) during the ovulation process. The ROS participate in follicle maturation and rupture for oocyte release. However, excessive production of such substances is potentially harmful to the granulosa cells as it may hinder ovulation and corpus luteum formation and even impair embryo quality due to changes in DNA. It may compromise the lipid peroxidation of the oocyte membrane as well[5,8]. Therefore, melatonin's antioxidant action may aid in the process[5,8,9]. Other melatonin roles reported in the studies were those as hormone receptor regulator and as an aid in adequate follicle growth.

Taketani et al.[9] evaluated ROS melatonin effects in a culture of follicle cells from healthy women undergoing in vitro fertilization (IVF). Melatonin regulated progesterone production in the cells and reduced ROS. These mechanisms may explain the data showing enhanced implant rate under melatonin treatment[17,19].

Melatonin receptors are located in the ovarian granulosa cells[1,7,10]. Perhaps the reduction in ROS is related to melatonin's intracellular signaling[7]. However, there is evidence that melatonin has other properties, which are independent of its receptors[11,18]. Nonetheless, during the ovulation process, the melatonin levels in the follicular fluid increase threefold over those in blood circulation[20]. The aim is possibly to regulate follicle growth and decrease reactive oxygen species, as well as to influence sex steroid production and action in the follicle microenvironment.

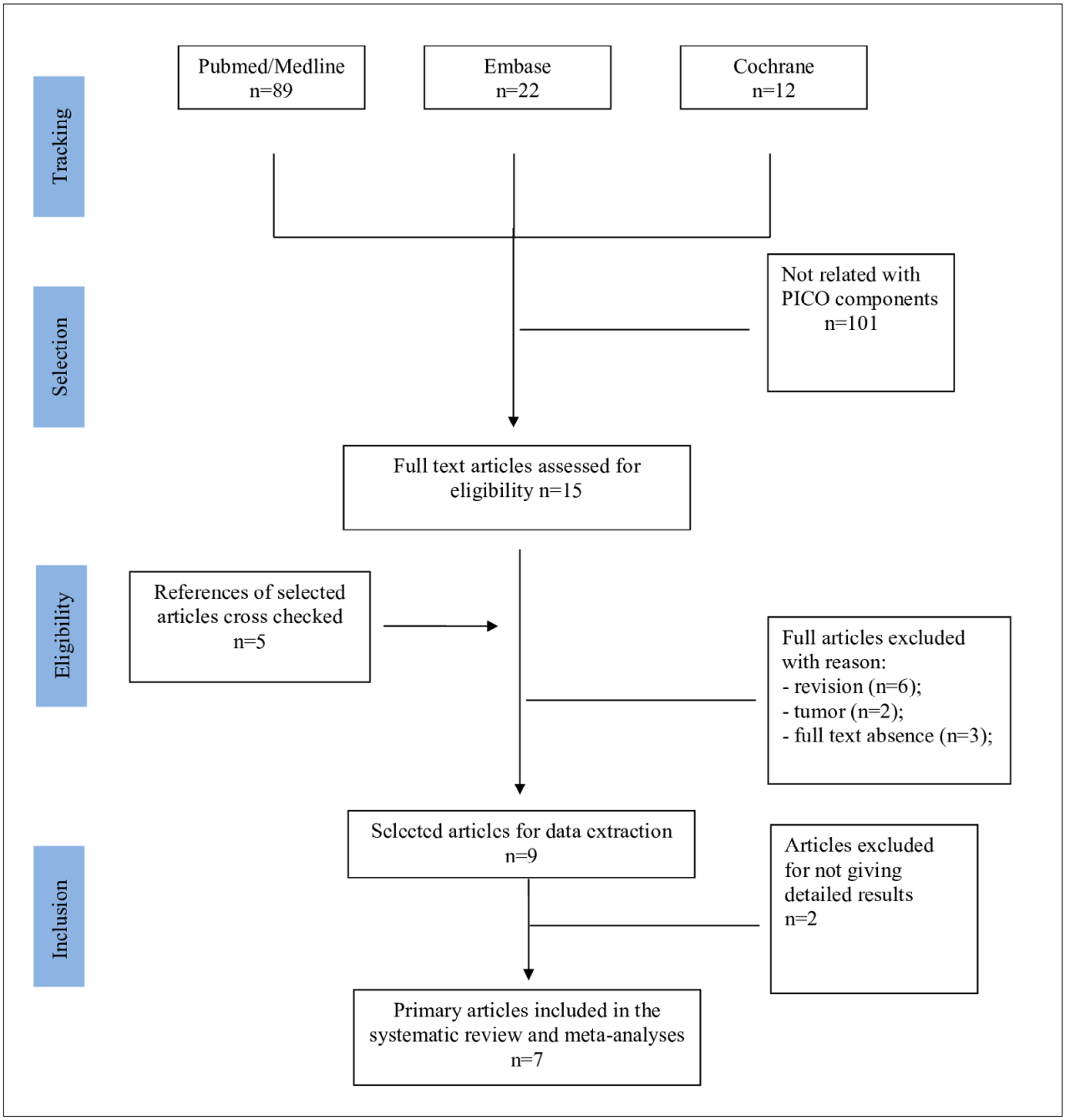
During follicle growth, melatonin's Gi-mediated intracellular signaling pathway, which influences the second messengers (AMPc and GMPc), and its Gs-mediated pathway, which affects PKC activity[1], possibly stimulate granulosa cell proliferation through MAPK activation. This potential mechanism was confirmed in vitro with melatonin treatment and ELK-1 phosphorylation, which is a dose-and-time-dependent action. During ovulation with a high melatonin concentration, the reverse occurs, i.e., a reduction in cell proliferation and in MAPK activation[10]. This is a property that may be important for egg release and enables cell apoptosis, acts as a brake on follicle growth, and reduces ROS[9].

Melatonin's influence on steroidogenesis seems to be dose-dependent, involving both central mechanisms and the ovarian microenvironment. Between 10pM and 100nM of melatonin, there is an increase

FIGURE 1. DATABASES USED AND SEARCH STRATEGIES.

Medline: ("Ovarian Function Tests" OR "Ovarian Reserve" OR "Ovarian Follicle" OR "Theca Cells" OR "Follicular Fluid" OR "Ovary" OR "Ovarian Tissue" OR "Granulosa Cells") AND ("Melatonin" OR "Melatonin Receptors")
Cochrane: Follicular Cells AND melatonin
Embase: 'ovarian follicles/exp AND (melatonin/exp OR ovary/exp).

FIGURE 2. ALGORITHM OF THE SELECTION OF THE STUDIES



in the luteinizing hormone (LH) receptor messenger RNA with no concomitant changes in the follicle-stimulating hormone (FSH) receptor gene expression, and there is also a reduction superior to 45% in gonadotropin-releasing hormone (GnRH) receptor messenger RNA[3,9,10,16]. This action has immediate repercussions in steroid production[11]. With respect to melatonin's direct action on the granulosa cells, it appears that melatonin is involved in the luteinization of the cells and in a rise in progesterone production[21]. This would be an important effect with a bearing on endometrial preparation and embryo receptivity. On the other hand, there is a study showing a reverse action[10]. A tentative explanation is that the heterogeneity of the stimulation protocols used in the studies influenced the number of gonadotropin receptors. Another point is the amount of melatonin on the granulosa cells and their time of exposure to it, which may have impacted steroidogenesis[10]. In general, via its receptor, melatonin can negatively influence estrogen production[16]. This could be important for the pituitary release of LH (peak) as well as for egg release[22].

Our study was hindered by a few limitations capable of affecting our results. For example, the adop-

tion of different stimulation protocols in the ovarian stimulation programs may have influenced both gonadotropin action and steroidogenesis. The time span between the beginning of cell culture and the melatonin treatment varied among the studies. This fact may have biased the analyses, given that gonadotropin receptors may be more abundant in the longer-span cultures and less so in those with a shorter time span. Moreover, melatonin action is known to depend on the interaction with gonadotropins, especially LH. A further limitation was the use of different protocols to evaluate melatonin.

Finally, melatonin seems to interfere in sex steroid production (progesterone increase and estrogen decrease) and in the reduction of free radicals in follicle cells after ovarian stimulation protocols by assisted reproduction techniques. However, it is necessary to confirm this melatonin action on the quality of both the granulosa cells and the oocytes.

CONCLUSIONS

We conclude that melatonin has actions in the production of sex hormones, in the improvement of antioxidant parameters, in the increase of cellular proliferation and in the oocyte quality. However, further studies are necessary to verify the actions of melatonin.

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Competing interests

The authors declare that they have no competing interests

Author Contributions

IPM - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

CML - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

MCPB - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published.

CCM - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published. –

CON - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published. –

RSS - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published. –

ECAV - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published

JCP - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

ECB - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

JMSJ - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

REUMO

A melatonina é conhecida por seus efeitos no sono e no sistema reprodutivo dos mamíferos. Este último tem receptores de melatonina tipos 1 e 2, que atuam para regular, entre outras coisas, o AMP cíclico. Apesar de todos os dados da literatura, ainda não há um conhecimento sólido ou uma compreensão clara da ação do hormônio na fisiologia das células foliculares ovarianas.

OBJETIVO: *Revisar e avaliar estudos da ação da melatonina na literatura sobre as células internas da granulosa/teca ovariana.*

MÉTODOS: *A revisão sistemática foi realizada de acordo com as recomendações do Prisma. As bases de dados primárias Medline e Cochrane foram consultadas com o uso de termos específicos. Não houve bar na língua ou ano de publicação.*

RESULTADOS: *Sete artigos sobre a ação da melatonina nas células da granulosa foram selecionados. O que se segue pode ser atribuído aos efeitos do hormônio: a) aumento de progesterona no meio de cultura; b) aumento da produção de estrogênio; c) ação antagônica no estrogênio; d) melhoria na qualidade celular, resultando em melhor embrião e maiores taxas de gravidez; e) melhor proliferação celular via MAPK; f) redução de radicais livres. No entanto, existem artigos controversos relatando redução na produção de progesterona.*

CONCLUSÃO: *A melatonina interfere na produção de esteroides sexuais, aumentando a produção de progesterona. Tal ação pode ajudar a melhorar a qualidade do oócito.*

PALAVRAS-CHAVE: *Melatonina. Ovário. Células da granulosa.*

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