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SECTIONS

ARTICLES

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Cardiovascular system and estrogen in menopause

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Women are known to have a lower incidence of cardiovascular disease than men of the same age, but this benefit disappears after menopause. Thus the risk of cardiovascular disease (CVD) increases greatly after menopause when estrogen levels decrease. Women are typically about ten years older than men at the first presentation of atherosclerotic coronary heart disease, and this appears to be related to the decline in postmenopausal ovarian hormone concentrations.

Estrogen-mediated vascular actions are mainly attributed to estradiol and exerted by estrogen receptors (ERα, ERβ, and G) through rapid and/or genomic mechanisms, but these effects depend on aging and vascular status. Thus estrogens can modulate vascular function by targeting estrogen receptors in endothelial and vascular smooth muscle cells. Estrogens induce the release of nitric oxide and prostacyclins, both vasodilators. They may also cause a reduction in endothelin and angiotensin II production, which are vasoconstrictors. In addition, estrogens also reduce inflammation and may reduce the secretion of pro-atherogenic cytokines such as tumor necrosis factor-alpha (TNF-α) and may increase prostaglandin, which reduces oxidative stress and also platelet activation.

In experimental studies, ERα-acting estradiol promotes the release of vasoactive compounds such as nitric oxide (NO) and prostacyclin and shifts the angiotensin axis to angiotensin production[1-7]. The mechanisms underlying estradiol vascular function also include anti-inflammatory and epigenetic modifications. 17β-estradiol alters the transcriptomic profile of endothelial cells, and miRNA involvement in vascular function regulatory pathways reinforces assumptions about estrogen vascular actions.

There is currently evidence that women of any age with vasomotor symptoms have a worse cardiovascular risk profile (increased risk of CVD, coronary heart disease, or ischemic stroke) compared with women without vasomotor symptoms. Women with vasomotor symptoms have significantly higher systolic and diastolic blood pressure, higher circulating total cholesterol levels, and a higher body mass index than women without symptoms.

However, data from the Women's Health Initiative (WHI) study point to an association between menopausal hormone therapy (THM) and cardiovascular risk (CV). However, analyses of post hoc subgroups that stratified participants according to their age and time of menopause paved the way for a better understanding of the relationship between estrogen and cardiovascular risk.

Thus, reviews that evaluated CV risk or benefit after estrogen administration, considering various factors, such as time, dose, route of administration, and formulation of MHT. Thus, MHT onset time was a critical factor in CV risk assessment. Consistent with the "time hypothesis", healthy symptomatic women who started THM younger than 60 years or within ten years of the onset of menopause were shown to have reduced risk of coronary heart disease (CHD) and mortality from all causes. In particular, MHT therapy has been associated with improved subclinical signs of

atherosclerosis. The risk of venous thromboembolism (VTE) was reduced when low doses of oral estrogen were used. In addition, transdermal hormone application significantly reduced CV risk compared with oral administration. The impact of MHT on the CV system was influenced by factors inherent to the specific regimen or individual patient. Therefore, the individualization of care is necessary[8-13]. Conclusion: Hormone therapy remains the most effective treatment for menopausal symptoms, but decisions are complex, requiring an assessment of benefits and risks and determining the best type, dose, and duration of treatment. Benefits outweigh risks for most women with bothersome symptoms of menopause or high risk of fracture if started when younger than 60 years or ten years after menopause onset. CV risk calculation should be considered by physicians to exclude patients with high CV risk, to whom MHT is contraindicated. Risk and benefit assessment in a patient-centered approach based on individual resources, health status, and personal preferences is important for safe and effective treatment.

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Hematological changes in Covid-19 infections

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The infection by COVID-19 (SARS-CoV-2) has been taking on proportions of pandemic characteristics. Preventive infection measures for this virus, as well as epidemiological, pathophysiological, diagnosis, and treatment knowledge in relation to it are extremely needed. The advances in diagnostic tests, whether in the detection of the antibody through "fast" tests, or in tests to identify the presence of the virus (using the technique of RT-PCR of respiratory samples from affected patients) are very important, as they help both in epidemiology, for case tracking and containment of outbreaks, as in the earlier diagnosis of the disease, leading to a favorable outcome, especially in severe cases. In this context, we are concerned about determining biomarkers that could be used in screening for diagnosis, as well as in monitoring the evolution of COVID-19 infections.

The complete blood count is the test used to approach infections that are very prevalent in Brasil, with emphasis on arboviruses, especially Dengue. The exam assesses hematopoietic lineage from a quantitative and qualitative viewpoint. In the Chinese population, studies have reported the presence of leukopenia on hospital admission, basically at the expense of moderate to severe lymphopenia and mild thrombocytopenia [2]. The review of studies that contained analyses of peripheral blood samples showed that a greater number of lymphopenic patients had the presence of reactive lymphocytes, of which a subset appeared to be lymphoplasmacytoid [2]. Thus, the monitoring of these hematological parameters is essential and can assist in the identification of patients who will need care in the Intensive care unit, as they presented a deeper lymphopenia, as well as a decrease in hemoglobin, absolute monocyte count and even tend to develop neutrophilia during hospitalization, with a peak in this period of ICU stay [FAN et al., 2020]. The presence of atypical or activated lymphocytes is not significant for the degree of infection, the main comorbidity of which is Acute Respiratory Distress Syndrome (ARDS). This contrasts with what is observed in severe dengue during the admission of patients from a referral hospital of Fortaleza, Brasil, and this parameter can have a screening function as a modulator of the evolution of the viral process in arboviruses [1]. The non-alteration of the erythroid lineage, such as hemoconcentration and a slight numerical alteration of platelets, when present, also contrasts with severe dengue [1]. Lymphopenia, which has also been documented in chikungunya [3], in covid-19, seems to be the most relevant peripheral hematopoietic alteration, its use being suggested as a severity biomarker of the infection. In this context, studies related to the evaluation of biomarkers of hematological parameters that can be used as screening for exam diagnosis, as well as monitoring the evolution of severe cases, when necessary.

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Advanced melanoma in adults: Pembrolizumab as a treatment option

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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.

PD-1 (programmed cell death protein 1) is a immunologic checkpoint that limits the activity of T cells (lymphocytes) in peripheral tissues. Pembrolizumab is a IgG4 monoclonal antibody, highly selective and humanized, which binds to the programmed cell death protein 1 receptor and blocks its interaction with the PD-L1 and PD-L2 ligands. A systematic review was performed using the PICO search system Adult patients with advanced melanoma (completely resectable or unresectable) without distant metastasis (patient) Treatment with pembrolizumab compared with another or no therapy (intervention) death (any cause); death or recurrence; and adverse events (outcome). The search process resulted in 323 papers, of which three were included. The details of the methodology and the results of this guideline are set out in Annex 1.

INTRODUCTION

PD-1 (programmed cell death protein 1) is a immunologic checkpoint that limits the activity of T cells (lymphocytes) in peripheral tissues. The PD-1 pathway is a immunological control checkpoint that can be coupled by tumor cells to inhibit immune surveillance of the active T cell.

Pembrolizumab is a IgG4 monoclonal antibody that is highly selective, humanized, and binds to the programmed cell death protein 1 receptor and blocks its interaction with the PD-L1 and PD-L2 ligands, which are expressed in antigen-presenting cells and may be expressed by tumors or other cells in the tumor microenvironment, assisting them in preventing of their detection and elimination by the immune system of the host.

The involvement of PD1 on the surface of lymphocytes by PD-L1 in melanoma cells provides inhibitory signals that negatively regulate the function of T cells.

The advent of monoclonal antibodies that target CTLA-4 (ipilimumab) or PD-1 (nivolumab and pembrolizumab) checkpoints increased the expectations for better outcomes in advanced melanoma. However, resistance remains an important issue. Genetic

mutations and deregulation of the immune system may be a factor in some patients and have a significant impact on the effectiveness of therapies.

RESULTS

Our study population included 2,393 patients with advanced melanoma who underwent pembrolizumab therapy (N=971) compared to ipilimumab (N=278), chemotherapy (N=179), or a placebo (N=505) and followed-up to measure the outcomes of death and adverse events at 6 or 12 months (Table 1).

Regarding the bias risk of the three studies included, two of them were not double-blind[3,4], and all had losses >20%, so the overall risk of the studies can be considered moderate (Table 2).

All studies assessed the outcome of death from any cause, and one of them assessed the composite outcome of death or recurrence (local, regional, or distant metastasis)[5] (Table 3).

TABLE 1. CHARACTERISTICS OF THE STUDIES INCLUDED

DESCRIPTIVE TABLE OF STUDY CHARACTERISTICS					
STUDY	Population	Intervention	Comparison	Out-comes	Time (medi-an, months)
SCHACHTER, 2017 KEYNOTE-006	834 patients >18 years (62 years median) with: melanoma confirmed histologically; unresectable; stage III or IV; without prior therapy with ipilimumab (previous treatment with one or two other therapies was allowed); wild-type or mutant Braf; Ecog 0 to 1; at least a measurable lesion per Recist version 1.1. Exclusion criteria: previous therapy with CTLA, PD-1, or PD-L1 inhibitors, ocular melanoma, cerebral metastasis, and severe autoimmune disease requiring corticosteroid therapy.	Pembrolizumab 10 mg/kg 2/2 weeks or 3/3 weeks (Q3W)	Ipilimumab 3 mg/kg 3/3 weeks, 4 cycles	OS SFP Adverse events	22.9
HAMID, 2017 KEYNOTE-002 Stage II	540 patients (median age of 62 years) with progressive unresectable melanoma in stage III-IV after ipilimumab or therapy with MEK or Braf inhibitor (or both), if positive mutant $Braf^{v600}$; Ecog status of 0 or 1; at least one measurable lesion. We excluded patients with active brain metastases or carcinomatous meningitis, active autoimmune disease, active infection requiring systemic therapy; HIV infection, active virus of hepatitis B or C, history of adverse events related to ipilimumab grade 4 or 3, or previous treatment with any other anti-PD-1 or anti-PD-L1 therapy.	Pembrolizumab 2 mg/kg, IV Q3W or 10 mg/kg Q3W	Chemotherapy chosen by the investigator (paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide)	OS Adverse events	28
EGGER MONT, 2018 KEYNOTE-O54	1,019 patients >18 years (54 years median) with metastatic cutaneous melanoma for regional lymph node, stage IIIA, IIIB, or IIIC, with complete resection and without distant metastasis. Complete regional lymphadenectomy was performed ≤13 weeks prior to the initiation of treatment. Exclusion criteria: Ecog >1(0-5); autoimmune diseases; uncontrolled infections; use of corticosteroids, and previous therapy for melanoma. 84% had a positive expression of PD-L1 (>1% of expression).	Pembrolizumab 200 mg IV every 3 weeks (Q3W), up to 18 doses, or until disease recurrence or unacceptable toxic effect	Placebo	OS SFP Adverse events Quality of life	15.1

Q3W = every 3 weeks; Ecog = Eastern Cooperative Oncology Group (minimum score of 0 and maximum of 5)

TABLE 2. DESCRIPTION OF THE BIASES OF THE STUDIES INCLUDED

Study/Year	Random	Blinded allocation	Double-blind	Losses	Prog. Charac-teristics	Outcome	ITT Analysis	Sample size cal-culation
Schachter, 2017								
Hamid, 2017								
Egger Mont, 2018								

ITT = intention-to-treat analysis. (blue) Low risk of bias (orange) Presence of bias (yellow) Unclear risk of bias

TABLE 3. STUDY RESULTS FOR THE OUTCOME OF DEATH

Study	Pembro patients/ events	Ipilimumab patients/ events	QT (paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide)	Placebo
Schachter, 2017(3) Keynote-006	IN 12 MONTHS 277/93 Death outcome 10 mg/kg; Q3W	278/133		
Hamid, 2017(4) Keynote-002	IN 12 MONTHS 180/85 Death outcome 2 mg/kg; Q3W		179/99	
Eggermont, 2017(5) Keynote 054	IN 6 MONTHS 514/101 Death or recurrence outcome 200 mg Q3W			505/142

Q3W = every 3 weeks

1. Pembrolizumab vs. Ipilimumab (1-year follow-up)

The study allows us to evaluate the outcome of death in up to 12 months, comparing pembrolizumab (10 mg/Kg, Q3W) with ipilimumab and shows a reduction of 14% (95% CI, 22 to 6%), favoring the use of pembrolizumab (p=0.0005); it is necessary to treat (NNT) seven patients (95% CI, 4 to 16) to prevent one death in this period (Figure 2).

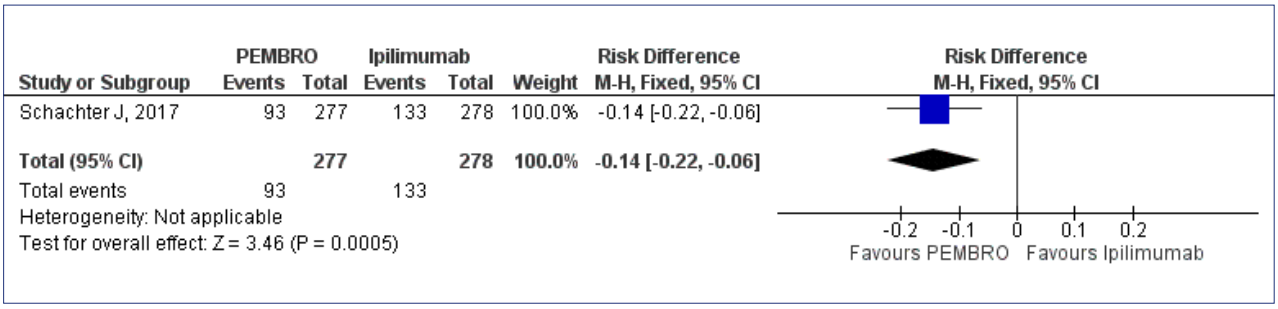

FIGURE 2. COMPARISON CHART: 1. PEMBROLIZUMAB *VERSUS* IPILIMUMAB IN THE TREATMENT OF ADVANCED MELANOMA, OUTCOME: DEATH IN UP TO 12 MONTHS.

2. Pembrolizumab vs. Chemotherapy (1-year follow-up)

Only one study compared pembrolizumab (2 mg/kg, Q3W) *versus* chemotherapy, as chosen by the investigator (paclitaxel plus carboplatin and paclitaxel, carboplatin, dacarbazine, or oral temozolomide), and allows to evaluate the outcome of death within 12 months.

There was no difference between the two therapies in relation to the risk of within 12 months [Absolute Risk Reduction (ARR) = -0.08 (-0.18 to 0.02); p=0.012; the result is not significant] (Figure 3).

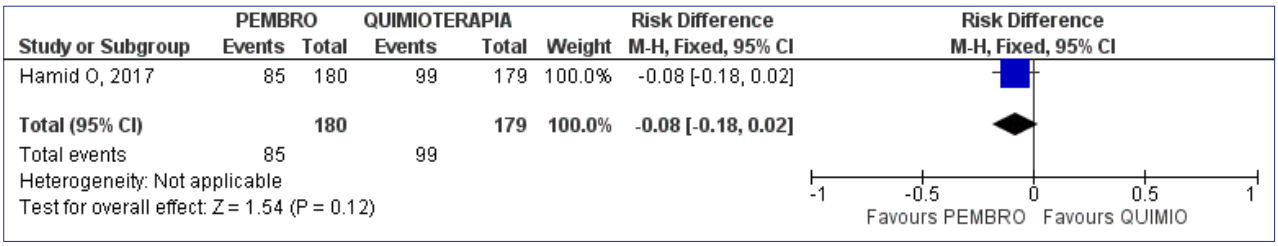

FIGURE 3. COMPARISON CHART: 2. PEMBROLIZUMAB *VERSUS* CHEMOTHERAPY IN THE TREATMENT OF ADVANCED MELANOMA, OUTCOME: DEATH IN UP TO 12 MONTHS.

3. Pembrolizumab vs. Placebo (6-month follow-up)

The study allows us to calculate the risk of death or recurrence within six months, comparing pembrolizumab (200 mg, Q3W) *versus* a placebo. There was a reduction in the risk of death or recurrence by 8% (IC 95%, 14% to 3%) favorable to pembrolizumab (p=0.001); it is necessary to treat (NNT) 12 patients (95%, CI 7 to 30) to avoid one recurrence or death in up to six months, Figure 4.

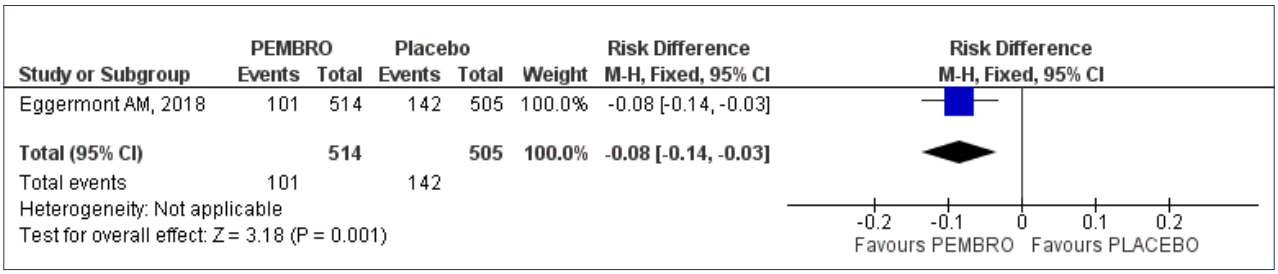

FIGURE 4. COMPARISON CHART: 3. PEMBROLIZUMAB VERSUS PLACEBO IN THE TREATMENT OF ADVANCED MELANOMA, OUTCOME: DEATH OR RECURRENCE IN UP TO 6 MONTHS.

ADVERSE EVENTS

All three comparisons of pembrolizumab[3-5] evaluated adverse events grade ≥3, regardless of the cause, in the population treated, Table 4. Pembrolizumab showed no difference in the number of adverse events grade ≥3 in comparison with ipilimumab (ARR=2.9, CI 95% -0.03 to 0.09; NNT=NS), however, it reduced the risk by 12.8% (95% CI, 45% to 21%) in comparison with chemotherapy (NNT=8, 95% CI, 5 to 22). In comparison with the placebo, pembrolizumab increased the risk of adverse events grade ≥3 by 13% (95% CI, 18% to 7.8%).

TABLE 4. ADVERSE EVENTS GRADE ≥III - ALL CAUSES - IN THE POPULATION TREATED

Study	Pembro patients/ events	Ipilimumab patients/ events	QT (paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide)	Placebo	ARR% or ARI% (CI 95%)	NNT or NNH (CI 95%)
Schachter, 2017 Keynote-006	277/46 10 mg/kg; Q3W	256/50			ARR=2.9 (−0.036 to 0.094)	NNT=NS
Hamid, 2017 Keynote-002	178/24 2 mg/kg; Q3W		171/45		ARR=12,8 (0.045 to 0.211)	NNT=8 (5 to 22)
Eggermont, 2018 Keynote-054	509/161 200 mg Q3W			502/93	ARI=−13.1 (-0.184 to -0.078)	NNH=8 (3 to 13)

ARR = absolute risk reduction; ARI = absolute risk increase; NNT = number needed to treat; NNH = number need to harm; CI = confidence interval

SYNTHESIS OF THE RESULTS FOR THE OUTCOME OF DEATH

Outcome or subgroup	Studies	Participants	Statistical Method	Estimated effect
1. Death iN up to 12 months Pembro vs. Ipilimumab	1[3]	555	Difference in risk (M-H, Fixed, 95% CI)	-0.14 [-0.22, -0.06]
2. Death in up to 12 months Pembro vs. Chemo	1[4]	359	Difference in risk (M-H, Fixed, 95% CI)	-0.08 [-0.18, 0.02]
3. Death or recurrence in up to 6 months Pembro vs. Placebo	1[5]	1,019	Difference in risk (M-H, Fixed, 95% CI)	-0.08 [-0.14, -0.03]

QUALITY OF EVIDENCE FOR THE OUTCOME OF DEATH

1. Pembrolizumab versus Ipilimumab

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Pembrolizumab	Ipilimumab	Relative (95% CI)	Absolute (95% CI)		
Death in up to 12 months												
1	RCT	severe [a]	not severe	not severe	not severe	None	93/277 (33.6%)	133/278 (47.8%)	RR 0.70 (0.57 to 0.86)	minus 14 per 100 (from minus 21 to minus 7)	⊕⊕⊕○ MODERATE	IMPORTANT

RCT: Randomized clinical trial; CI: Confidence interval; RR: Risk ratio

Explanations

a. A loss greater than 20%

2. Pembrolizumab versus Chemotherapy

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Pembrolizumab	Chemo	Relative (95% CI)	Absolute (95% CI)		
Death in up to 12 months												
1	RCT	severe [a]	not severe	not severe	severe [b]	none	85/180 (47.2%)	99/179 (55.3%)	RR 0.87 (0.70 to 1.05)	7 less per 100 (from 17 less to 3 more)	⊕⊕○○ LOW	IMPORTANT

RCT: Randomized clinical trial; CI: Confidence interval; RR: Risk ratio

Explanations

a. A loss greater than 20%

b. The result was not significant. Small sample size

3. Pembrolizumab versus Placebo

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecise	Other considerations	Pembrolizumab	Placebo	Relative (95% CI)	Absolute (95% CI)		
Death in up to 12 months												
1	RCT	severe [a]	not severe	not severe	not severe	None	101/514 (19.6%)	142/505 (28.1%)	RR 0.70 (0.56 to 0.87)	minus 8 per 100 (from minus 12 to minus 4)	⊕⊕⊕○ MODERATE	IMPORTANT

RCT: Randomized clinical trial; CI: Confidence interval; RR: Risk ratio

Explanations

a. A loss greater than 20%

SYNTHESIS OF EVIDENCE

In patients with unresectable melanoma; stage III or IV; without prior therapy with ipilimumab; with no ocular melanoma or cerebral metastasis; wild-type or mutant Braf, pembrolizumab (first or second line) in comparison with ipilimumab: reduces the risk of death by 14% (NNT=7, 95% CI, 4 to 16), in up to 12 months. The quality of the evidence that supports this result is moderate.

In patients with progressive unresectable melanoma in stage III-IV, after ipilimumab or therapy with MEK or Braf inhibitor (or both), if positive for mutant $Braf^{v600}$, pembrolizumab in comparison with chemotherapy: showed no statistical difference in the risk of death in up to 12 months. The quality of the evidence that supports this result is low. However, pembrolizumab reduces the risk of adverse events in grade ≥III - quality of evidence low.

In patients with cutaneous melanoma metastasis to a regional lymph node; stage IIIA, IIIB, or IIIC; with complete resection and regional lymphadenectomy; without distant metastasis and prior therapy, pembrolizumab in comparison with a placebo: Reduces the risk of death or recurrence by 8% (NNT=12, 95% CI, 7 to 30), in up to six months. The quality of the evidence that supports this result is moderate.

DISCUSSION

The evidence currently available on the efficacy and safety of pembrolizumab as the first or second line of treatment for advanced melanoma is based on three randomized clinical trials[3-5].

The effectiveness results presented by the studies show that pembrolizumab decreased the risk of death in cases of unresectable melanoma in comparison with ipilimumab[3], as well as in adjuvant therapy for stage III melanoma with lymph node involvement, with complete resection, compared with a placebo[5]; however, the magnitude of the benefit in the long term is not clear. For stage III-IV progressive unresectable melanoma, after ipilimumab or therapy with MEK or Beaf inhibitor (or both), if positive for mutant $Braf^{v600}$, pembrolizumab in comparison with chemotherapy showed no statistically significant reduction in the risk of death; however, this result should be considered with caution due to the high risk of bias, indicating a low quality of evidence[4].

In this review, we chose to use, whenever possible, the branch of the study that used a dose of 2 mg/kg of pembrolizumab, Q3W, if there was a branch that used 10 mg under the same conditions[4].

Considering the three studies, the rates of patients with positive PD-L1 tumor ranged from 83.3% to 54% and negative PD-L1 from 26.7% to 11.5%.

We did not consider the results of PD-L1 subgroup analyses of the studies in this review, due to the risk of false-positive results, avoiding the following question: is there indeed a significant difference in the treatment effect or it is merely a random occurrence (considering the absence of prior sample size definition and subsequent statistical power for this difference)?

ANNEX I

Clinical question

What is the impact of pembrolizumab on the outcomes of overall mortality (death from any cause) and adverse events in the treatment of patients with advanced melanoma when compared to chemotherapy alone?

Structured question

P	Adult patients with advanced melanoma (completely resected or unresectable) without distant metastasis
I	Treatment with pembrolizumab compared with another or no therapy
C	-
O	Death (any cause); death or recurrence; and adverse events

Eligibility criteria

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.

No restriction was made regarding the year of publication or language.

The following outcomes were excluded: quality of life, objective response, progression-free survival; subgroup analysis will not be evaluated (PD-L1 in tumor tissue) and cycles every two weeks.

Randomized clinical trial studies were included.

Search for papers

Database

The search for evidence will be conducted on Medline virtual scientific information database, and, when necessary, manually in the references found from references.

The search in these databases was performed by the month of June 2019, and a systematic review was performed according to the Prisma recommendations.

Search strategy

PubMed: Melanomas OR Malignant melanoma OR Malignant Melanomas) AND Pembrolizumab AND Random*;

CENTRAL / Cochrane: (Melanomas OR melanoma) AND Pembrolizumab

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.

Results application - External validity

We will extract the following data from the studies: name of the author and year of publication, study population, intervention and comparison methods, absolute number of deaths and adverse events, time of follow-up.

Randomized clinical trials will have their risk of biases analyzed according to the following criteria: randomization, blinded allocation, double-blinding, losses, prognostic characteristics, presence of relevant outcome, time for the outcome, method for outcome measurement, sample size calculation, early interruption, presence of other biases.

The results will be presented as the difference in the risk of death or adverse events between pembrolizumab therapy and another or no treatment. The confidence level adopted was 95%.

The results of the studies included will be meta-analyzed by RevMan 5.3[1], and the difference in overall risk will be the final measure used to support the synthesis of evidence that will answer the clinical question of this review.

The quality of evidence will be graded as high, moderate, low, or very low using the Grade instrument[2] and taking into account the risk of bias, the presence of inconsistency, vagueness or indirect evidence in the meta-analysis of the outcomes of death and adverse events, and the presence of publication bias.

Results

The search for evidence retrieved 323 papers, of which six were selected based on their title and abstract on immunotherapy with pembrolizumab, for the treatment of patients with advanced melanoma, in comparison with ipilimumab, chemotherapy, or a placebo. The six studies that met the eligibility criteria were then were accessed for analysis of their full text. Of the six studies, three were selected to support this assessment[3-5]; the grounds for exclusion and the list of studies excluded are available in the references, Figure 1, and Table 5.

The selection of retrieved from the virtual databases of scientific information is detailed in the flowchart below:

FIGURE 1. FLOWCHART

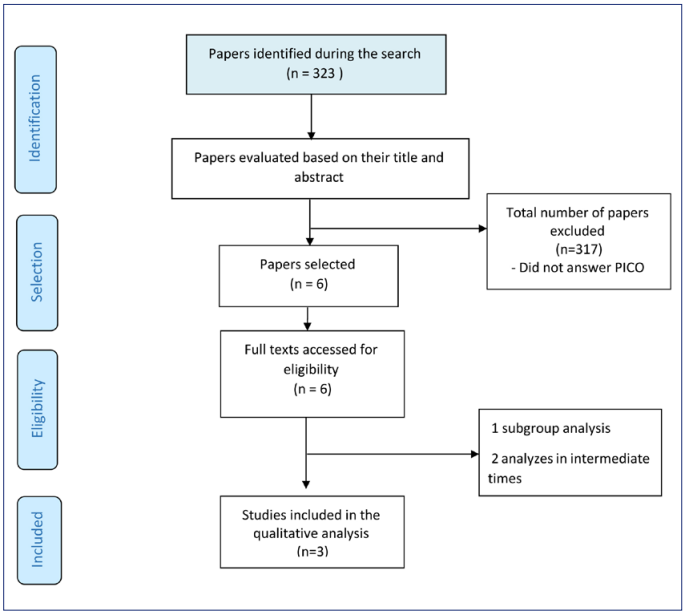

TABLE 5. STUDIES EXCLUDED AND REASON FOR EXCLUSION

Study	Reason for exclusion
Carlino MS, 2018	Subgroup analysis
Ribas A, 2015	Analysis in an intermediate time
Robert C, 2015	Analysis in an intermediate time

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.

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PAPERS EXCLUDED

(REASONS IN TABLE 9 – ANNEXES)

Carlino MS, Long GV, Schadendorf D, Robert C, Ribas A, Richtig E, et al. Outcomes by the line of therapy and programmed death-ligand 1 expression in patients with advanced melanoma treated with pembrolizumab or ipilimumab in KEYNOTE-006: A randomised clinical trial. Eur J Cancer 2018;101:236-243. PMID: 30096704

Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 2015;16:908-18. PMID: 26115796

Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015 25;372:2521-32. PMID: 25891173

Urinary lithiasis: evaluation of the use of laser vs. Pneumatic ureteral lithotripsy

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Question: In the treatment of urinary lithiasis by ureterolithotripsy, is the use of a laser energy source superior to the conventional one (pneumatic)?

Answer: In patients with urinary lithiasis and stones <20 mm affecting the ureter. There is no difference in the procedure time and the need for ureteral stent between the two types of treatment (laser and pneumatic ureterolithotripsy). The laser treatment offers increased rates of therapeutic success and stone free rate outcomes and reduces the risk of complications when compared with pneumatic ureterolithotripsy[1].

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Substernal Goiter: a case to remember

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SUMMARY

Goiter is a localized or generalized thyroid hypertrophy. It can remain within the cervical region or grow down until it invades the mediastinum. The signs and symptoms depend on the size and location of the goiter. Although drugs and radioactive iodine are often used to treat thyroid disease, the presence of symptomatic substernal goiter is a clear indication for surgery. Death or postoperative complications rarely occur.

We present a case of a 71-year-old man with recurrent thyroid pathology in the form of substernal goiter and hyperthyroidism even after partial thyroidectomy. The importance of this relates to the clinical evolution, volume, and location of the goiter as well as the surgical and pharmacological approach.

KEYWORDS: *Goiter. Goiter, substernal/surgery. Hyperthyroidism.*

INTRODUCTION

A 71-year-old man, with istmo-lobectomy of the left thyroid, was referred with hypothyroidism. In 4 months, he recovered from a euthyroidism state under levothyroxine 50 µg. Cervical ultrasound showed a heterogeneous right lobe with two small hypoechoic nodules of 5 mm. Two years later, in the Emergency Department, he presented persistent irritative cough and dysphonia with a month of evolution and without dysphagia, difficulty breathing, or fever. Upon examination, there were no palpable thyroid or cervical adenopathies, oropharynx without alterations, blood pressure of 142/70 mmHg, heart rate of 99 bpm, temperature of 37ºC, eupneic on ambient air, rhythmic on cardiac auscultation, vesicular murmurs on pulmonary auscultation, and absence of peripheral edema. He started systemic corticosteroid therapy due to irritative cough and dysphonia. Blood tests showed hyperthyroidism [TSH < 0.01 mUI/L (0.350 5.500 mUI/L), Free T4 2.2 ng/dL (0.9 - 1.8 ng/dL), Free T3 4.6 pg/mL (2.0 - 4.2 pg/mL)] and calcitonin <1.00 pg/ml (0.40 - 18.90 pg/mL). The patient started propylthiouracil 100 mg tid. A chest radiograph showed an enlargement of the mediastinum. Cervico-thoracic computed tomography revealed a solid mediastinal mass originated on the lower half of the right lobe and the isthmus of the thyroid, which was compressing the left brachiocephalic vein (*Fig. 1* and *Fig.2*). Thyroid scintigraphy showed marked hypertrophy

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of the right lobe with an extensive hypoactive area in the lower half and an extensive area with a slight uptake of the prolonged radiopharmaceutical. Bronchofibroscopy showed an extrinsic compression of the trachea. The patient underwent thyroidectomy without complications. Histological analysis revealed a benign follicular nodule. After thyroidectomy, the patient remained in euthyroidism with levothyroxine 100 µg.

DISCUSSION

There is a strong correlation between incidences of substernal goiter and cervical goiter and endemic regions[1,2]. The incidence of substernal goiter is difficult to assess, but 2.6-21% of these patients undergo thyroidectomy[3]. This variation reflects the lack of a definitive definition of substernal goiter. About 85-95% of substernal goiters are benign and represent up to 7% of mediastinal tumors[3]. Other mediastinum lesions, including thymomas, lymphomas, dermoid cysts, pleuropericardial cysts, and neurogenic tumors, must be considered in the differential diagnosis.

Although 5 to 50% of the patients are asymptomatic, symptoms such as dyspnea, stridor, and dysphagia are common and result from substernal goiter compression of the trachea and/or esophagus[4]. Superior vena cava syndrome and progressive hoarseness are less common symptoms.

Hyperthyroidism is often associated with substernal goiter. Although drugs and radioactive iodine are often used to treat thyroid disease, the existence of substernal goiter, particularly if symptomatic, is a clear indication for surgery[3,5,6]. In most cases, this type of goiters can be excised successfully through a transcervical approach, although transthoracic approaches are occasionally required[7,8]. Such as our clinical case, recurrence is not rare after partial thyroidectomy[1,9]; therefore, some authors recommend systematic total thyroidectomy, even if the contralateral lobe appears healthy[3,10].

Certain radiological findings may alert the surgeon to the possible need for such an approach. Computed tomography (CT) scanning is currently the most useful tool in the preoperative assessment of patients' anatomy. CT assesses the extension toward the aortic arch[1,11], locates the goiter (anterior, posterior, or mixed) and any stenosis as well as pathological interaction with any other organs and the esophagus in particular[1,12].

As highlighted in this case, patients undergoing thyroid surgery should have preoperative thyroid function studies as well as appropriate medical management of any thyrotoxicity to avoid thyroid storm[10].

Postoperative mortality usually implicates cardio-respiratory failure[13], or sometimes a hemorrhagic, infectious or thyrotoxic event, or even pulmonary embolism. The most frequent complications are

FIGURE 1. SUBSTERNAL GOITER

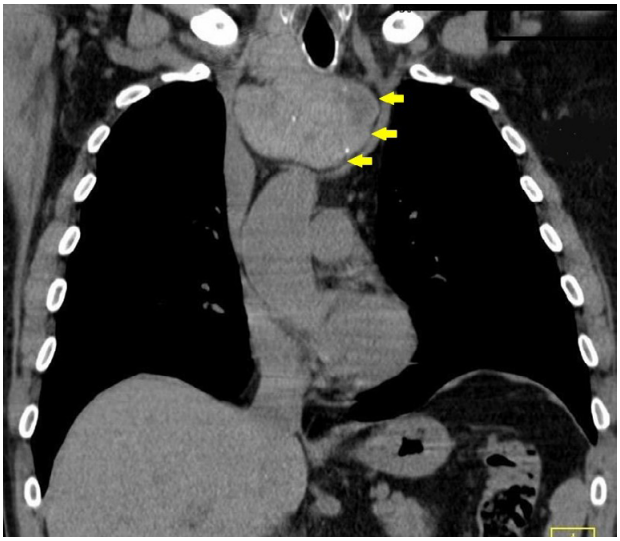
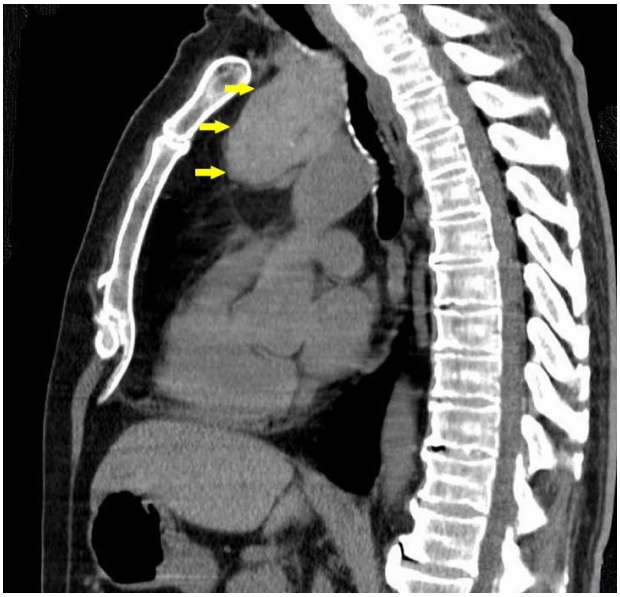


FIGURE 2. CERVICO-THORACIC COMPUTED TOMOGRAPHY WITH A SOLID MEDIASTINAL MASS ORIGINATED ON THE LOWER HALF OF THE RIGHT LOBE AND THE ISTHMUS OF THE THYROID



vascular and tracheoesophageal wounds. Recurrent nerve lesions are rarely recognized before extubation. Despite all of this, death or postoperative complications rarely occur[6].

After surgery, supplementation with levothyroxine is required. The prognosis is generally good.

Disclosure of interest

The authors report no conflict of interest.

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Authors contributions

Sara Simões Macedo collected data, performed the analysis, and wrote the paper; Mónica Teixeira performed the analysis and wrote the paper; Andreia Correia wrote the paper; Cátia Cabral wrote the paper.

RESUMO

O bócio é a hipertrofia da glândula tiroide localizada ou generalizada. Esta pode localizar-se na região cervical ou crescer através do mediastino. Os sinais e sintomas dependem do tamanho e da localização do bócio. Embora os fármacos e o iodo radioativo sejam frequentemente usados para tratar doenças tireoidianas, a presença do bócio subesternal sintomático é uma clara indicação para a cirurgia. A morte ou complicações pós-operatórias são raras.

Apresentamos o caso de um homem de 71 anos com recorrência de patologia tireoidiana sob a forma de bócio subesternal e hipertireoidismo após tireoidectomia parcial. A importância desse caso relaciona-se com a evolução clínica, o volume e a localização do bócio e a abordagem cirúrgica e farmacológica desse tipo de patologia.

PALAVRAS-CHAVE: *Bócio. Bócio subesternal/cirurgia. Hipertireoidismo.*

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Far-East "Orhon" Inscriptions (720-735 AD) in the view of Andrology

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SUMMARY

INTRODUCTION: *The stone monuments named as "Orhon" inscriptions located in Middle Asia are considered the first written Turkish findings. Our aim was to discuss the contents and physical appearance of the monuments according to the andrological perspective.*

METHODS: *These inscriptions were composed of three stone monuments built in the years 720-735 AD, in honor of three Khagans (Ruling leaders).*

RESULTS: *Although the theme of the writings emphasizes the male-dominant ruling style of the antique Middle Asian migratory tribes, we claim that the most interesting point was that the phallus had a secret role in the perspective of the stone monuments.*

CONCLUSION: *The trilogy of power, state authority, and erection was monumentalized in 8th-century inscriptions. The signs of Andrology should be sought in history, archeology, and art to expand the esthetic horizon of modern medical sciences.*

KEYWORDS: *Andrology/history. Far East. Science in the Arts.*

The "*Orhon"* Inscriptions (720-735 AD) at the monumental carved rocks located inside what is currently Mongolia are the first known written Turkish findings regarding their history. This was noteworthy considering that antique Middle Asian tribes had a migratory lifestyle, rather than a settled one, and based on animal herd economy. These kinds of local groups are still present in territorial areas. Based on records from available archives, the first findings about *Orhon* inscriptions were published by a traveler whose full name is Alâeddin Atâ Melik Cüveynî. He was born in the northeast region of today's State of Iran, which belonged to the State of *Khorosan* in the past, and, in 1226, wrote a work entitled "*Tarikh-i Jahangushayi Juvaini*- Tarih-i Cihanguşa (History of World Conqueror)"[1,2]. After this, the historical written value was forgotten for a long time. The original monuments were rediscovered in the 18th century by a Swedish military officer named Von Strahlenberg when he was imprisoned by the Russian Army in the region. Subsequently, it was published in 1730 in Stockholm to be presented into the Modern Science of the World[1]. This topic in written form drew the attention of modern Turkish authorities in Istanbul when it was first published by a newspaper, "Ikdam", in 1895. According to *Ergin*'s reviews about *Orhon* findings, these inscriptions definitely involved the term of monument both materially and spiritually, and they are also a type of

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art-themed writings exuberantly written with senses of gratefulness towards the authorities of the government of the time[3].

The *Orhon* Inscriptions were made up of three monuments, which were built between the years 720 and 735, in honor of three *Khagan* (Ruling leader) whose names were *Kul Tigin*, *Bilge Khagan*, and *Tonyukuk*[1]. While *Tonyokuk's* was established for himself, *Bilge Khagan* did it for *Kül Tigin*, and the next *Khagan* provided the inscription for Bilge *Khagan*[1]. While these inscriptions were narrating Turkish Nation's fight for freedom in an epic language, they were also important for people who were governing the state to identify their responsibilities. This case was a primitive specific example of having governors monitored by the public, what we call democracy in our modern world.

In this study, my aim was to interpret possible reflections of these inscriptions in terms of andrology regarding content and shape. With some exceptions of female administrative figures, such as Istemi *khan Hatun (*khan Hatun*; appellation for a high-rank female official)* who were beyond that era as mentioned in the inscriptions, it was not wrong to interpret that the mentality at the root of Turkish state governments was male-dominant[3]. Also, the antinomy of "Fatty bull – Scrawny bull"[1,3], which was repeated in some parts of the inscriptions, can be noted as a reflection of the male-dominant ruling system in a representative way of male sexual authority. Furthermore, as it was reported in the inscriptions, fears of losing the state governance as a result of wars and having the ruling class female members captured by authorities of the enemy mentioned in terms of sexuality were explicitly and exaggeratedly expressed with statements as: *"The hero rode his horse and lanced nine men and did not let them to conquere the ruling center. My nursing mother and my mother, elder sisters, daughter-in-laws, princesses would almost have been odalisques (female slaves) altogether."*

FIGURE 1. MONUMENT OF *BILGE KHAGAN*.

I think that the descriptions on the monuments, in terms of Andrology, as explained in the context above, actually compose an entirety with shapes of the monuments as well. It will definitely be unfair if the monuments of *Orhon* Inscriptions were just regarded as simple stone monuments. All three of them stand like they make connections between the *Yer Tanri* (The Gods of the ground which are types of geographic entities like mountains, rivers, forests, etc.) and *Gok Tanri* (The God of the sky, which is the most common belief) with their geometric shapes of compound rectangles and cylinders. However, old Turkish populations prioritized political authority instead of religion phenomena[4]. Describing the geometric combination of the monument of *Bilge Khagan* as an erect penis would not be an exaggeration (Figure 1). Moreover, in social complex *Tonyukuk,* the explicit similarity between some worn body pieces without a head as an erected penis can easily be detected by close inspection (Figure 2). These kinds of erect phallic monuments as worshipping items had been used almost in the same period in Europe, and interestingly, early Christianity also tolerated these prehistoric *menhirs** by adding a Celtic cross at the top[5]. Indians also used phallic *stone menhirs* with a special name of 'Lingam'[6]. Prehistoric Far East cultures such as the Japanese also had similar stone phalluses for aesthetic reasons rather than religious. Thus, this point of view intersects with antique Asian Turkish tribes[7]. A few centuries later, in the same territory of the present "Karakorum" Mongolia as the first capital of Genghis Khan as a middle Asian leader, a fantastic stone phallus was laid down across green hills, which probably symbolized the female

FIGURE 2. ANTIQUE SOCIAL COMPLEX OF *TONYUKUK*. ATTENTION TO STANDING STATUE THAT CARVED AS IN THE FORM OF PHALLUS.

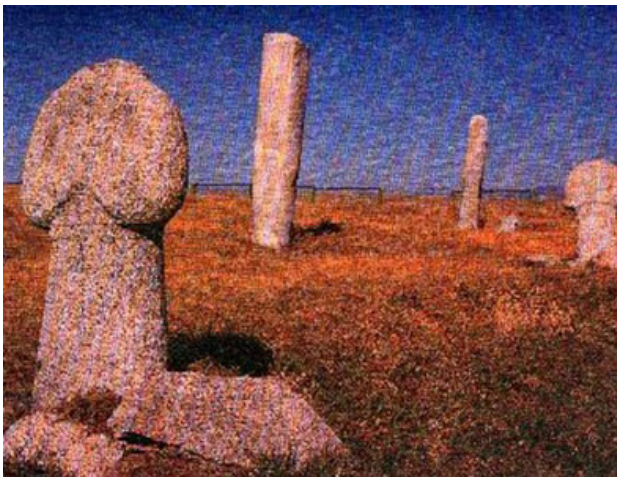

FIGURE 3. A LARGE PHALLIC STONE FOUNDED BY GENGHIS KHAN IS LAYING THROUGH THE GREEN HILLS WHICH POSSIBLY REPRESENTS THE FEMALE GENITALS. KARAKORUM, MONGOLIA

genitals, to improve the renewing of the environment (Figure 3)[8].

In another speculation about *the Tonyukuk* monument, even the detail at the intersection of the plump glans penis and corpus penis was explicit in standing style of the human body without a head, in the figure. Furthermore, the erect penis seemed circumcised. Based on that evidence, the present study supports the hypothesis that advocates that circumcision was also common among prehistoric Middle Asians who lived before Islam. Although the first written report of circumcision is by *Herodotus* (5th BC), the Greek historian and father of History, in antique Egypt, Mattelaer[9] reported that circumcision is supposed to be one of the oldest surgical practices of the humankind, with traces of it even in Neolithic cave paintings.

In conclusion, this study believes that the trilogy of power, state authority, and erection was monumentalized in the 8th century *Orhon* Inscriptions, as has been reported in other cultures of premodern times. The evidence of Andrology should be followed throughout history, archeology, art to expand the aesthetic horizon of modern medical sciences.

Footnote

*A *menhir* (from Brittonic languages: maen or men, «stone» and hir or hîr, «long»), standing stone, orthostat, lith is a large human-made upright stone, typically dating from the European middle Bronze Age. They can be found solely as monoliths, or as part of a group of similar stones. https://en.wikipedia.org/wiki/Menhir

P.S. Figure 1, 2, and 3 are from references 3 and 8 with kind permissions of Altun G; chairman of Bogazici Press, Istanbul, and Mattelaer JJ; EAU Historical Committee, Arnhem, the Netherlands respectively.

RESUMO

INTRODUÇÃO: *Os monumentos de pedra denominados inscrições "Orhon", localizados na Ásia Central, são considerados os primeiros achados escritos da Turquia. Nosso objetivo foi discutir os conteúdos e a aparência física dos monumentos de acordo com o ponto de vista andrológico.*

MÉTODOS: *Essas inscrições foram compostas de três monumentos de pedra construídos nos anos 720-735 d.C., para as honras de três Khagans (líderes de governo).*

RESULTADOS: *Embora o tema dos escritos fosse enfatizar o estilo dominante masculino das antigas tribos migratórias da Ásia Média, alegamos que o ponto mais interessante era que o falo tinha um papel secreto nas perspectivas dos monumentos de pedra.*

CONCLUSÃO: *A trilogia de poder, autoridade estatal e ereção foi monumentalizada em inscrições do século VIII. Os sinais da andrologia devem ser buscados na história, na arqueologia e na arte relacionada, no sentido de ampliar o horizonte estético das ciências médicas modernas.*

PALAVRAS-CHAVE: *Andrologia/história. Extremo Oriente. Ciência nas artes.*

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ISSUE ARTICLE

Coronavirus: a clinical update of Covid-19

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SUMMARY

INTRODUCTION: *A covid-19 pandemic decreed by WHO has raised greater awareness of it.*

EPIDEMIOLOGY: *The infection reached the mark of 350,000 patients in 33 countries and affected as comorbidities the presence of comorbidities and advanced age.*

TRANSMISSIBILITY: *The transmissibility calculated so far is similar to the H1N1 epidemic, but with lower mortality rates.*

PHYSIOPATHOLOGY: *The SARS-CoV-2 virus, of the Coronaviridae family, has the capacity for cellular invasion through the angiotensin-converting enzyme 2 does not have a lower respiratory epithelium and in the cells of the small intestine mucosa.*

CLINICAL MANIFESTATIONS: *a presentation can be divided into mild (fever, fatigue, cough, myalgia, and sputum) and severe (cyanosis, dyspnoea, tachypnea, chest pain, hypoxemia and need for clinical measurement) and has an estimated estimate of 2%.*

DIAGNOSIS: *allows the detection of viral load in CRP-TR of patients with high clinical suspicion.*

TREATMENT: *based on supportive measures and infection control. In severe cases, the use of medications such as hydroxychloroquine and azithromycin or medication can be promising. Take care to avoid the use of corticosteroids. There are no restrictions on the use of resources and ACEIs / ARBs.*

KEYWORDS: *coronavirus, covid-19, SARS-CoV-2, pandemic, update.*

INTRODUCTION

Originally discovered in poultry in the 1930s, several coronaviruses cause respiratory, gastrointestinal, liver, and neurological disease in animals. Only seven coronaviruses are known to cause disease in humans. Four of these (229E, OC43, NL63, and HUK1) more often cause symptoms of a common cold. On rare occasions, there may be a severe infection of the lower respiratory tract, such as pneumonia, especially in children, the elderly, and immunocompromised patients.

The three coronaviruses that cause the most severe respiratory infection in humans, sometimes even fatal, are considered zoonoses and are described below:

SARS-CoV-2 is the new coronavirus identified on 31/12/2019 as the etiological agent of the disease caused by coronavirus 2019 (Covid-19) described in Wuhan, China.

Mers-Cov was identified in 2012 as the cause of the Middle East Respiratory Syndrome (MERS).

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SARS-CoV was identified in 2002 as the cause of an outbreak of Severe Acute Respiratory Syndrome (SARS).

It is believed that Covid-19 (SARS-CoV-2) originated in Chiroptera mammals (bats), since they have been reported to a local animal trade in Wuhan, and due to its close genetic similarity to infectious coronavirus in this genre.

EPIDEMIOLOGY AND RISK FACTORS

Only 71 days after the discovery of covid-19 (and 59 days from its genetic sequencing), on 11/03/2020, The World Health Organization declared a pandemic. A total of 33 countries have registered covid-19 cases, with at least 350,000 cases and 15,000 deaths. A large global economic impact has been estimated due to the saturation of health systems and quarantine state, with an expected GDP of 24% recession in the most affected quarter in the US.

The average age of infected individuals ranges between 49 and 56 years, and the conditions in rare in individuals younger than 20 years (children, in general, are asymptomatic). Severity increases with age, and the mean age of deaths in Italy (the second epicenter of the pandemic) is 79.5 years.

Among the known risk factors for severe symptomatic presentation are cardiovascular (hypertension) and pulmonary (smoking, asthma) comorbidities and advanced age (over 60 years).

TRANSMISSIBILITY

Transmissibility is mainly by droplets - close interpersonal contact (less than 2m - 6 feet - for a prolonged time) - and fomites. Studies in the USA with 445 contacts of infected individuals found a rate of social contagion of 0.45%. Fecal-oral transmission is possible (due to the presence of ACE2 in the gastrointestinal tract and the presence of positive RT-PCR in stool samples); however, it has yet to be documented.

A very important quantification of viral transmissibility is the basic reproduction number, which is usually denoted by R_0 (pronounced "R-naught"). The epidemiological definition of R_0 is the average number of people who contract a disease from a contagious person. It applies specifically to a population of people who previously were free of infection and were not vaccinated. There are three possibilities for the potential spread or decline of disease, depending on the value of R_0:

If R_0 is less than 1, each existing infection generates less than 1 new infection. In this case, the disease will decrease and eventually disappear.

If R_0 is equal to 1, the disease will remain alive, but there will be no epidemic.

If R_0 is greater than 1, the cases will grow exponentially and cause an epidemic or even a pandemic.

As far as we currently know, the R_0 value calculated for 2019-nCoV is significantly greater than 1. The preliminary estimate of a 1.4-2.5 R_0 was presented on the WHO declaration about the 2019-nCoV outbreak on January 23, 2020. Studies had estimated the average R_0 for 2019-nCoV in the initial stage of the outbreak ranging from 3.3 to 5.5 (likely lower than 5, but above 3 with increased rate), which was slightly higher than those of SARS-CoV (R_0: 2-5), indicating that 72-75% of transmissions must be avoided to stop the growing trend. In contrast, previous studies had suggested that the R_0 for MERS-CoV is less than 1, which means that it is unlikely to cause a pandemic. Super-spreading events have been implicated in the transmission of 2019-nCoV, as was the case with SARS-CoV and MERS-CoV, but its relative importance remains unclear, and it is difficult to trace the super-spreaders. In addition, R_0 can change seasonally according to climate or annual gatherings.

Transmissibility seems to be reduced in hot and humid climates.

The table 1 compares the current major contagious diseases and covid-19 regarding mortality and R_0.

Virus	Case Fatality Rate %	R_0
2019-nCoV	3	1.4-5.5[a]
SARS-CoV	10	2-5
MERS-CoV	40	<1
Avian H7N9 (2013)	40	<1
H1N1 (2009)	0.03	1.2-1.6
H1N1 (1918)	3	1.4-3.8
Measles Virus	0.3	12-18
Rhinovirus	<0.01	6
Ebola Virus	70	1.5-2.5
HIV	80[b]	2-4
Small Pox Virus	17	5-7

a - WHO: 1.4-2.5; S. Zhao et al.: 3.3-5.5; J. Read et al.: 3.6-4.0; M. Shen et al.: 4.5-4.9.
b - Without therapy

PHYSIOPATHOLOGY

It has been reported that the coronaviruses, including covid-19, have their mechanism of infection by binding to the ACE2 (angiotensin-converting enzyme

2) protein. Notably, ACE2 is abundantly present in humans in the epithelia of the lung and small intestine, and the coronaviruses can infect the upper respiratory and gastrointestinal tracts of mammals.

Studies indicate that the viral load detected in asymptomatic patients was similar to that found in symptomatic patients; however, the viral loads in patients with serious diseases were higher than those of patients with mild to moderate presentations. In addition, higher viral loads were detected in the nasal mucosa than in the oropharynx. This suggests the effectiveness of an upper swab of the nasal mucosa, which presents less risk to the professional in charge of sample collection.

The incubation period ranges from 2 to 14 days (average of 5.2 days). The average time between the first symptoms and the development of acute respiratory distress syndrome (ARDS) is 8 days. A possible explanation for such rapid and serious deterioration in the cytokine release syndrome (CRS), or 'cytokine storm', an overproduction of immune cells and cytokines, which leads the system of multiple organs to fail and causes fatal damage to the tissues of the lungs, kidneys, and heart.

CLINICAL PRESENTATIONS

It is estimated that most individuals are asymptomatic or present only mild symptoms (85%), including fever, fatigue, cough, myalgia, and sputum. There may be anosmia (initial symptom), headache, odynophagia, and runny nose. Severe cases (15%) may include chest pain, dyspnea, cyanosis, tachypnea, signs of respiratory distress, hypotension, decompensation of underlying diseases, and lymphopenia, which must be treated in a hospital bed. RR >30 bpm, SatO2 <93%, PaO2/FiO2<300 were indicators of poor prognosis and progression for mechanical ventilation (risk factors for mechanical ventilation: hypertension, diabetes mellitus, and age over 65 years). The mortality rate is around 2.9% (95% CI 1,4-4,3%), lower than SARS (10%).

In severely immunosuppressed patients (transplanted), the initial presentation may be gastrointestinal (diarrhea and fever), progressing to respiratory involvement in 48h.

The most common finding on computed tomography scans of the chest in patients was ground-glass opacity nodules with peripheral and lowe-lobe bilateral involvement. Such findings may occur even in asymptomatic patients, but are more common in patients with covid-19-related pneumonia. The presence of a fine fibrotic layer (fine reticular opacities) indicates a good prognosis of the disease, with progression in remission.

Convalescence lasts for 1 to 3 weeks for mild cases and from 2 to 6 weeks for severe cases.

DIAGNOSIS

Clinical suspicion is based on the presence of fever and respiratory symptoms (cough, dyspnea). The presence of compatible epidemiology (contact with a suspected or confirmed case, travel to an endemic location in the previous 14 days) increases suspicion of covid-19 at the expense of other respiratory syndromes and should be an indication for a RT-PCR test.

The diagnosis is possible through a positive RT-PCR or the presence of high clinical suspicion (compatible clinical signs + favorable epidemiology) associated with a CT scan with bilateral peripheral glass opacities of the lower lobes.

TREATMENT

The treatment is supportive (antipyretics and hydration). General measures such as oxygen supplementation if SatO2 is less than 94%, maintenance of the MBP between 65-70 mmHg (in cases of shock, supply isotonic hydration at 30 ml/kg, proceeding to vasoactive drugs if refractory), protective mechanical ventilation (tidal volume 4-8 mL/kg, plateau pressure <30 cmH2O), intermittent nocturnal sedation for early weaning, prevention of DVT/PE (stimulate ambulation, use of compression tights/intermittent pneumatic compression, low-molecular-weight heparin), prevention of nosocomial infection (pneumonia associated with mechanical ventilation, infections due to catheters), prevention of decubitus ulcers (change of decubitus every 2h) are essential and must be implemented in cases of Covid-19. Upon medical discharge, the patient must be instructed on possible clinical worsening 5-8 days after the onset of symptoms.

Look for epidemiological measures of infection control management. Mild cases should be treated on an outpatient basis, with home isolation, and instructing all household individuals on sanitary practices (the patient must be restricted to the bedroom, with the door closed and well ventilated, fomites must be sanitized with soap and water or alcohol 70° by the patient, minimal agitation and handling of clothing, frequent

sanitation of hands by the patient and other household members, quarantine of all household members for 15 days). Severe cases must be hospitalized in isolation, 2 meters away from other suspected cases, and with the necessary precautions. NIV should be used with caution: although it is effective in preventing the intubation of patients with respiratory distress, it can cause dispersion of droplets. The use of PPE by health professionals (disposable caps and gowns, face shields, gloves, and N95 masks) is indicated, and respiratory masks should be provided to symptomatic patients already in the hospital.

Early clinical trials with small samples were promising for the use of hydroxychloroquine + azithromycin and remdesevir (an antiviral drug developed against the Ebola Virus).

Hydroxychloroquine (HQ) and chloroquine (CQ) are immunomodulators implicated in the inhibition of lysosomal activation of antigen-presenting dendritic cells and in the suppression of TRL binding, thus attenuating the production of IL-1, IFN-1, and TNF. The first action would reduce the excessive secretion of cytokines, delaying the overactivation of the immune system triggered by the disease. In addition to this role in modulating the immune response, HCQ and CQ inhibit binding to the receiver and fusion of the membrane, the two main steps necessary for the cell entry by coronaviruses: interfering in the glycosylation of the angiotensin-converting enzyme 2 (ACE2) (the cell receptor of SARS-CoV) and blocking the binding of the virus to the host cell. In addition, they significantly raise the endosomal pH, interrupting the action of proteases and activation of the endosome for virus endocytosis. It is recommended that a dose of 400 mg twice a day of hydroxychloroquine sulfate administered orally, followed by a maintenance dose of 200 mg twice a day for 4 days. Gastrointestinal responses, such as vomiting and diarrhea, are the most common adverse effects of these two drugs. Patients with exposure to CQ exceeding 5 years suffer severe side effects, such as retinopathy, circular defects (bulls-eye maculopathy), and cardiomyopathy. There is no contraindication to HQ during pregnancy. (Figure 1)

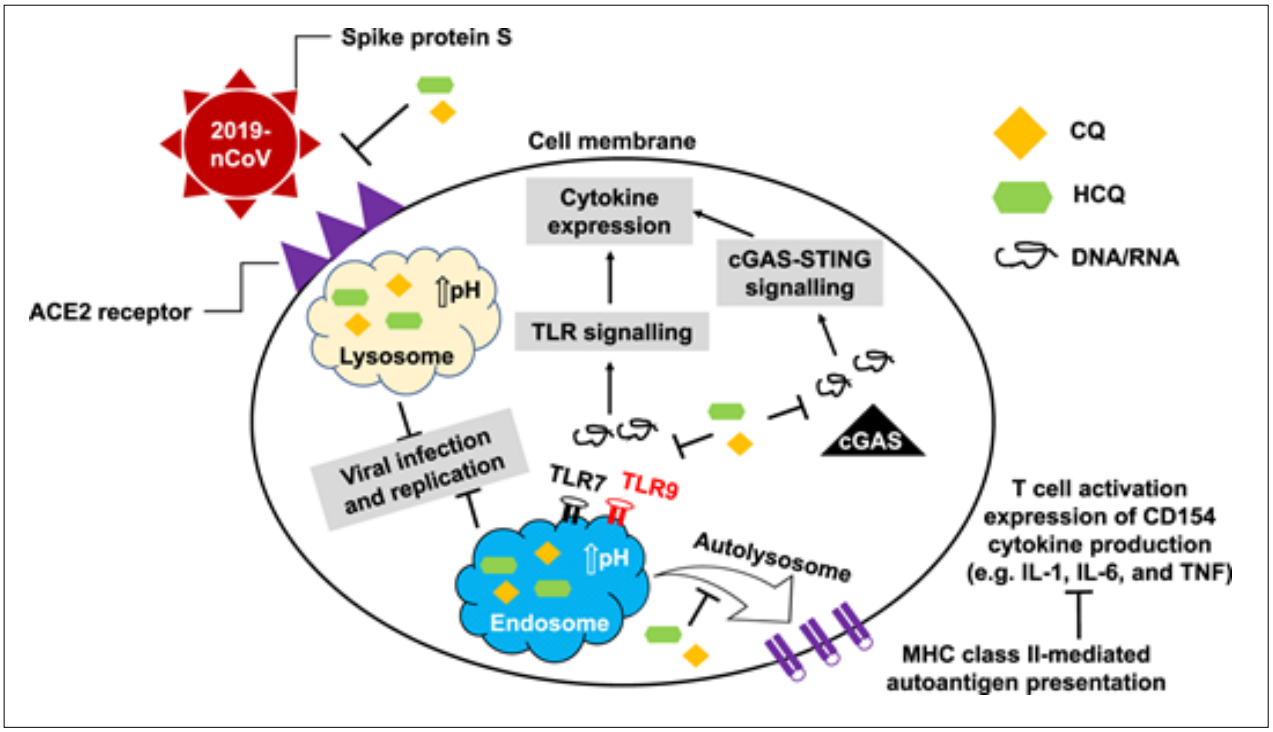

FIGURE 1. A graphic illustration of the antiviral mechanisms of CQ and HCQ. Both chemicals may interfere in the glycosylation of ACE2 and reduce the efficiency of binding between ACE2 in host cells and spike protein (S) on the surface of the coronavirus. They can also increase the pH of endosomes and lysosomes, thus preventing the process of binding of the virus with host cells and its subsequent replication. When HCQ enters APCs, it prevents antigen processing and the presentation of autoantigen mediated by class II MHC in T cells. The subsequent activation of T cells and the expression of CD154 and other cytokines are suppressed. In addition, HCQ interrupts the interaction of DNA / RNA with TLRs and the nucleic acid sensor cGAS and, therefore, the transcription of proinflammatory genes cannot be stimulated. As a result, the administration of CQ or HCQ not only blocks the invasion and replication of coronaviruses but also mitigates the risk of a cytokine storm.

Remdesevir is a promising drug, along with HQ. An antiviral drug, it has been linked to the reduction of viral load and decreased severity of the condition. A dose of 200 mg in D1, followed by 100 mg/day for 9 days in single daily doses, is recommended.

The use of corticosteroids has been associated with a worse clinical outcome and is not recommended. Its use in initial presentations was related to a higher viral load, and its use during the period of severe pneumonia was related to delayed clearance of the viral load, as well as the decompensation of diabetes mellitus and presence of psychosis.

However, it should be considered in the presence of septic shock refractory to vasopressors and with suspected adrenal insufficiency.

Measures such as self-quarantine or temperature control at borders should not be very effective since half of the infections are asymptomatic, and 44% of the patients do not present fever. Currently, there is a consensus regarding the closing of schools and restrictions of social gatherings (including the closure of workplaces) to limit population movements and introduce the so-called sanitary cordons (quarantines at a cities or region scale). There is less consensus regarding which measure should be the first to be implemented, in which combination, and when. The World Health Organization recommends universal testing with subsequent isolation of positive asymptomatic patients (effective measure to control the spread of the virus).

The association between the use of ACE inhibitors and increased infectivity and severity of SARS-CoV-2 has also been described; however, it is known that cardiovascular diseases are still the number one cause for deaths worldwide; therefore, it is not advisable to suspend such medication.

DIFFERENTIAL DIAGNOSES IN PEDIATRICS

Current data indicate that symptomatic pediatric cases are rare, as well as severe cases in individuals without comorbidities younger than 20 years. Therefore, in the face of a pediatric respiratory syndrome case, one of the differential diagnoses below should be raised:

→ Acute viral rhinopharyngitis:

It is the most common infectious disease during childhood (children younger than 5 years usually present from 5 to 8 episodes per year).

The most common etiologic agents are: rhinovirus, coronavirus, respiratory syncytial virus (RSV), influenza, parainfluenza, coxsackie, and adenovirus. The transmission is by droplets and direct contact with the mucous membranes, and the incubation period is of 2-5 days. The affected individual presents a risk of contagion in the one-day period before the onset of symptoms and two days after it.

The clinical presentation lasts from 5 to 7 days and includes rhinorrhea, nasal obstruction, dry coughing, sneezing, pharyngitis, and fever of variable intensity. There may be hyperemia of the tympanic membrane, changes in sleep, and irritability in infants. The adenovirus and RSV may generate lower airway infection (leading to tachypnea, retractions, and grunting). The influenza virus is implicated in a more severe clinical presentation, with diarrhea, abdominal pain, prostration, high fever, and myalgia, which puts the clinical presentation of a common cold (coryza, nasal obstruction, cough, and pharyngitis) in the background. Both RSV and rhinovirus can trigger asthma episodes. There may be complications with acute otitis and sinusitis due to the obstruction of the Ostia of the paranasal sinuses and eustachian tube secondary to the inflammatory process or indiscriminate use of decongestants.

The diagnosis is clinical and should be differentiated from hepatitis A (onset of jaundice, increased direct bilirubin and transaminases), measles (intense cough, conjunctivitis with photophobia, exanthema, and enanthema of Koplik), pertussis (severe cough, and may cause vomiting and dyspnea, duration of 6 to 10 weeks, absence of vaccination), rhinitis (ARDS caused by winter, positive family history and personal history of atopy, remission after use of nasal corticosteroids), streptococcal pharyngitis, and mononucleosis (both with intense hyperemia of the oropharynx, petechiae on the palate, hypertrophy of the tonsils and purulent plates). The detection of the virus is unnecessary.

The treatment consists of rest during the febrile period, nasal instillation of isotonic saline solution (3-5 mL in each nostril every 2 hours), and antipyretic medication. In adults with influenza A, one can resort to amantadine 200 mg every 24h (pre-contact prophylaxis with 70-80% of effectiveness, equally effective treatment with a reduction of the symptomatic period). In children older than 12 years or weighing over 40 kg and adults with suspicion of influenza type A or B, oseltamivir 75

mg can be used every 12h for 5 days (pre-contact prophylaxis with 92% efficiency, reduces the severity and duration of symptoms, can be used in children over one year). The use of probiotics and vitamin C in high doses (>1g/day) has proven effective in the prevention of common colds in athletes, as well as in the reduction by 20% of the duration of the condition in children. Caretakers should be instructed on the need for hand hygiene at home and to return in case of clinical worsening.

→ Acute sinusitis

Defined as a bacterial infection of the paranasal sinuses, with a duration of fewer than 30 days. Acute sinusitis is rare in small children since the maxillary and ethmoidal sinuses are reduced before the age of two years, and the frontal and ethmoidal sinuses develop from the age of 4 years.

The most frequent etiological agents are *Moraxella catarrhalis, Hamophilus influenza, and Streptococcus pneumoniae.*

Acute sinusitis is suspected when there is the persistence of ARDS exceeding 10 days or recurrence after clinical improvement. The symptoms include nasal obstruction, purulent rhinorrhea, halitosis, coughing with worsening at night, fever, and frontal tension headache (may also present as pain in teeth). There may be osteomyelitis, periorbital cellulitis (a sign of inflammation on the ethmoid bones), meningitis, thrombosis of the cavernous sinus, and brain abscess.

The diagnosis is clinical, and an x-ray of the paranasal sinuses is usually unnecessary. It must be differentiated from adenoiditis (snoring, epistaxis, acute otitis media) and nasal foreign body (obstruction and asymmetrical rhinorrhea, history of foreign body insertion). It may be necessary to have a specialized assessment if sinusitis lasts for over 90 days or in case of recurrent sinusitis. It may be necessary to have a computed tomography of the skull in refractory cases or in case of suspected complications.

The treatment consists of rest, antipyretics, nasal instillation of isotonic saline solution every 2h, topical or systemic corticosteroids (in case of a history of asthma), and antimicrobial agents. The first choice is amoxicillin clavulanate 875+125 mg every 12h for 7 days (or 10 days in case of resistance factors). Other options are cefuroxime, clarithromycin, or azithromycin. In the absence of a response after 48h, consider doubling the dose or changing the medication. Instructions to avoid decongestants and diving during periods of ARDS, avoid smoking (active and passive), and proper treatment of allergic rhinitis.

Children with allergic rhinitis can present chronic or repetition sinusitis.

→ Streptococcal pharyngitis (SAP)

SAP is an acute infection of the oropharynx by *Streptococcus pyogenes* (group A beta-hemolytic). It is transmitted via droplets, or direct contact with patients presents incubation of two to five days and represents 15% of ARDS.

The most common clinical presentation is high fever, odynophagia with sudden onset, pharyngeal hyperemia, hypertrophy of the tonsils and tonsillar exudate, associated or not with prostration, cervical adenopathy, vomiting, and abdominal pain. It can complicate with cervical lymph node abscesses, acute otitis media, post-streptococcal glomerulonephritis, reactive arthritis, rheumatic fever, and sepsis.

The diagnosis is clinical, and a quick test can be used. It must be differentiated from scarlet fever (strawberry tongue, exanthema with signs of Pastia - increase of the flexural exanthema, and Filatov - perioral pallor), viral pharyngitis (coryza, absence tonsil hypertrophy, and exudate), diphtheria (predominant diarrhea, grayish-white plates in the oropharynx that may invade the uvula) and infectious mononucleosis (generalized adenopathy, exanthema after the use antimicrobial agents).

The treatment is based on rest, infusion of warm saline solution in the oropharynx, antipyretics, and antibiotics (the drug of choice is benzathine penicillin G 600 thousand or 1.2 million IU IM single dose, for <27 kg and >27 kg, respectively). The treatment is less painful if a heated solution used for dilution. In the case of allergy to penicillin, the drug of choice is erythromycin estolate 40 mg/kg/day in 2-3 administrations. Instruct the patient to return in case of dysphagia and muffled/nasalized voice (tonsillar abscess), dyspnea, exanthema, or clinical worsening. Instruct to be absent from nursery/school and work activities for 48h after starting the treatment.

→ Viral croup

This is subglottic laryngitis, a viral infection with a variable degree of congestion and obstruction of the airways. It is often caused by RSV and parainfluenza

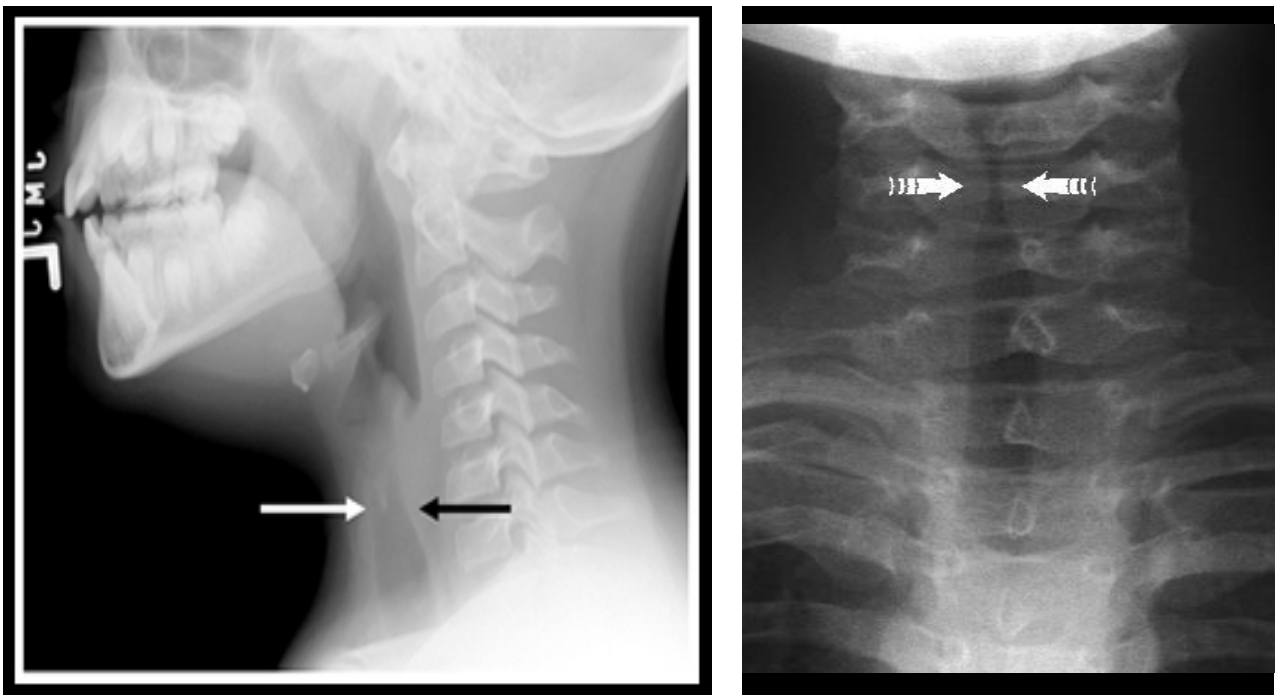

FIGURE 2. CERVICAL RADIOGRAPHY SUGGESTIVE OF VIRAL CROUP

I and II, presents a natural history of partial obstruction of the airways for 2-3 days, with remission after 5 days.

The clinical manifestations include prodromes (coryza, dry cough, nasal obstruction, and fever) and laryngitis (hoarse cough, dysphonia/aphonia, and respiratory stridor). There may be signs of respiratory failure (nasal flaring, subcostal retraction, tachypnea, cyanosis/paleness) and can evolve to airway obstruction and death.

The diagnosis is clinical and can be aided by radiography (laryngeal narrowing with pencil-point sign). It must be differentiated from a foreign body (sudden onset of airway obstruction, with cyanosis and cough), a congenital malformation of the airway (repeat laryngitis), spasmodic laryngitis (absence of prodromes, nocturnal onset, spontaneous regression, personal history of GERD, and improvement with humidification) and allergic laryngeal edema (history of exposure to allergens or drugs, presence of angioedema and other stigmata of anaphylaxis).

The treatment is based on the humidification of the environment and hydration, as well as symptomatic treatment. In severe cases (progressive or at rest stridor, signs of respiratory failure, toxemia), use inhaled corticosteroids and consider tracheal intubation.

RESUMO

INTRODUÇÃO: *A pandemia de covid-19 decretada pela OMS suscita maior conhecimento acerca da doença.*

EPIDEMIOLOGIA: *A infecção atingiu a marca de 350.000 pacientes em 33 países e levantou como fatores de risco a presença de comorbidades e a idade avançada.*

TRANSMISSIBILIDADE: *A transmissibilidade calculada até o momento é similar à da epidemia de H1N1, contudo, com taxa de mortalidade inferior.*

FISIOPATOLOGIA: *O vírus SARS-CoV-2, da família Coronaviridae, tem capacidade de invasão celular através da enzima conversora de angiotensina 2 presente no epitélio respiratório inferior e nas células da mucosa do intestino delgado.*

MANIFESTAÇÕES CLÍNICAS: *A apresentação pode ser dividida em leve (febre, fadiga, tosse, mialgia e escarro) e grave (cianose, dispneia, taquipneia, dor torácica, hipoxemia e necessidade de ventilação mecânica) e tem mortalidade estimada de pouco mais de 2%.*

DIAGNÓSTICO: *Dá-se pela detecção da carga viral no PCR-TR de pacientes com alta suspeição clínica.*

TRATAMENTO: *Baseado em medidas de suporte e de controle de infecção. Em casos graves, uso de medicamentos como hidroxicloroquina e azitromicina ou remdesivir podem ser promissores. Evitar o uso de corticosteroides. Não há evidências suficientes para abster-se do uso de ibuprofeno e IECAs/BRAs.*

PALAVRAS-CHAVE: *Coronavírus. Covid-19. Pandemia. Atualização.*

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ORIGINAL ARTICLE

Musculoskeletal injuries in taekwondo athletes: a nationwide study in Portugal

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SUMMARY

BACKGROUND: *Taekwondo is a martial art that emphasizes blows using the feet and fists, and it is characterized by direct and continuous body contact, which subjects their practitioners to a higher number of injuries. This study aimed to determine the incidence of musculoskeletal injuries in Portuguese taekwondo athletes and analyze its associated factors.*

METHODS: *The sample included 341 taekwondo athletes, aged between 4 and 62 years (18.77±12.77 years), 237 (69.5%) were male, and 104 (30.5%) female. A questionnaire was administered at a national level in taekwondo training and competitions via interview.*

RESULTS: *One hundred and thirty-two (38.7%) taekwondo athletes reported having suffered an injury since they began their practice, totaling 294 injuries. Seventy-six (22.3%) athletes had an injury in the previous 12-months period, with a total of 112 injuries. There were 2.15 injuries per 1,000 hours of taekwondo training. The most common of all injuries was muscle injury (strain, contusion) (58.6%), in the foot and fingers (18.9%). The attack technique (28.8%) was the most prevalent injury mechanism. Adult athletes presented a higher risk of sustaining taekwondo-related injuries than adolescents (odds ratio = 3.91; 95%CI: 1.13-13.55; p=0.032), and athletes who trained more than 1 hour had a risk 4.20 times greater (95%CI: 1.44-12.29; p=0.009) than those who trained up to 1 hour per session.*

CONCLUSIONS: *Injuries were frequent among Portuguese taekwondo athletes, with specific body areas affected, mainly caused by the attack technique. It is necessary to create injury prevention strategies, including specific training and the use of protective equipment.*

KEYWORDS: *Epidemiology. Incidence. Wounds and Injuries. Prevalence. Martial Arts.*

INTRODUCTION

Martial arts are antique forms of combat often used for different fighting styles. Karate and taekwondo emphasize blows using the feet and fists. Jiu-Jitsu and judo emphasize maneuvers that use the arms, joint locks, and projection techniques. Mixed martial arts (MMA) are styles that blend the techniques mentioned above[1].

Taekwondo is a Korean martial art and combat sport characterized by its emphasis on dynamic kicking techniques delivered from a mobile stance[2-4]. As of 2018, the global membership of the World Taekwondo Federation stands at 208 national member associations, spanning five continents[5].

Taekwondo, like other martial arts, has grown in popularity and has unarguably become one of the most commonly practiced martial arts in the world[2,3].

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The increase of physical demands in combat sports requires athletes to workout close to the maximum exhaustion limits, and the characteristics of these martial arts (competitive aspects characterized by a high direct and continuous body contact) cause their practitioners to be more prone to injuries[6].

Altarriba-Bartes et al.[7] evaluated 48 taekwondo athletes of the Spanish national team during two Olympic periods, and verified the occurrence of 1,678 injuries in a period of 8 years, concluding that the practice of taekwondo is related to a high risk of injury.

Lystad et al.[8] performed a meta-analysis and estimated an overall competition injury incidence rate of 79.3 per 1,000 athlete-exposures after adjusting to level of play, sex, and average age, and found that the most frequently injured body regions were the lower limbs and the head/neck region, while the most common types of injuries were contusions and joint sprains.

To our knowledge, there is no literature with regard to Taekwondo injuries in Portugal. As such, this study aimed to determine injury epidemiology and risk factors for musculoskeletal injury in Portuguese taekwondo athletes, as well as their type, location, and mechanism of injury and analyze the associated risk factors in order to develop appropriate preventive strategies.

METHODS

A retrospective cohort study was conducted to gather data on injuries at a national level. This study was approved by the Research in Education and Community Intervention (RECI) research unit.

Population

The study population consisted of Portuguese taekwondo athletes, who participate or not in competitions, of both sexes and any age.

The inclusion criteria were specified taekwondo athletes who had practiced taekwondo for at least one year, at least two times per week, who freely agreed to participate and signed the informed consent form (if under 18, the consent was signed by their guardian), and were present during the data collection days.

Data on the number of non-federated athletes in Portugal is unknown. Because of that, we used the latest data from the Portuguese Taekwondo Federation, from 2016. The sample size was determined using a population of approximately 4,127 federated taekwondo athletes in Portugal[9], an estimated injury prevalence of 60% (as reported in national and international studies)[10,11] and assuming an error margin of 5% with a 95% confidence interval (CI). Based on these, the minimum sample size was 341 taekwondo athletes[12].

Instrument

Based on a previous exploratory study[13], a specific questionnaire entitled "Taekwondo Injuries" was used. This previously developed questionnaire was applied to a sample of athletes of other martial arts (judo and jiu-jitsu practitioners). For our study, this questionnaire was evaluated by a group of three experts (with different backgrounds, namely Physiotherapist Ph.D. in Epidemiology, President of the Portuguese Taekwondo Federation, and taekwondo coach) and a pre-test was carried out (on ten athletes). Minor adjustments were made to clarify some issues, such as changing the order of the questions and adding a limit to describe a maximum of only three injuries fully. Data were collected between March and June 2017 in clubs/schools and in taekwondo championships.

The first part of the questionnaire involved items concerning age, gender, region, graduation, dominant upper and lower limbs, years of taekwondo practice, training regularity per week, hours of training sessions, performing other sports regularly at least twice a week, participation in competitions, and the type of competition [form (Taegeuk/Poomsae) or combat].

The second part of the questionnaire focused on the occurrence/presence of injuries resulting from taekwondo practice in three different periods: 1. at the time of data collection; 2. over the whole taekwondo practice (lifetime prevalence); and 3. over the last 12 months.

The athletes who presented an injury in the past year had to describe its characteristics: the number of injuries; type and location; the moment of injury occurrence; if treatment was performed and, if so, the kind of treatment; the mechanism of injury and the technique that caused the injury. The categories of variables can be seen in the Tables (Results section). It was only possible for respondents to specify the characteristics of a maximum of three injuries (those considered most serious, or that required more time for recovery).

An injury was defined as any condition or symptom that occurred as a result of taekwondo practice and had at least one of the following effects: the athlete had to stop the taekwondo activity (training, competition) for at least one day; the athlete didn't have to

stop the activity, but had to change the activity (to fewer hours of practise or training, a lower intensity of effort, or was less able to perform certain gestures or manoeuvres/techniques); the athlete sought advice or treatment from health professionals to address this condition or symptom[14].

The questionnaire was applied in a single moment by the researcher, and in the form of an interview (a structured interview), so as to allow to clarify the doubts without interfering with the respondents' opinions or producing biased answers.

Data analysis

Firstly, descriptive statistics were obtained regarding all variables in the study. Secondly, the injury proportion (IP) and injury rate (IR) were calculated. To determine the IP, the total number of participants who had at least one injury during the past 12 months was divided by the total number of participants. The IR refers to the total number of injuries divided by the total time for which the athlete was exposed to risk (usually per 1,000 hours). The calculation of the total hours of training per 12-month was done by multiplying the total average hours of training per week by the average number of times of training per week, multiplied by the 12-month period (42 weeks)[15].

In our study, we described the injury rate as a measure of prevalence. Although this is not the typical means of reporting the injury rate in sports, it has been reported similarly in other sports studies[16].

The influence of the variables on the presence of injury was assessed using binary logistic regressions, based on the Enter methods, and crude and adjusted odds ratios (by sex and age), and respective confidence intervals were calculated.

A final multivariate model was developed, using the Forward Likelihood Method, and its validity, quality of fitting and predictive capacity were assessed by Omnibus and Hosmer-Lemeshow tests, and the Nagelkerke correlation coefficient.

In all inferential analyses, statistical significance was set at 0.05.

The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS), version 24.0.

RESULTS

Our sample comprised 341 taekwondo athletes (minimum sample size estimated), aged between 7 and 62 years (18.87±12.67 years), 237 (69.5%) were male, and 104 (30.5%) were female.

The data were collected at a national level, 72 (21.1%) athletes belonged to the northern region of the country, 110 (32.3%) to the central region, and 159 (46.6%) to the southern region.

Regarding belt rank, 13 (3.8%) athletes had white belt, 13 (3.8%) white/yellow, 39 (11.5%) yellow, 40 (11.7%) yellow/green, 64 (18.8%) green, 26 (7.6%) green/blue, 27 (7.9%) blue, 18 (5.3%) blue/red, 26 (7.6%) red, 4 (1.2%) red/black, e 71 (20.8%) black belt.

FIGURE 1.

FIGURE 2.

Two hundred and ninety-two (85.6%) athletes were right-handed, 27 (7.9%) were left-handed, and 22 (6.5%) used both sides. Two hundred and sixty-two (76.8%) used the right side as the dominant side, 44 (12.9%) used the left side, and 35 (10.3%) both sides of the lower limb to practice taekwondo.

One hundred and thirty two (38.7%) taekwondo athletes had been practicing it for 1 and 2 years, 86 (25.2%) between 3 and 4 years, 35 (10.3%) between 5 and 6 years, 25 (7.3%) between 7 and 8 years, 14 (4.1%) between 9 to 10 years, and 49 (14.4%) individuals practiced taekwondo for over 10 years.

The duration of each training session ranged from 45 to 180 minutes (88.86±26.71 min), and the weekly training frequency ranged from 1 to 7 times (2.45±0.89 times a week).

One hundred and ninety-three (56.6%) individuals participated in taekwondo championships, and 148 (43.4%) never participated in one. Regarding the type of competition, 59 (30.6%) athletes participated in forms competitions, 67 (34.7%) of combat, and 67 (34.7%) of both types of competition.

Individuals who participated in competitions trained 1 to 6 times a week (2.68±0.99 times), with a duration of between 45 and 180 minutes (95.28±24.01 min) per session. Athletes who did not compete trained between 1 and 7 times a week (2.14±0.61 times), with a duration between 60 to 180 minutes (80.47±27.78 min).

One hundred twenty-seven (37.2%) individuals practiced another type of sport on a weekly basis (at least 2 times a week).

Twenty-four (7%) athletes said they were injured at the time of the data collection, and 132 (38.7%) reported having suffered some type of injury since the beginning of their taekwondo practice. Forty-eight (14.1%) individuals referred having sustained one injury, 40 (11.7%) reported two injuries, 10 (2.9%) three injuries, and 34 (10%) four or more injuries (totaling 294 injuries during their entire careers).

For the 12-month period, 76 (22.3%) athletes referred to having an injury related to taekwondo training or competition, with 48 athletes (14.1%) reporting only one injury, 21 (6.2%) reporting two injuries, 6 (1.8%) three injuries, and only 1 (0.3%) four or more injuries (totaling 112 injuries).

An injury proportion of 0.23 (CI 95%: 0.18-0.27) per athlete per 12 months was calculated. The injury rate obtained was 2.15 injuries per 1,000 hours of taekwondo training.

The average number of injuries per practitioner (total number of injuries / total number of athletes) was 0.33, and the average of injuries per injured athletes (total number of injuries / number of injured athletes) was 1.47.

Table 1 shows the relative and absolute frequencies of the type and location of the injuries. Each participant could describe a maximum of three injuries; the following table accounts for 111 injuries in total (only one individual mentioned four or more injuries).

Most of the practitioners (n=100; 90.1%) reported that the injury occurred during the training, 8 (7.2%) during the competition, 1 (0.9%) during the warm-up, and 2 (1.8%) during the cool-down.

Eighty-eight (79.3%) athletes received some type of treatment to treat the injuries. Of the 88 (100%) who received treatment, 34 (38.6%) rested or took medication, 24 (27.3%) received physiotherapy, 8 (9.1%) were immobilized, 5 (5.7%) received surgery, 3 (3.4%) non-conventional therapies, and 14 (15.9%) another type of treatment.

Table 2 shows the injury causes and techniques that caused them.

Table 3 shows the relationship between the occurrence of injury in the last 12 months and gender, age group, participation in a competition, years of taekwondo practice, weekly frequency of training, duration of training per session, and belt rank obtained based on the application of the binary logistic regression model. We chose to divide belt rank into the following categories: beginners/intermediates (included white, yellow, and green belts) and advanced (included blue, red, black belts).

The final model was considered mathematically valid, but with a relatively weak predictive capacity, because the values obtained in the Omnibus test states that all coefficients of the equation are not null (p=0.000), the values of Hosmer-Lemeshow confirm that there are significant differences between predicted and observed values (p=0.147) and the Nagelkerke value obtained shows that the model is able to explain about 13% of the variance recorded in the dependent variable (R2=0.130). However, statistical significance was observed in some variables analyzed in the binary logistic regression.

It was found (adjusted by sex and age) that adult taekwondo athletes had 3.91 times higher probability (95% CI: 1.13-13.55; p=0.032) of having an injury than adolescent athletes, and taekwondo athletes who trained more than 1 hour had 4.20 times more

TABLE 1. AREA AND TYPE OF INJURY

Area of injury	Fracture	Muscle injury (strain, contusion)	Joint injury (cartilage, meniscus, ligament injury/ sprain, luxation)	Tendinopathy	Others	Total
Face	-	-	-	-	2 (40%)	2 (1.8%)
Thorax/ chest/ribs	-	1 (1.6%)	-	-	-	1 (0.9%)
Lumbar spine	-	1 (0.9%)	-	-	-	1 (0.9%)
Shoulder	-	-	3 (13%)	4 (28.6%)	-	7 (6.3%)
Elbow	-	-	2 (8.7%)	-	-	2 (1.8%)
Wrist	-	2 (3.1%)	-	1 (7.1%)	-	3 (2.7%)
Hand & fingers	1 (25%)	-	-	-	-	1 (0.9%)
Pelvis	-	7 (10.9%)	1 (4.3%)	1 (7.1%)	-	9 (8.1%)
Thigh	-	16 (25%)	-	1 (7.1%)	-	17 (15.3%)
Knee	-	6 (9.4%)	5 (21.7%)	2 (14.3%)	-	13 (11.7%)
Leg	-	11 (17.2%)	3 (13%)	-	3 (60%)	17 (15.3%)
Ankle	-	4 (6.3%)	8 (34.8%)	5 (35.7%)	-	17 (15.3%)
Foot & fingers	3 (75%)	17 (26.6%)	1 (4.3%)	-	-	21 (18.9%)
Total	4 (3.6%)	65 (58.6%)	23 (20.7%)	14 (12.6%)	5 (4.5%)	111 (100%)

TABLE 2. INJURY CAUSES AND TECHNIQUES

Cause of injury	N (%)
Direct impact with another athlete	21 (18.9%)
Falling	13 (11.7%)
Breaking techniques	3 (2.7%)
Attack	32 (28.8%)
Counterattack	4 (3.6%)
Defense	4 (3.6%)
Others	34 (30.6%)
Injury technique	N (%)
Ap Chagi	7 (6.3%)
Dollyo Chagi	17 (15.3%)
Yop Chagi	6 (5.4%)
Naeryo Chagi	9 (8.1%)
Twit Chagi	1 (0.9%)
Mondollyo Dollyo Chagi	3 (2.7%)
Bandal Chagi	17 (15.3%)
Block (Maki)	1 (0.9%)
Other	35 (31.5%)
Does not remember	15 (13.5%)
Total	111 (100%)

probability of injury (95% CI: 1.44-12.29; p=0.009) than those who trained up to 1 hour per session.

DISCUSSION

To our knowledge, this is the largest Portuguese national survey to date to investigate taekwondo injuries in all regions of the country (North, South, Center, and Lisbon area, except the islands), involving different ages. There were 112 injuries reported in the previous year by 76 (22.3%) Portuguese athletes.

The data of other studies revealed a higher percentage of injured athletes, compared to those observed in the present study. Lystad et al.[10] evaluated 152 Australian amateur taekwondo athletes, aged 12 years or more, and found that 88 athletes (57.9%) reported having incurred one or more injuries during the previous 12 months, totaling 307 injuries. The study of Kazemi et al.[11] evaluated 28 Canadian male and female Taekwondo athletes competing in a national tournament, and the results showed that 79% of athletes reported having experienced an injury. The discrepancy between the findings may be related to differences in style of combat, level of skill and experience, physical build, and training methods. For example, in the study by Kazemi et al.[11] all of the athletes of the sample were black belts.

Another study by Kazemi et al.[17] verified that the competitor experience level influences the presence of single versus multiple injuries, as black belt competitors sustained significantly more multiple injuries (28.99%) than color belts (9.09%).

The types of sampling methods and data collection used in previous studies have limitations that could affect the results obtained. The non-randomized sampling method used does not represent the population studied. In the study by Lystad et al.[10], an online questionnaire was applied, and the higher prevalence of injuries obtained in this last study could be explained by the fact that the questionnaire was filled out mainly by athletes who had an injury and possibly felt more motivated to answer the questionnaire. In the study by Kazemi et al.[11], the athletes

TABLE 3. RELATIONSHIP BETWEEN THE EVENT, THE PRESENCE OF INJURY, AND VARIABLES ON NON-MODIFIABLE SAMPLE FACTORS AND TAEKWONDO PRACTICE CHARACTERISTICS

Variables	OddsRatiocrude (CI 95%); p-value	Odds Ratioadjusted** (CI 95%); p-value	Odds Ratioadjusted*** (CI 95%); p-value
Gender (male*) female	1.07 (0.62-1.85); 0.817	---	---
Age group (children*) adolescent (adolescent*) adult	3.67 (1.24-10.84); 0.019 10.19 (3.46-30.02); ≤0.001	---	1.69 (0.50-5.67); 0.399 3.91 (1.13-13.55); 0.032
Participated in championships (no*) yes	1.53 (0.89-2.59); 0.117	1.11 (0.63-1.97); 0.715	---
Years of practice (< 5 years*) ≥ 5 years	2.66 (1.47-4.81); 0.001	1.72 (0.91-3.23); 0.095	---
Weekly training (≥ 3 times*) up to 2 times	1.27 (0.75-2.15); 0.376	1.03 (0.59-1.79); 0.929	---
Duration of training per session (up to 1 hour*) more than 1 hour	7.55 (2.95-19.36); ≤0.001	4.47 (1.52-13.18); 0.007	4.20 (1.44-12.29); 0.009
Belt rank (beginner/intermediate*) advanced	2.92 (1.72-4.96); ≤0.001	1.71 (0.95-3.09); 0.072	---

* Class reference; ** adjusted for gender and age group (Enter model); *** Forward LR model

were invited to participate by the current author and his assistants.

Another fact that can explain these results differences in prevalence values between these studies could be the type of competition that the athletes in the studies participated in. In the study by Lystad et al.[10], there was no information about how many athletes participated in form or combat competitions; in our study, 31% of athletes participated in form competitions, which does not cause injuries by direct contact. We suppose that if Lystad's study[10] involved a greater number of athletes who participated in combat competitions, they would be more likely to be injured because of the direct contact with another athlete, and this would explain the difference in the injury prevalence values obtained in these studies.

Regarding to injury rate, the value obtained in this study was 2.15 injuries per 1,000 hours of taekwondo training, which differs from those found in the systematic review carried out by Pieter et al.[18], which revealed that the values of injury rates for elite men varied from 20.6/1,000 athlete-exposures to 139.5/1,000 athlete-exposures and, for elite women, the rates varied from 25.3/1,000 athlete-exposures to 105.5/1,000 athlete-exposures (95% CI 89.8 to 121.1). Another study[19] evaluated injuries in a Canadian National Taekwondo Championships (n=318), and the overall rate of injuries was 62.9/1,000 athlete-exposures. These studies were also based on injuries in elite black belts, not color belts.

This difference between these studies mentioned above can be explained because the denominator data might have different numbers according to the study. To calculate a valid injury rate, the number of injuries experienced (numerator data) is linked to a suitable denominator measure of the amount of athletic exposure to the risk of injury; consequently, a rate consists of a denominator and a numerator over a period of time[20]. The denominator value can be the number of athletes in a club or team, the number of games played, the number of minutes played, or the number of player appearances. The choice of denominator affects the final value[20]. Another factor that may explain the differences found between these studies and ours may be the type of sample studied, involving only competitive athletes, whereas our data also involved amateurs and children.

The most common types of injuries found in this study were muscle injuries with strains and contusions (58.6%), and joint injuries involving cartilage and meniscus, and ligament structures, sprains, and luxations (20.7%). These findings can be explained by the greatest numbers of offensive and defensive techniques that are the main cause of this injury type[21,22].

Furthermore, the strain could happen as the result of kicking actions above the waist or above the opponent's head to obtain the highest scores in taekwondo[21].

Ji[23] evaluated 512 taekwondo athletes, and the data showed that contusions (48.4%), strains (13.5%), and sprains (11.4%) were the most frequent injury types. Altarriba-Bartes et al.[7] found similar results, with contusions (29.3%), cartilage (17.6%), and joint (15.7%) injuries the most common types. Kazemi and Pieter[19] data revealed that the most common type of injury in men were sprains and, in women, contusion. In another study, Kazemi and Pieter[19] observed that sprains/strains (45%), followed by contusions, fractures, and concussions were the most common types.

The most frequent locations of injuries observed in this study were the foot and fingers (18.9%), thigh, leg, and ankle (15.3% each one). This finding could be explained by the nature of taekwondo, which uses mostly kicks to score points during combats[21].

Results found by the Ji study[23] revealed that the foot was the location of more injuries (16%), following by the knee (14.8%), ankle (13.8%), and thigh (11.1%). Altarriba-Bartes et al.[7] study revealed that knee (21.3%), foot (17.0%), ankle (12.2%), and thigh (11.4%) were the areas most affects by injuries. Several studies showed that the most common injury location was the lower limb[s8,11,18,19,24]. Some studies do not specify the area of the body affected, including the lower limbs, upper limbs, trunk, and head, making it difficult to compare with the data obtained in this study.

Taekwondo athletes are equipped with a padded trunk protector, protective padded headgear, protective gloves, and shin guards[7,25]; although there was protective equipment for taekwondo practice, it was observed during the data collection that athletes did not use protective material during training, only when in combat. In addition, although there are also foot protectors, some athletes do not have this equipment, and it does not provide specific protection for the toes. There is no protective material for the thigh either, one of the sites referred to as the most injured. Future studies should verify if athletes use protective equipment during training to verify their effectiveness in reducing the number of injuries.

Regarding the moment when injuries occurred (training or competition), most of the athletes (90.1%) reported that the injury occurred during training, which was consistent with the results of Lystad et al.[10] and Kazemi et al.[11] due to non-use of the protective material during training, as mentioned.

Most athletes in this study received some sort of treatment (79.3%), the most used were resting or medication (38.6%), and physiotherapy (27.3%). Similar results were found by Lystad et al.[10], with 60.3% of athletes receiving some treatment and physiotherapy the most used treatment.

According to our study data, the offensive technique was the more prevalent cause of injury (29%), followed by a direct impact with another athlete (19%). The Altarriba-Bartes et al.[7] study revealed that the main injury mechanism in taekwondo was through direct contact. The data obtained by Yiemsiri et al.[24] showed that the most common mechanism of injury in men was from delivering a kick and in women were both from receiving a kick and delivering a kick, whereas the literature review study by Pieter et al.18 reported turning kick. The injury mechanism classification in these studies is different, making a more specific comparison difficult.

Future studies should use a standardized injury classification system, including the same classification for the mechanism of injury and the technique that caused it.

Regarding the risk factors for sustaining injuries, this study found that adult taekwondo athletes had 3.91 more probability of suffering an injury than adolescent athletes, and taekwondo athletes who trained more than 1 hour had 4.20 times more probability of injury than those who trained up to 1 hour per training session. The data obtained by binary logistic regression using the odds ratio crude (non-adjusted) revealed that athletes with more years of practice, with a longer duration of the training, and at more advanced levels are more likely to have an injury. The athletes who have more years of practice and who are at more advanced levels are generally older, and the training load (longer duration) may also be related to the likelihood of developing injuries as it implies greater work stress. Thus, these results can be explained by the different dynamics and skillsets required for performance in advanced levels and also by the specific competition time and training methods associated with different weight classes[26].

The study by Lystad et al.[10] showed that neither age, gender, nor level of practice were related to the occurrence of injury; similar results obtained by Lystad et al.[10] revealed no relationship between sustaining an injury and demographic variables as gender, age, and years of experience. However, the data from Altarriba-Bartes et al.[7] revealed that chronological age and weight category could be considered risk factors for sustaining injuries in elite taekwondo athletes.

This study contains some limitations, including the data collection approach of an interview relying on the memory of the participant. This memory bias occurs when there is a memory differential in information for cases and controls and is a limitation of retrospective studies. Furthermore, as reported injuries were not evaluated by health professionals, the reliability of the injury classification may be questionable.

CONCLUSIONS

This is the first nationwide study in Portugal investigating taekwondo-specific injuries. There were 1.16 injuries per 1,000 hours of taekwondo training, and 22% of the participants suffered at least one injury over a 12-month period. The most common injuries were muscle injuries (strains and contusions), and joint injuries (cartilage and meniscus injuries, ligament rupture, sprains and luxation), most involving the foot and fingers, thigh, leg and ankle, and the offensive technique and direct impact with another athlete were the principal mechanisms of injuries.

This type of study can help devise injury prevention strategies during training and competition, such as some modifications to the competition rules and mandatory use of protective equipment since competition protection equipment is rarely used during training sessions. The lack of security equipment in training and competitions associated with psychosocial aspects are among the risk factors that can increase injuries in taekwondo practice.

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RESUMO

INTRODUÇÃO: *O Taekwondo consiste numa arte marcial que enfatiza os golpes com os pés e punhos, sendo caracterizada pelo contato corporal direto e contínuo, fatores que podem ocasionar lesões. O objetivo do estudo foi determinar a incidência de lesões musculoesqueléticas em atletas portugueses de taekwondo e analisar os fatores associados.*

MÉTODOS: *A amostra foi constituída por 341 atletas de taekwondo, com idades entre 4 e 62 anos (18,77±12,77), sendo 237 (69,5%) do sexo masculino. O instrumento de medida consistiu num questionário, aplicado sob a forma de entrevista, em nível nacional.*

RESULTADOS: *Cento e trinta e dois (38,7%) atletas relataram terem sofrido lesões desde que iniciaram a prática, totalizando 294 lesões. Setenta e seis (22,3%) atletas referiram presença de lesões no período de 12 meses, totalizando 112 lesões. Foram registradas 2,15 lesões por 1.000 horas de treinamento de taekwondo. O tipo de lesão mais frequente foi a lesão muscular (57,7%) e as localizadas no pé e dedos (18,9%). A técnica de ataque (28,8%) foi o mecanismo de lesão mais prevalente. Os adultos apresentaram maior risco de sofrer lesões comparados aos adolescentes (odds ratio = 3,91; IC 95%: 1,13-13,55; p=0,032), e os atletas que treinaram mais de uma hora tiveram um risco de 4,20 (IC 95%: 1,44-12,29; p=0,009) do que aqueles que treinaram até uma hora por sessão.*

CONCLUSÕES: *Os dados do estudo revelaram que as lesões foram frequentes em atletas portugueses de taekwondo, com áreas corporais específicas afetadas, e causadas principalmente pela técnica de ataque. Torna-se necessário elaborar estratégias de prevenção de lesões, incluindo treinamentos específicos e uso de material de proteção.*

PALAVRAS-CHAVE: *Epidemiologia. Incidência. Ferimentos e lesões. Prevalência. Artes marciais.*

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ORIGINAL ARTICLE

Does periodontitis affect mean platelet volume(MPV) and plateletcrit (PCT) levels in healthy adults?

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SUMMARY

OBJECTIVE: *Periodontitis may stimulate infectious and immune response and cause the development of atherogenesis, coronary heart disease, and myocardial infarction. The aim of this study was to compare the plateletcrit (PCT) and mean platelet volume (MPV) levels derived from complete blood count (CBC) tests in patients suffering from stage 3 periodontitis with those of healthy individuals without periodontal disease.*

METHODS: *The study included 57 patients (28 females and 29 males) with Stage 3 Periodontitis and 57 volunteering individuals (31 females and 26 males) who were periodontally healthy. The age of study participants ranged from 18 to 50 years. Their periodontal condition was investigated with probing depth (PD), clinical attachment level, bleeding on probing, and plaque index. Leukocyte (WBC) and erythrocyte count (RBC), hemoglobin (Hb) and hematocrit (HCT) levels, mean corpuscular volume (MCV) and red cell distribution width (RDW), thrombocyte count, mean platelet volume (MPV), plateletcrit (PCT), and neutrophil and lymphocyte counts were evaluated based on the CBC test results of the study participants.*

RESULTS: *PCT, WBC, Neutrophil, and MPV values were found to be significantly higher in the periodontitis group ($p<0.05$). There were no significant differences in RBC counts, Hb, HCT, MCV, RDW, and platelet and lymphocyte counts between the two study groups ($p>0.05$).*

CONCLUSIONS: *PCT and MPV levels may be a more useful marker to determine an increased thrombotic state and inflammatory response in periodontal diseases.*

KEYWORDS: *Blood cell count. Cardiovascular diseases. Inflammation. Leukocyte count. Periodontitis. Blood platelets. Risk factors.*

INTRODUCTION

Periodontitis is an inflammatory disease of chronic nature in the supporting tissues of the teeth. More than one factor is involved in its etiology; however, biofilms of dysbiotic plaques are the main cause of the disease. During the course of periodontitis, the supporting tissues of the teeth are progressively destructed[1]. The harmful effects of periodontitis are not only restricted to the oral cavity but also have an impact on the general health status of the individual. Microorganisms and/or their products and the inflammatory mediators can access the systemic circulation through the ulcerated pocket epithelium and initiate

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a systemic acute-phase response by activating the immune system. Periodontitis may cause bacteremia, endotoxemia, and low-grade systemic inflammation, and it is potentially associated with the impairment of well-being by causing, among others, cardiovascular diseases (CVDs), adverse pregnancy outcomes, respiratory system diseases, and diabetes mellitus[2].

Periodontal inflammation can exacerbate systemic conditions through the pathological changes caused by leukocytes. Leukocytes, especially neutrophils, produce a number of specific molecules directly responsible for the inflammatory response, which can be a risk factor for atherosclerosis and cardiovascular complications[3]. The other major component of the blood is the platelets, which are closely associated with inflammation. When they are activated, pro-inflammatory mediators are released, and pro-inflammatory receptors are exposed. This, in turn, causes platelets to bind to WBC and endothelial cells. Pathogens existing in the periodontal tissues may readily stimulate platelets and WBC, and this activation might be involved in aggravating atherothrombosis[4].

Several studies in the literature have reported that high levels of systemic inflammation markers were detected in periodontitis compared to healthy controls. Among these, total white blood cells (WBC), neutrophils, lymphocytes, serum globulin, C-reactive protein, and platelets were listed[5]. Furthermore, it has been suggested that elevated levels of these markers, particularly high leukocyte counts and high levels of C-reactive protein, as well as increased platelet activation, can help establish the relationship between periodontitis and cardiovascular diseases (CVD)[6].

Complete blood count (CBC) tests are commonly used in clinical practice. Plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are indices specific to platelet morphology and proliferation kinetics, and these parameters can be derived from CBC[7]. In the literature, white blood cell (WBC) and platelet counts, PDW, and MPV have been used in several studies to investigate the association between periodontal disease and CVD; however, the results were contradictory. As far as we have observed, the association of PCT with periodontal diseases has not been investigated yet. PCT is an index providing information on the total platelet mass. The following formula is used to calculate PCT: PCT = Platelet count X MPV/10000[8]. The levels of PCT normally vary in range from 0.22% to 0.24%. Therefore, assessing the PCT value can give us more accurate information about inflammation and increased thrombogenic events[8].

In this study, we aimed to investigate PCT levels in the CBC of patients suffering from stage 3 periodontitis as compared to those of healthy individuals without periodontal disease.

METHODS

Approval by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University was obtained prior to the conduct of the study. The study was conducted in compliance with ethical standards according to the current version of the Declaration of Helsinki. After informing the eligible volunteers of the objective and procedures of the study, written consent was obtained from the subjects to enroll them in the study. The sample size was calculated considering Type I errors (0.05), targeted power (0.80), and effect size(0.50) due to the PCT value ($p<0.05$). The minimum sample size required was calculated as 51.

The study participants comprised 57 patients (28 females and 29 males) with Stage 3 Periodontitis and 57 periodontally healthy subjects (31 females and 26 males). The age range of the participants was between 18 and 50 years. Since individuals older than 50 years might have already developed atherosclerotic processes or might have comorbid diseases, they were excluded from the study as these potential conditions could interfere with the results of the complete blood count tests. Patients were diagnosed with Stage 3 Periodontitis or were determined to be periodontally healthy based on the criteria proposed by the International Workshop for Classification of Periodontal Diseases and Conditions in 2017[1]. The study was conducted at the Department of Periodontology between July 2018 and December 2018.

Inclusion and exclusion criteria

Patients were included in the study if they were systemically healthy and found to have a probing depth (PD) of ≥6 mm and interdental clinical attachment level (CAL) of ≥ 5 mm, if they had tooth loss of ≤4 teeth due to periodontitis, and if they had radiographically detected bone loss reaching the mid-third of the root and beyond. Periodontally healthy subjects were included in the study if they had no radiographically detected bone loss and if they had no sites with clinical attachment loss and no sites with probing depth (PD) of >3 mm in their oral cavity.

Individuals were excluded if they had a history of cardiovascular disease, diabetes mellitus, hypertension, upper respiratory tract infections, smoking, hypo/hyperthyroidism, chronic renal failure, malignancy, any hematological abnormalities, or any medication use such as antiplatelet agents, anticoagulants, antihyperlipidemic, angiotensin-converting enzyme inhibitors, and steroids. Individuals who had been treated for periodontitis in the past 6 months were also excluded.

Clinical examination

All clinical parameters were evaluated by a single experienced periodontist (G.U), and a calibration exercise was performed to obtain acceptable interexaminer reproducibility. Periodontal examinations were performed with a Williams probe (Hu-Friedy, Chicago, IL, USA). The clinical parameters of PD, plaque index (PI), and clinical attachment level (CAL) were measured for every tooth present in the oral cavity. The measurements were performed at six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual), and the results were recorded in approximation to the nearest whole millimeter. The distance from the bottom of the pocket to the cementoenamel junction was defined as CAL, which was measured and recorded. The mean PD and the mean CAL values were calculated by dividing the total score of all teeth by the total number of teeth examined during the study. The periodontal probe was carefully and gently introduced into the gingival sulcus to calculate the percentage of BOP, even one site with BOP was recorded as (+) for each individual tooth.

Blood examinations

CBC and platelet volumes were tested simultaneously with optical and impedance measurements (Cell Dyn 3700; Abbott Diagnostics, Lake Forest, Illinois, USA). Platelet count, the levels of hemoglobin (Hb), hematocrit (HCT), red blood cells (RBC), red cell distribution width (RDW), mean corpuscular volume (MCV), neutrophil and lymphocyte counts, total number of WBC, MPV, and PCT were recorded for each patient.

STATISTICAL ANALYSIS

Statistical analyses were conducted with the SPSS software (SPSS 20.0 for Windows, IBM Co, Chicago, IL, USA). The distribution of the variables in study groups was analyzed by the Kolmogorov-Smirnov test. Normally distributed variables were compared by the t-test, and the results were expressed as mean ± standard deviation. Variables that did not conform to a normal distribution were compared with the Mann-Whitney U test. The chi-square test was used for comparing nonparametric variables between the study groups. A p-value, lower than 0.05, was accepted to indicate a statistical significance.

RESULTS

There were no significant differences in age and gender between the study groups (p>0.05). PD and CAL were found to be statistically different between the groups (*p<0.001*) (Table 1).

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY GROUPS

Baseline characteristics	Stage III Periodontitis Mean± SD (n=57)	Control Mean± SD (n=57)	p
Age (years)	37.4±7.0	35.6±7.0	NS
Male/female	29/28	26/31	NS
PD(mm)	6.10±0.72	1.89±0.42	<0.001
CAL(mm)	6.21±0.81	1.85±0.40	<0.001
BOP(%)	63±15	14±4	<0.001
PI	82±17	15±5	<0.001

SD:Standard deviation, PD: Probing Depth, CAL: Clinical attachment level, BOP: Bleeding on probing, PI: Plaque index

TABLE 2. LABORATORY DATA OF STUDY GROUPS

	Periodontitis Mean± SD (n=57)	Control Mean± SD (n=57)	p
WBC, (u/mm^3)	8.02±2.68	6.87±1.30	0.004
RBC, (u/mm^3)	4.87±0.56	4.96±0.58	0.403
Haemoglobin (gr/dl)	13.92±1.76	14.29±1.69	0.258
Haematocrit (%)	41.78±4.94	42.67±4.83	0.337
MCV (fL)	85.82±6.50	86.17±5.19	0.748
RDW (%)	14.80±1.88	15.20±1.51	0.212
Platelet counts (k/mm^3)	258.52±51.11	240.48±54.58	0.073
MPV,(fL)	8.75±1.32	8.22±1.08	0.021
PCT (%)	0.223±0.04	0.196±0.04	0.001
Neutrophil, (u/mm^3)	4.79±2.36	3.99±1.08	0.021
Lymphocyte, (u/mm^3)	2.46±1.14	2.14±0.56	0.060

SD:Standard deviation., WBC: White blood cells, RBC: Red blood cells, MCV: Mean Cell Volume, RDW: Red cell distribution width, MPV: Mean platelet volume, PCT: Plateletcrit.

PCT was found to be higher in the periodontitis group, and this difference was statistically significantly higher (p=0.001) (Table 2). The periodontitis group was found to have statistically significant higher levels of WBC count and MPV compared to the control group (p=0.004, p=0.021; respectively). PCT, MPV, and WBC distribution of the control and periodontitis group are shown in Figure-1. There were no statistically significant differences between the two groups regarding the other parameters investigated in the study, including the RBC count, HB and HCT levels, MCV, and RDW *(p>0.05)(Table 2).* The correlations between the parameters were also tested in the two groups (N=114). In Table 3, the statistically significant correlations between the parameters investigated are shown.

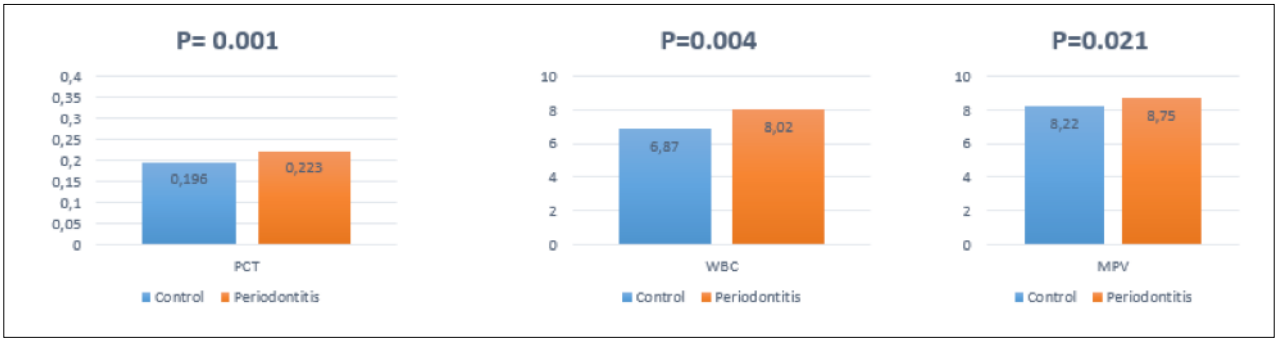
DISCUSSION

Recent studies demonstrated that periodontitis and systemic diseases such as cardiovascular diseases and diabetes were strongly related. Aggravated systemic inflammatory responses and reactions to maintain homeostasis may be the key factors to provide insight into the relationship between periodontal disease and systemic conditions[9].

It is known that infections increase WBC and neutrophil counts, and it has been proposed that these increases might be linking infections with systemic diseases, including CVDs[10]. In our study, an increase in the WBC and neutrophil counts were detected in the periodontitis group compared to the healthy controls. The presence of a high number of leukocytes in the systemic circulation makes the blood more viscous, facilitating the adherence of the circulating cells to the endothelial lining of the blood vessels. This latter change also increases the viscosity of the blood. Decreased blood flow might be involved in the development of CVD. Similar to our results, Kumar et al.[11] reported that patients suffering from periodontitis had high WBC counts compared to controls, and the differences were statistically significant. Conversely, the study by Rao et al.[12] showed no statistically significant differences in WBC count between both groups.

Platelets play a crucial role in managing vascular integrity and regulating hemostasis, and they are involved in the fundamental biological process of chronic inflammation associated with disease pathology, thrombosis, and atherogenesis[13]. Platelet activity can be evaluated with platelet indices such as MPV, platelet counts, PDW, and PCT. Previous studies have

FIGURE 1. PCT, WBC AND MPW DISTRIBUTIONS OF CONTROL AND PERIDONTITIS GROUPS



MPV: Mean platelet volume, PCT: Plateletcrit, WBC: White blood cells

TABLE 3. THE STATISTICALLY SIGNIFICANT CORRELATIONS BETWEEN BLOOD COUNT PARAMETERS AND CLINICAL PERIODONTAL PARAMETERS

	WBC		MPV		PCT		Neutrophil	
	r	P value	r	P value	r	P value	r	P value
Mean PD	0.221*	0.019	0.253**	0.007	0.344**	p<0.001	0.202*	0.032
Mean CAL	0.237*	0.012	0.251**	0.007	0.344**	p<0.001	0.221*	0.019
BOP	0.210*	0.026	0.271**	0.004	0.359**	p<0.001	0.195*	0.040

Correlation analysis was performed using Pearson's correlation analyses. ** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level. MPV: Mean platelet volume, PCT: Plateletcrit, WBC: White blood cells. PD: Probing Depth, CAL: Clinical attachment level, BOP: Bleeding on probing

shown that platelet counts increase in cardiovascular diseases and vascular complications[14,15]. In our study, we detected a higher platelet count in the periodontitis group, but the difference was not statistically significant. This higher platelet count may be explained due to dental plaque bacteria, including the periodontal pathogen *Porphyromonas gingivalis*, which induces platelet activation and aggregation[6]. Similarly, several studies in the literature observed higher platelet counts in periodontitis patients[4,16]. Differently, Kumar et al.[11] reported statistically lower platelet counts in the periodontitis group.

In recent years, it has been reported that MPV can also be used as a marker of inflammation in different inflammatory diseases[17]. Different studies in the literature have reported that MPV has a positive or negative correlation with inflammatory activity[17,18]. Thus, Ekici et al.[19] reported a strong association between MPV and angiographic severity of coronary artery disease. In our study, MPV value was found to be higher in the group of patients with periodontitis. Czerniuk et al.[18] reported similar findings. On the other hand, some studies in the literature have demonstrated decreased MPV in periodontitis[20,21], and others concluded that MPV values were not statistically different between the periodontitis groups and periodontally healthy groups[22,23]. In the studies we have mentioned, there are different and contradictory results regarding MPV and platelet counts. These might be due to the different severity of periodontitis in the studies and differences in the technological methods and modes of measurements used. Also, genetic and environmental factors may affect platelet indices of different populations. Also, in our study, there were no statistically significant differences between the two groups in RBC count, HB, and HCT levels. Conversely, Rao et al.[12] found that the mean Hb level in the periodontitis group was statistically lower compared to the control group.

In the literature, some studies concluded that PCT is a reliable indicator for the diagnosis and treatment of several diseases[24,25]. According to our findings, PCT was significantly higher in the periodontitis group and positively correlated to periodontal clinical parameters that described the severity of periodontal disease. This data might provide more accurate insight into the platelet mass and function in periodontal diseases. There was increasing evidence that platelet indices, including MPV and PCT, were found to be significantly associated with vascular risk factors[26]. In the study by Aslan et al.[27], 230 patients with carotid artery disease were included, and high PCT levels were demonstrated to be statistically higher in patients with major adverse cardiac and cerebrovascular events. Furthermore, a study showed that there was a strong relationship between PCT and saphenous vein disease and slow coronary flow[28]. Therefore, high PCT levels in systemically healthy individuals with periodontitis may pose a risk for systemic diseases such as atherosclerotic events.

Study limitations

The limitation of our study is the small patient population (57 patients) and the cross-sectional study design. Since the participants did not undergo coronary angiography, we were unable to demonstrate the relationship between PCT and coronary artery disease directly. Further prospective and randomized studies with larger stratified populations are needed to reveal possible effects of periodontitis on CVDs.

CONCLUSION

As far as we know, this is the first clinical study to investigate PCT values derived from CBC in patients with periodontitis. We conclude that periodontitis may elevate WBC, MPV, and PCT levels compared to healthy control patients. Furthermore, the prevention and treatment of periodontitis may decrease serum mediators and markers of acute-phase response and may be beneficial in the control of atherosclerosis and other systemic inflammatory diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

Authors' contributions

Concept, study design, and project management were done by Dr. Erdal and Dr. Ustaoglu; statistics and writing were done by Dr. Inanir and Dr. Ustaoglu.

Conflict of interest

The authors declare that there is no conflict of interest and funding support. The Clinical Research Ethics Committee of the Bolu Abant İzzet Baysal University approved this study.

RESUMO

OBJETIVO: *A periodontite pode estimular a resposta infecciosa e imunitária e causar o desenvolvimento da aterogênese, doença coronária e infarto do miocárdio. O objetivo deste estudo foi comparar os níveis de plaquetócrito (PCT) e de volume médio de plaquetas (VMP) derivados dos testes de hemograma completo (CBC) em doentes que sofrem de periodontite de fase 3 com os de indivíduos saudáveis, sem doença periodontal.*

MÉTODOS: *O estudo incluiu 57 doentes (28 mulheres e 29 homens) com periodontite de fase 3 e 57 voluntários (31 mulheres e 26 homens) que eram periodontalmente saudáveis. A idade dos participantes do estudo variou de 18 a 50 anos. A condição periodontal dos participantes do estudo foi investigada com profundidade de sonda (PD), nível de ligação clínica, hemorragia na sonda e índice de placas. Contagem de leucócitos (WBC) e eritrócitos (RBC), níveis de hemoglobina (Hb) e hematócrito (HCT), volume corpuscular médio (VCM) e largura de distribuição das células vermelhas (RDW), contagem de trombócitos, volume plaquetário médio (MPV), plaquetócrito (PCT) e contagem de neutrófilos e linfócitos foram avaliados com base nos resultados do teste CBC dos participantes do estudo.*

RESULTADO: *Verificou-se que os valores de PCT, WBC, neutrófilos e MPV eram significativamente mais elevados no grupo da periodontite (p<0,05). Não houve diferenças significativas nas contagens de glóbulos vermelhos, Hb, HCT, MCV, RDW; nem nas contagens de plaquetas e linfócitos entre os dois grupos estudados (p>0, 05).*

CONCLUSÃO: *Os níveis de PCT e MPV podem ser um marcador mais útil para determinar um estado trombótico aumentado e a resposta inflamatória em doenças periodontais.*

PALAVRAS-CHAVE: *Contagem de células sanguíneas. Doenças cardiovasculares. Inflamação. Contagem de leucócitos. Periodontite. Plaquetas. Fatores de risco.*

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ORIGINAL ARTICLE

Two criteria of oral glucose tolerance test to diagnose gestational diabetes mellitus

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SUMMARY

OBJECTIVE: *To evaluate two different criteria, one or two cut-off values, of oral glucose tolerance test with 75g of glucose for the diagnosis of gestational diabetes mellitus.*

METHODS: *A cross-sectional study involving 120 records of pregnant women who received prenatal care at the service of a Brazilian university was carried out. Bivariate analysis of obstetric and perinatal outcomes was performed using the chi-square test.*

RESULTS: *Considering criterion I, 12.5% of patients were diagnosed with gestational diabetes mellitus. Patients were 3.57 times more likely to have a large fetus for the gestational age at birth (p=0.038). Using criterion II, gestational diabetes mellitus was diagnosed in 5.8% of patients, macrosomia was 7.73 times more likely to be found in the presence of gestational diabetes mellitus (p=0.004), and a large fetus for the gestational age at birth was 8.17 times more likely (p=0.004).*

CONCLUSIONS: *There was a difference in the prevalence of gestational diabetes mellitus between the two criteria analyzed. The new criterion proposed increased prevalence.*

KEYWORDS: *Diabetes, gestational. Diagnostic techniques and procedures. Glucose tolerance test. Perinatal care.*

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized by a carbohydrate intolerance with onset or diagnosed during pregnancy[1]. The stress caused by pregnancy, with other genetic and/or nutritional factors, are the main causes of GDM due to the increase of insulin counter-regulatory hormones[1].

In addition to clinical and obstetric complications for the patient, during pregnancy and at birth, children from pregnant women with GDM present a higher risk of neonatal hypoglycemia, macrosomia, polyhydramnios, abnormal presentation, birth injury, malformation, and perinatal death[1,2]. Prenatal exposure to GDM is a predictor of glucose intolerance and obesity in adolescence. However, associations with the long-term effects of GDM on infants continue to be investigated[3]. Moreover, GDM is a predisposing factor for the development of Type 2 diabetes mellitus (DM) 5 years after pregnancy among those women[4].

The incidence of GDM varies depending on the population studied and the different diagnostic criteria[1]. The overall prevalence of GDM is 3-5%, reaching up to 18%. In Brasil, the estimated prevalence ranges from

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2.4% to 7.2%[5]. In the United States, the disease affects 1.4% to 2.8% of women at low risk, and 3.3% to 6.1% of women present some risk factor[6]. A review published covering the last 10 years on the evolution of GDM prevalence suggests that it will increase significantly[6].

Therefore, GDM is a common obstetric complication, and its prevention is a public health priority[6]. There are many options for screening and diagnosing this disease, and the disagreement of the methodology applied by different services reflects on the results of different studies. This incongruity leads to diversified protocols of investigation of GDM[7-9]. Although not diagnosed with GDM, many patients have elevated blood glucose levels, which would make them possible subjects with diabetes according to some other investigation criterion[9]. This is a problem that requires further elucidation. However, what appears to be a consensus among the different criteria used is the association of GDM with its risk factors[1,5,7,9].

The Hyperglycemia Adverse Pregnancy Outcomes (HAPO) research group, conducted a study on the adverse results of increased maternal blood glucose during pregnancy. It was demonstrated that high blood sugar levels lead to increased frequency of newborns (NB) with birth weight above 90th percentile of the expected for the gestational age (GA) and increased incidence of cesarean delivery and neonatal hypoglycemia. It also suggests that maternal hyperglycemia, not diagnosed as GDM, is associated with neonatal abdominal fat deposition, mediated by fetal insulin production[3,4].

Women who underwent proper treatment for GDM had a reduced number of macrosomic fetuses. However, some authors claim that there is a modest benefit in identifying and treating women with GDM, when related to long-term complications developed over time, such as Type 2 DM and obesity[10-12].

The American Diabetes Association (ADA) defines GDM as diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation[13]. Getting a pattern on issues concerning screening time, diagnostic tests, and the appropriate glycemic cut-offs that should be used to define GDM is still a problem[13-15].

Recently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG), in accordance with the American Diabetes Association (ADA), published a consensus derived from the HAPO study. The goal was to find the exact cut-off point linking maternal hyperglycemia with adverse perinatal events, which would allow for the possibility of revising the diagnostic criteria for GDM[14-16]. Such consensus suggests that all pregnant women, regardless of the existence of risk factors for the development of GDM, should undergo an oral glucose tolerance test (OGTT) with dosages of fasting plasma glucose (FPG), 1-h and 2-h after ingestion of 75g of glucose, between 24-28 weeks of pregnancy. It proposed cut-off points at 92 mg/dl, 180 mg/dl, and 153mg/dl, respectively[15]. According to these criteria, if at least one of these values is equal to or above those limits, GDM will be diagnosed. The Brazilian Federation of Gynecology and Obstetrics Association (FEBRASGO), supported by the Brazilian Diabetes Society (SBD), suggested the use of another criterion, with the same OGTT of 75g of glucose, measured between 24-28 weeks. GDM would only be present if two values were equal or higher than 95 mg/dl, 180 mg/dl, and 155 mg/dl, respectively[17].

Other screening methods are used worldwide. The Canadian Diabetes Association (CDA) recommends all women to be screened with a 1-h glucose measurement after a 50-g oral glucose load between 24 and 28 weeks of gestation, followed by the 2-h 75-g OGTT if the threshold has been surpassed[18]. This two-steps approach, commonly used in the USA, is supported by the American College of Obstetricians and Gynecologists (ACOG)[19] and recommended by the National Institutes of Health (NIH)[20]. Overall, the one-step approach should be preferred due to its simplicity of execution, greater patient adherence, accuracy in the diagnosis of GDM, and closeness to the international consensus[15]. The International Diabetes Federation (IDF) addresses some attention to this aspect by suggesting that selective screening should be considered only in particular epidemiological and clinical conditions, and local cost-effectiveness[21].

As noted, there is no consensus on the best method for screening and diagnosing GDM. Apparently, the proposal advocated by some entities (FEBRASGO/SBD) is that if fewer cases of GDM are diagnosed, fewer treatments would be carried out and, consequently, worse outcomes could emerge. The elucidation of the best 2-h 75-g OGTT diagnostic criteria for GDM may help define adequate prenatal care protocols, minimizing the risks for pregnant women and their children. Therefore, this study aims to evaluate the difference in GDM prevalence with two different 2-h 75-g OGTT criteria and the difference between the obstetric and perinatal outcomes in cases where there is a disagreement between the methods.

METHODS

A cohort study was carried out to test the association between obstetric and neonatal outcomes with two different criteria to diagnose GDM. All records of pregnant patients who consulted at the Gynecology and Obstetrics Ambulatory at the *Universidade do Sul de Santa Catarina* (UNISUL) and delivered their babies at the *Regional Hospital de São José*, both institutions located in the Metropolitan area of Florianópolis/SC, Brasil, for a one-year period. Those who presented 75g glucose OGTT at 24-28 weeks of pregnancy were included. Women with Types 1 or 2 DM prior to pregnancy were excluded, once the screening protocol would not be performed on them.

The Ethics Committee on Human Research of UNISUL approved this study under protocol number 12.094.4.01.III. All the procedures were explained in detail to the participants, and informed consent was signed.

As the incidence of GDM was uncertain, the sample size was calculated based on the Pregnancy Risk Assessment Monitoring System (PRAMS)[22] with a range from 1% to 25%, depending on the ethnicity and diagnostic criteria used. Therefore, using a 25% positive exposure rate, a confidence level of 95% (error type I = 5%), a power of 80% (error type II = 20%), and an equal non-exposure and exposure rate, a minimum sample size of 118 pregnant women was estimated. After analyzing the medical records, it was noted that 120 patients received both prenatal and delivery care. The outcomes that were considered to be compared with GDM diagnoses were macrosomia, polyhydramnios, NB weight according to gestational age at birth, fetal abdominal circumference (FAC), and type of delivery.

Outpatient medical records gathered information about the risk factors for GDM, laboratory values of FPG and OGTT, presence of polyhydramnios, and measurement of the fetal abdominal circumference. The body mass index (BMI) was calculated based on the anthropometric data on the first prenatal visit (weight/height^2). The measurement of FAC was obtained from the third-trimester ultrasound (34-36 weeks) and categorized with the Hadlock table for FAC[23].

The hospital records offered information concerning the presence of macrosomia, NB weight according to gestational age at birth, and type of delivery[24]. NB with a weight greater than or equal to 4 kg were considered macrosomic[18,19]. NB with weight below the 5th percentile, between the 5th and 95th percentile, and above the 90th percentile expected for gestational age were classified as small, appropriate or large, respectively, and according to Hadlock table for estimated fetal weigh[25].

The gestational age was calculated by the reliable last menstrual period and/or ultrasound in the first trimester of pregnancy. When there was disagreement between the two parameters, the highest gestational age was considered.

The investigation of GDM during prenatal care is a routine procedure in this service, performed in all patients with no previous diagnosis of type 1 and Type 2 DM. The OGTT is performed on all patients with FPG blood tests higher than 85 mg/dl in the first trimester of pregnancy and with any risk factor for GDM. The OGTT is carried out with 75g of glucose, between 24-28 weeks of gestation, following 3 stages (FPG, 1-h, and 2-h after glucose intake). The cut-off points are 95 mg/dl, 180 mg/dl, and 155 mg/dl), respectively. If two or more results are equal to or greater than the cut-off point, GDM is considered as diagnosed. According to this protocol, after diagnosed with GDM, the patient is referred for appropriate treatment and monitoring.

Data from the OGTT were evaluated and interpreted as two diagnostic criteria for GDM. Criterion I (IADPSG / ADA)[14-16]: GDM was diagnosed when at least one value was equal to or greater than the cut-off for FPG (92 mg/dl), 1-h (180 mg/dl), and 2-h (153 mg/dL) OGTT, respectively. Criterion II (FEBRASGO / SBD) [17]: GDM was diagnosed when at least two or more values were equal to or greater than the cut-off for FPG (95 mg/dl), 1-h (180 mg/dl), and 2-h (155 mg/dL) OGTT, respectively. Posteriorly, the outcomes were compared to the presence of GDM according to both criteria, and to those patients diagnosed with GDM under criterion I but not under criterion II (Figure 1).

Statistical analysis was performed using SPSS – version 18.0. Qualitative variables were described in absolute and relative frequencies, and quantitative variables were categorized for further bivariate analysis. The chi-square was used to test the homogeneity of proportions. The significance level was $p < 0.05$. Odds ratios with 95% confidence intervals (95% CI) were estimated.

RESULTS

In the study population, the mean age was 27.3 years (±7.0). Regarding the skin color, 86.7% of patients were white, 9.2% were black, and the remaining 7.4%

were of other ethnicities. None of the patients reported being illiterate, 32.5% completed high school, and 2.6% had joined college.

The analysis of the risk factors for GDM showed 62.5% of patients aged 25 years old or more, 54.2% with BMI ≥ 25 kg/m², 29.2% with family history of DM, 0.8% used corticoid, 0.8% used thiazide diuretic, 1.7% with polycystic ovarian syndrome, 4.2% with systemic arterial hypertension, 30.0% with excessive weight gain, and 4.2% with preeclampsia. About the risk factors during a previous pregnancy, 3.3% of the patients had had GDM, 3.3% had had macrosomia, 18.3% had experienced fetal or neonatal death, and 6.7% had undergone preterm labor.

Considering criterion I, 12.5% of patients would be diagnosed with GDM. Under this criterion, large fetus for the gestational age at birth were 3.57 times (95% CI 1.01-12.59) more likely in patients with GDM (p=0.038) (Table 1).

Using criterion II, GDM was diagnosed in 5.8% of the patients. These patients belonged to the treated group, according to the service protocol. Under this criterion, macrosomia was 7.73 times (95% CI 1.51-39.49) more likely in the presence of GDM (p=0.004), and large fetus for the gestational age at birth were 8.17 times (95% CI 1.58-42.32) more likely in these patients (p=0.004) (Table 1).

After sorting out patients diagnosed as GDM by criterion I, but not by criterion II, no outcome associated with the presence of GDM was found (Table 1).

TABLE 1. ASSOCIATION BETWEEN OBSTETRIC AND PERINATAL CHARACTERISTICS AND THE PRESENCE OF GDM BY CRITERION I (IADPSG£ / ADA¥), CRITERION II (FEBRASGO□ / SBD□) AND CRITERION I (IADPSG£ / ADA¥) BUT NOT BY CRITERION II (FEBRASGO□ / SBD□). PALHOÇA, SC, BRASIL (N = 120).

Obstetric and perinatal characteristics	GDM* by Criterion I			p-value	OR† (CI 95%)	GDM* by Criterion II			p-value	OR† (CI 95%)	GDM* by Criterion I - Criterion II			p-value	OR† (CI 95%)
	Yes	No	Total			Yes	No	Total			Yes	No	Total		
	n (%)	n (%)	n (%)			n (%)	n (%)	n (%)			n (%)	n (%)	n (%)		
Macrosomia															
Yes	5 (38.5)	8 (61.5)	13 (10.8)	0,061	3.30 (0.96-11.34)	3 (23.1)	10 (76.9)	13 (10.8)	0.004	7.73 (1.51-39.49)	2 (20.0)	8 (80.0)	10 (8.8)	0.512	1.73 (0.33-9.06)
No	17 (15.9)	90 (84.1)	107 (89.2)		1.00	4 (3.7)	103 (96.3)	107 (89.2)		1.00	13 (12.6)	90 (87.4)	103 (91.2)		1.00
Polyhydramnios															
Yes	-	1 (100.0)	1 (0.8)	0,643	2.21 (0.07-67.79)	-	1 (100.0)	1 (0.8)	0.167	8.00 (0.25-259.50)	-	1 (100.0)	1 (0.9)	0.480	3.23 (0.10-100.60)
No	22 (18.5)	97 (81.5)	119 (99.2)		1.00	7 (5.9)	112 (94.1)	119 (99.2)		1.00	15 (13.4)	97 (86.6)	112 (99.1)		1.00
NB‡ weight/GA§															
SGA‖	-	6 (100.0)	6 (5.0)	0.073	0.42 (0.02-7.81)	-	6 (100.0)	6 (5.0)	0.640	2.04 (0.10-43.25)	-	6 (100.0)	6 (5.3)	0.682	0.55 (0.03-10.34)
AGA¶	17 (16.7)	85 (83.3)	102 (85.0)		1.00	4 (3.9)	98 (96.1)	102 (85.0)		1.00	13 (13.3)	85 (86.7)	98 (86.7)		1.00
LGA**	5 (41.7)	7 (58.3)	12 (10.0)	0.038	3.57 (1.01-12.59)	3 (25.0)	9 (75.0)	12 (10.0)	0.004	8.17 (1.58-42.32)	2 (22.2)	7 (77.8)	9 (8.0)	0.459	1.87 (0.35-9.99)
FAC††															
Percentile < 5	14 (16.3)	72 (83.7)	86 (71.7)	0.319	0.61 (0.23-1.62)	3 (3.5)	83 (96.5)	86 (71.7)	0.073	0.26 (0.06-1.24)	11 (12.8)	72 (87.2)	83 (73.4)	0.941	0.96 (0.28-3.27)
Percentile 5-95	8 (24.2)	25 (75.8)	33 (27.5)		1.00	4 (12.1)	29 (87.9)	33 (27.5)		1.00	4 (12.1)	25 (87.9)	29 (25.7)		1.00
Percentile > 95	-	1 (100.0)	1 (0.8)	0.801	1.56 (0.05-51.06)	-	1 (100.0)	1 (0.8)	0.451	3.63 (0.11-126.40)	-	1 (100.0)	1 (0.9)	0.511	3.13 (0.09-109.30)
Type of delivery															
Vaginal	9 (13.6)	57 (86.4)	66 (55.0)		1.00	2 (3.0)	64 (97.0)	66 (55.0)		1.00	7 (10.9)	57 (89.1)	64 (56.6)		1.00
Cesarean	13 (24.1)	41 (75.9)	54 (45.0)	0,142	2.01 (0.78-5.14)	5 (9.3)	49 (90.7)	54 (45.0)	0.148	3.27 (0.61-17.54)	8 (16.3)	41 (83.7)	49 (43.4)	0.403	1.59 (0.53-4.73)

£International Association of Diabetes and Pregnancy Study Groups, ¥American Diabetes Association, *Gestational Diabetes Mellitus, †Odds Ratio, ‡Newborn, §Gestational Age, ||Small for Gestational Age, ¶Appropriate for Gestational Age, **Large for Gestational Age, ††Fetal Abdominal Circumference

DISCUSSION

One of the strengths of this study remains in the fact that its application occurred in a reference center of prenatal care, with a standard screening method for GDM, and accessible to a diverse population. However, there was no pattern regarding the origin of the test results because OGTT was analyzed at different laboratories.

A higher diagnostic of GDM was noted when criterion I (IADPSG/ADA) was used, as expected since its cut-off points are lower, and it needs only one abnormal measure. This result was also found in other international studies[9,14,15,26-28]. In the study by O'Sullivan et al.[26] a GDM tracking was performed using criterion I and compared to the results of criterion II. They found a higher prevalence of diagnoses by the criterion of IADPSG, and also a combination of these diagnoses with an increased incidence of adverse maternal and neonatal outcomes. Jenum et al.[28] performed a universal screening of pregnant women at 28 weeks and found a prevalence of GDM 2.4 times higher with criterion I when compared to criterion II.

The results of this study have shown there is a difference in the prevalence of GDM defined by these two different criteria. Criterion I increased the diagnoses of GDM by 2.21 times when compared to criterion II, similar to the studies [cited9,14,15,26-28]. This finding raises a question regarding the need for intervention in patients who would be diagnosed by criterion I but currently are not diagnosed as GDM by criterion II.

The outcome of macrosomia had low prevalence among the subjects of this study. However, when related to the diagnostic of GDM by both criteria, there was a statistical difference in patients who were diagnosed by criterion II (p=0.004). This finding is consistent with other international studies[26,27]. O'Sullivan et al.[26] assessed the impacts of the new diagnostic criteria for GDM proposed by IADPSG, but there was no significant association with macrosomia. Wendland et al.[29] conducted a systematic review of the association

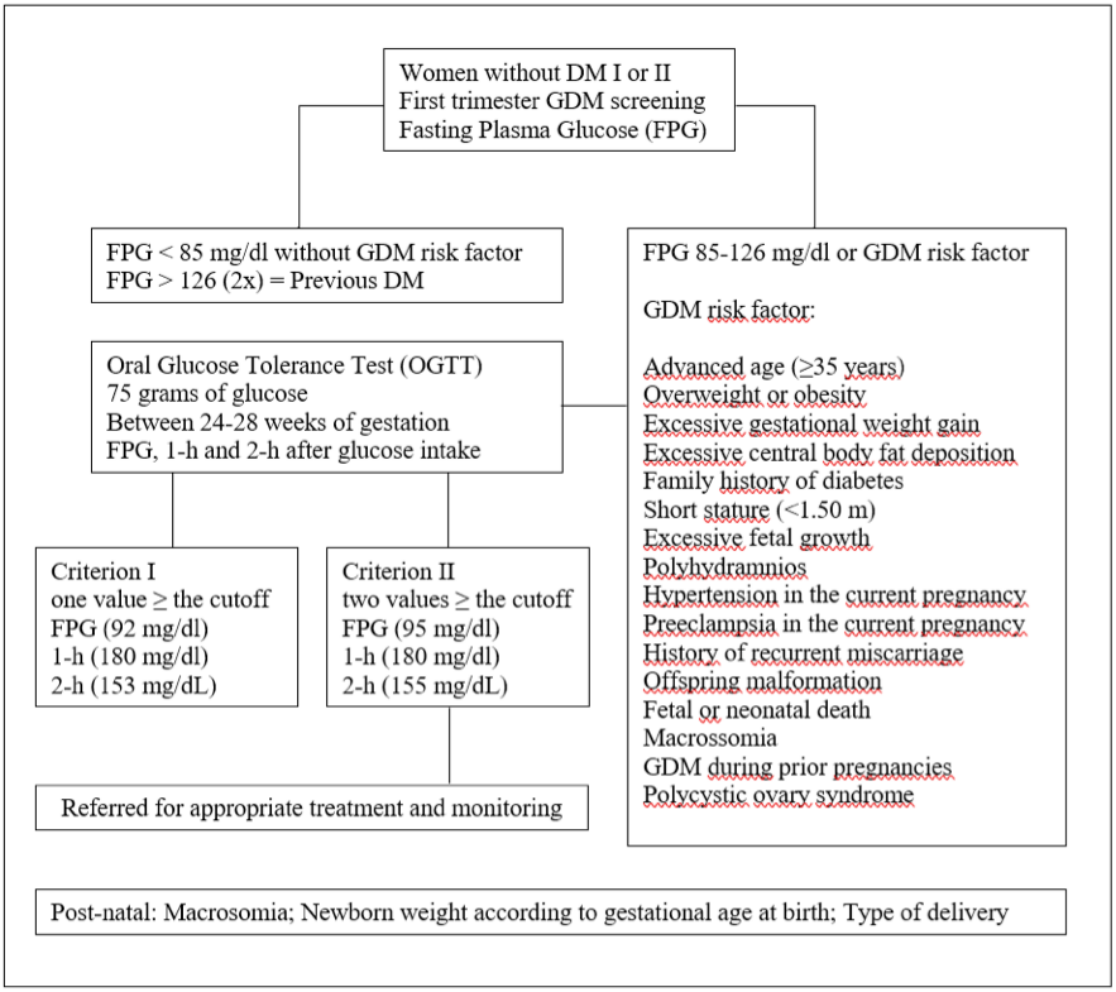

FIGURE 1. FLOW CHART OF PARTICIPANTS THROUGHOUT THE STUDY.

between GDM and macrosomia. Results showed that patients diagnosed by criterion II were more likely to present macrosomia, and those diagnosed by criterion I but no longer by criterion II, were not associated with that outcome.

The research by O'Sullivan et al.[26] reported a statistically significant association of GDM and polyhydramnios. The current study showed that regardless of the criteria used, there is no association between GDM and this obstetric feature, regardless of the criterion used.

The NB greater weight, according to gestational age, was significantly associated with the presence of GDM by criteria I and II. When the association between NB weight/GA and the diagnostic of GDM by criterion I and not by criterion II was analyzed, no difference was found. This result is also reported in other studies[2,26,29]. One study reported an increased prevalence of large NB for their gestational age in pregnant women with GDM[26]. Wendland et al.[29] and Koning et al.[30] described the same association by both criteria.

FAC was not found to be statistically associated with the presence of GDM by both criterion I and II. Besides, this study did not identify any description with FAC above the 95th percentile, showing no relationship with GDM.

The predominant mode of delivery in this study was cesarean. According to O'Sullivan et al.[26], the cesarean rate increased significantly in patients with GDM. Wendland et al.[29] had shown that pregnant women diagnosed with GDM by criterion II presented an increased risk of cesarean delivery compared to pregnant women diagnosed by criterion I. The present analysis did not find any statistically relevant association between both criteria.

The strength of this research was the use of the same patients to evaluate different criteria. Thus, the results were elaborated on the same sample population, reducing the bias of group differences. Otherwise, it was limited to a restrict sample, even knowing that it might be inferred to other populations.

Criterion I of a 2h 75g OGTT increased the prevalence of GDM, but patients diagnosed by this criterion alone, which no longer were diagnosed by criterion II, did not present any outcomes. Therefore, patients diagnosed by criterion I, but not by criterion II, during pregnancy, could receive unnecessary interventions. These patients would undergo unnecessary diets or use hypoglycemic agents. The outcomes verified in patients by criterion I were the same as those of criterion II, regarding the idea that criterion II might possibly continue to be chosen as GDM screening protocol.

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Authors declare no conflicts of interest

Authors' contributions

Rodrigo Dias Nunes – Design, data collection, analysis, and interpretation of data, review, and approval of the final version of the article.

Mayara Eloisa Flôres – Design, data collection, analysis, and interpretation of data, review, and approval of the final version of the article.

Mayara Seemann – Analysis and interpretation of the data, review, and approval of the final version of the article.

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Jefferson Traebert – Analysis and interpretation of the data, review, and approval of the final version of the article.

RESUMO

OBJETIVO: *Avaliar dois critérios distintos, um ou dois valores de corte, do teste oral de tolerância à glicose com 75 g de glicose para o diagnóstico de diabetes mellitus gestacional. Métodos: Estudo transversal envolvendo 120 prontuários de gestantes que realizaram pré-natal em um ambulatório de uma universidade brasileira. Análise bivariada dos resultados obstétricos e perinatais foi realizada pelo teste do qui-quadrado. Resultados: Considerando o critério I, 12,5% das pacientes foram diagnosticadas com diabetes mellitus gestacional. As pacientes apresentaram uma chance 3,57 maior de ter um feto grande para a idade gestacional (p=0,038). Utilizando o critério II, o diabetes mellitus gestacional foi diagnosticado em 5,8% das pacientes. Mediante esse critério diagnóstico, a chance de macrossomia foi 7,73 vezes mais provável na presença de diabetes mellitus gestacional (p=0,004) e a chance de um feto grande para a idade gestacional foi 8,17 vezes maior de ocorrer (p=0,004). Conclusões: Observou-se diferença na prevalência de diabetes melittus gestacional entre os dois critérios analisados, sendo que o novo critério proposto aumentou a prevalência.*

PALAVRAS-CHAVE: *Diabetes gestacional. Técnicas e procedimentos diagnósticos. Teste de tolerância à glicose. Assistência perinatal.*

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ORIGINAL ARTICLE

Investigating gender differences for effectiveness and side effects of varenicline during smoking cessation treatment

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SUMMARY

Varenicline is a useful pharmacological option for smoking cessation. Unfortunately, there is a lack of studies on its effectiveness, retention, and side effects in low- and middle-income countries. The present study aimed to investigate gender differences regarding these outcomes in a Brazilian clinical sample (n = 124). The 12-week treatment protocol included six consultations with a psychiatrist and six sessions of cognitive-behavioral therapy. All subjects received varenicline on the first evaluation, following the standard posology for 12 weeks and instructions to stop smoking after the second week of treatment. Both Mini-International Neuropsychiatric Interview (MINI) Plus and Fagerstrom Test for Nicotine Dependence were applied at baseline. The UKU-Side Effects Rating Scale was administered at weeks 3, 7, and 11, and the Questionnaire of Smoking Urges-Brief at weeks 1, 5, and 9 to ascertain the side effects of the medication and craving, respectively. At the end of the 12-week treatment, abstinence was biochemically assessed. At months 6 and 12 after the treatment, follow-up telephone interviews were conducted to access nicotine abstinence. Short- and long-term abstinence and retention rates did not differ between genders. However, women presented more side effects than men, especially in the second half of the treatment. Increased dream activity, reduced duration of sleep, constipation, and weight loss were the most notable side effects. Despite women reporting more side effects than men, this difference did not influence the treatment success rates.

KEYWORDS: *Smoking. Varenicline. Female. Women.*

INTRODUCTION

Worldwide, 1.1 billion people are estimated to be smokers, and 80% of these smokers live in low- and middle-income countries[1]. Smoking is a public health issue, especially in these countries where the prevalence is not falling as it is in wealthy regions. This high prevalence tends to cause morbidity and mortality in lower-income regions. The epidemic of smoking among men had peaked and is now declining; however, the prevalence of smoking among women is rising in Brasil, where more than 10 million women are smoke[rs1,2].

Women who smoke have worse health outcomes than men[3]. Thus, providing evidence-based (pharmacological and non-pharmacological) smoking cessation

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treatment for women is a current public health issue. Investigating gender differences on varenicline outcomes is of utmost importance, as it is currently the most effective stand-alone medication available[4] and seems to perform better in women[5].

Despite reporting similar levels of nicotine dependence in psychometric measures, gender differences are observed in treatment settings[6]. Women tend to receive less pharmacological treatment[7], even though they seek treatment more often[8], and when on medication, report more side effects[9]. In addition, women report cravings more frequently, present a higher level of response to negative emotions[10], and concern about weight gain[11]; all these factors contribute to an increased risk for relapse and may play an essential part in the fact that women are less successful in trying to quit smoking than men, particularly in the late abstinence period[12].

Nicotine replacement therapy (NRT) seems to be more effective in men[13], but there is no consensus regarding gender differences when using bupropion[14]. In a recent meta-analysis, varenicline was more effective in women at 12 months of treatment. This discrepancy may have attenuated the differences usually seen in smoking cessation treatments[5]. This is a hypothesis that needs to be better understood and replicated in samples that include individuals from low- and middle-income countries.

Varenicline is an appropriate option for smoking cessation in women. However, there is a lack of studies on its effectiveness, retention, and side effects in low- and middle-income countries. The present study aimed to investigate gender differences regarding treatment effectiveness, retention, and side effects for varenicline during 12 weeks of treatment and 6- and 12- month follow up in a Brazilian clinical sample.

METHODS

Sample

Participants were sampled among individuals voluntarily seeking treatment for nicotine dependence at the Institute of Psychiatry, Medical School, University of São Paulo, Brasil. Recruitment occurred from January 2009 to January 2011. The inclusion criteria were: being 18 years old or older, smoking at least ten cigarettes a day for the past year, and being nicotine-dependent according to the ICD-10 criteria. The exclusion criteria were the following: meeting criteria for current DSM-IV axis I disorder other than nicotine dependence, renal impairment, and women who were pregnant or nursing. A total of 145 subjects sought treatment, and 124 were eligible to participate in this study. Twelve individuals refused, and nine did not meet the ICD criteria. All participants provided written informed consent, and the procedures were approved by Hospital das Clinicas, Medical School Ethics Committee (protocol number 0226/08).

Procedure

The treatment protocol was composed of 12 weeks. The participants were assessed every 2 weeks by a trained psychiatrist. At each assessment, a questionnaire about smoking status ("have you been smoking in the prior 14 days?," "how many cigarettes do you smoke daily?," and "how many cigarettes have you smoked since your last appointment?" – if a participant reported having a puff, it was considered as one cigarette), use of medication ("did you take your medication in the last 2 weeks?", at each visit participants received medication for 2 weeks and showed the blister of the prior 2 weeks), side effects, general health issues, and psychiatric symptoms was completed by the patients. Carbon Monoxide (CO) was measured in every evaluation for comparison with the smoking self-report. Participants with expired CO readings between 0-6 ppm were considered "not smoking"; between 7-10 ppm may have smoked; and those with more than11 ppm were considered to be "smoking".

All subjects received varenicline on the first evaluation (titrated to 1 mg twice daily for 8 days) for 12 weeks and were instructed to stop smoking during the second week of treatment. They attended six sessions of group behavioral therapy conducted by trained clinical psychologists. The UKU-Side Effects Rating Scale (UKU) was applied at weeks 3, 7, and 11 and the Questionnaire of Smoking Urges-Brief (QSU) at weeks 1, 5, and 9 to ascertain side effects of the medication and craving, respectively. At months 6 and 12 after the treatment, follow-up telephone interviews were conducted to access nicotine abstinence.

Instruments

The Mini-International Neuropsychiatric Interview (M.I.N.I.) Plus is a short-structured instrument for psychiatric diagnosis. It has similar results as the Structured Clinical Interview DSM Disorders (SCID) and Composite International Diagnostic Interview (CIDI)[15]. The M.I.N.I was applied at the first evaluation

to rule out any psychiatry disorder other than nicotine dependence.

The Fagerstrom Test for Nicotine Dependence (FTND) is a simple, widely used test to assess the severity of nicotine dependence[16].

UKU Side Effects Rating Scale was created to assess and rate the side effects of psychopharmacological medications[17]. Every question can be scored from 0 to 3 (none to severe side effects); total scores can vary from 0 to 150 points.

The Questionnaire of Smoking Urges-Brief (QSU-Brief)[18] - is a craving assessment scale composed of 10 questions that the individual answers using a 7-point scale Likert scale. The QSU-B can be analyzed through the total sum of points.

Statistical Analysis

Data were summarized using frequency-distribution tables based on gender for all categorical variables. Survival analysis using the Kaplan–Meier method and the Wilcoxon test was applied to examine the association between gender and dropout over time. The χ^2test and Fisher's exact test were used to analyze abstinence at different moments of the treatment and at treatment conclusion (retention). All subjects who dropped out of the treatment were considered as relapsing in the survival analysis model, which considered these individuals missing data through censoring. All analyses were conducted using SAS version 9.2 and SPSS version 20. A statistical significance level of 5% was adopted.

RESULTS

The study sample included 124 participants, of which 79 (63.7%) were women, and the mean ages were 36.5 for men and 40.3 for women (p=0.07). Men started smoking at age 15.6 (average) and women at

TABLE 1. SOCIODEMOGRAPHIC VARIABLES BY GENDER

	Female (n)	%	Male (n)	%	p
Marital Status					0.89
Married	29	61.7	18	38.3	
Separated/Divorced	7	58.33	5	41.67	
Widowed	1	100	0	0	
Religion					0.18
Catholic	26	53.06	23	46.94	
Spiritualism	8	61.54	5	38.46	
Protestant	5	55.56	4	44.44	
Atheist	2	66.67	1	33.33	
No formal religion	35	77.78	10	22.22	
Others	3	75	1	25	
Race					0.13
White	56	66.67	28	33.33	
African American	7	77.78	2	22.22	
Mixed	10	58.82	7	41.18	
Others	4	33.33	8	66.67	
Education					0.71
< Middle School	5	83.33	1	16.67	
< High School	8	72.73	3	27.27	
Some college	31	60.78	20	39.22	
College graduate or higher	35	62.5	21	37.5	
Working Status					0.67
No	18	69.23	8	30.77	
Yes	61	62.24	37	37.76	
Monthly income (in Reais –Brazilian Currency)					<0.01
< 700	5	100	0	0	
700 to 3499	37	82.22	8	17.78	
3500 to 6999	20	47.62	22	52.38	
> 7000	17	53.13	15	46.88	

14.8 (p=0.31). The demographic characteristics are displayed in Table 1. These characteristics did not differ significantly between gender, except for a higher monthly income among men (p<0.01). There were no significant gender differences on FTND at baseline; craving reports (QSU score) at weeks 1, 5, and 9; smoking self-report ratings and CO breath measures among participants throughout the treatment.

In this study, no gender difference for treatment completion and dropout was observed. A total of 28 (62.2%) men and 45 (57%) women completed treatment. Likewise, there was no difference between gender in treatment dropout when survival analysis was used (Figure 1). Forty-nine patients who dropped out were reached by telephone. Among these, 18 participants (36.7%) missed more than one psychotherapy or psychiatric evaluations, 11 (22.4%) failed to give reasons for quitting treatment, 11 (22.4%) discontinued treatment because they could not give up smoking, 6 (12.2%) dropped out because of side effects, and 3 (6.1%) had severe side effects and were excluded or chose to leave the study. Among these three participants, two developed severe depression, both having a previous history of depression (more than one year before the study) and one developed severe gastrointestinal symptoms.

No significant gender differences were found for abstinence at the end of the treatment (12 weeks, 62% for men versus 54% for women), and the telephone follow-ups. After 6 months, 38% of the women and 51% of the men remained abstinent. After 12 months, 30% of the women and 38% of the men remained abstinent.

There were significant gender differences regarding side effects in almost all the assessments. Women had higher total UKU scores than men (8.7, 8.2, and 7.0 points versus 6.3, 3.8, and 2.9, respectively) in weeks 3, 7, and 11 ($p \leq 0.05$). The most common side effects for both genders were: nausea/vomiting, reduced duration of sleep, increased dream activity, headaches, sleepiness, tension, and reduced salivation (see table 2). Considering all the assessments, women presented more increased dream activity, reduced duration of sleep, constipation, and weight loss. Gender differences in side effects at each evaluation are shown in Table 2. No differences were found during the assessment on week 3. On week 7, women presented more increased dream activity and constipation. On week 11, women showed more nausea/vomiting.

TABLE 2. GENDER DIFFERENCES OF SIDE EFFECTS IN A BRAZILIAN CLINICAL SAMPLE OF SMOKING CESSATION USING VARENICLINE (N = 124)

	Week 3			Week 7			Week 11		
	Men	Women	p	Men	Women	p	Men	Women	p
Nausea/ vomiting	27 (67.5)	42 (72.4)	0.85	15 (57.7)	30 (75)	0.27	5 (26.3)	19 (55.9)	0.05*
Reduced sleep time	15 (37.5)	25 (43.1)	1	4 (15.4)	14 (35)	0.15	3 (15.8)	8 (23.5)	0.73
More dreams	15 (37.5)	31 (53.4)	0.44	5 (19.2)	20 (50)	0.02*	3 (15.8)	12 (35.3)	0.21
Headaches	15 (37.5)	23 (39.6)	0.85	3 (11.5)	11 (27.5)	0.22	2 (10.5)	13 (38.2)	0.06
Sleepiness/Sedation	8 (20)	17 (29.3)	0.65	1 (4.8)	5 (12.5)	0.39	2 (10.5)	7 (20.6)	0.46
Reduced salivation	7 (17.5)	21 (36.2)	0.13	4 (15.4)	10 (25)	0.54	1 (5.3)	9 (26.5)	0.07
Asthenia	6 (15)	18 (31)	0.17	1 (4.8)	7 (17.5)	0.14	1 (5.3)	8 (23.5)	0.13
Constipation	7 (15)	13 (22.4)	1	1 (4.8)	12 (30)	0.01*	2 (10.5)	7 (20.6)	0.46
Orthostatic Dizziness	8 (15)	14 (24.1)	1	4 (15.4)	4 (10)	0.70	1 (5.3)	3 (8.8)	1
Tension/ Inner unrest	5 (12.5)	2 (3.4)	0.10	4 (15.4)	10 (25)	0.54	2 (10.5)	8 (23.5)	0.30
Increased sleep time	5 (12.5)	12 (20.7)	0.59	1 (4.8)	5 (12.5)	0.39	1 (5.3)	7 (20.6)	0.23
Polyuria/Polydipsia	5 (12.5)	4 (6.9)	0.30	2 (7.7)	7 (17.5)	0.46	0 (0)	4 (11.8)	0.29
Concentration Problems	4 (10)	13 (22.4)	0.28	2 (7.7)	6 (15)	0.47	2 (10.5)	8 (23.5)	0.29
Failing Memory	4 (10)	12 (20.7)	0.29	2 (7.7)	8 (20)	0.29	1 (5.3)	8 (23.5)	0.13
Depression	4 (10)	14 (24.1)	0.19	0 (0)	5 (15)	0.15	1 (5.3)	5 (14.7)	0.40
Weight loss	1 (2.5)	2 (3.4)	1	0 (0)	11 (27.5)	<0.01*	0 (0)	1 (2.9)	1
Weight gain	3 (7.5)	6 (10.34)	1	1 (4.8)	9 (22.5)	0.07	2 (10.5)	5 (14.7)	1
Increased sweating	2 (5)	2 (3.4)	0.64	0 (0)	6 (15)	0.07	0 (0)	2 (5.9)	1
Increased salivation	3 (7.5)	3 (5.17)	0.67	1 (4.8)	4 (10)	0.64	2 (10.5)	1 (2.9)	0.29

*p≤ 0.05

DISCUSSION

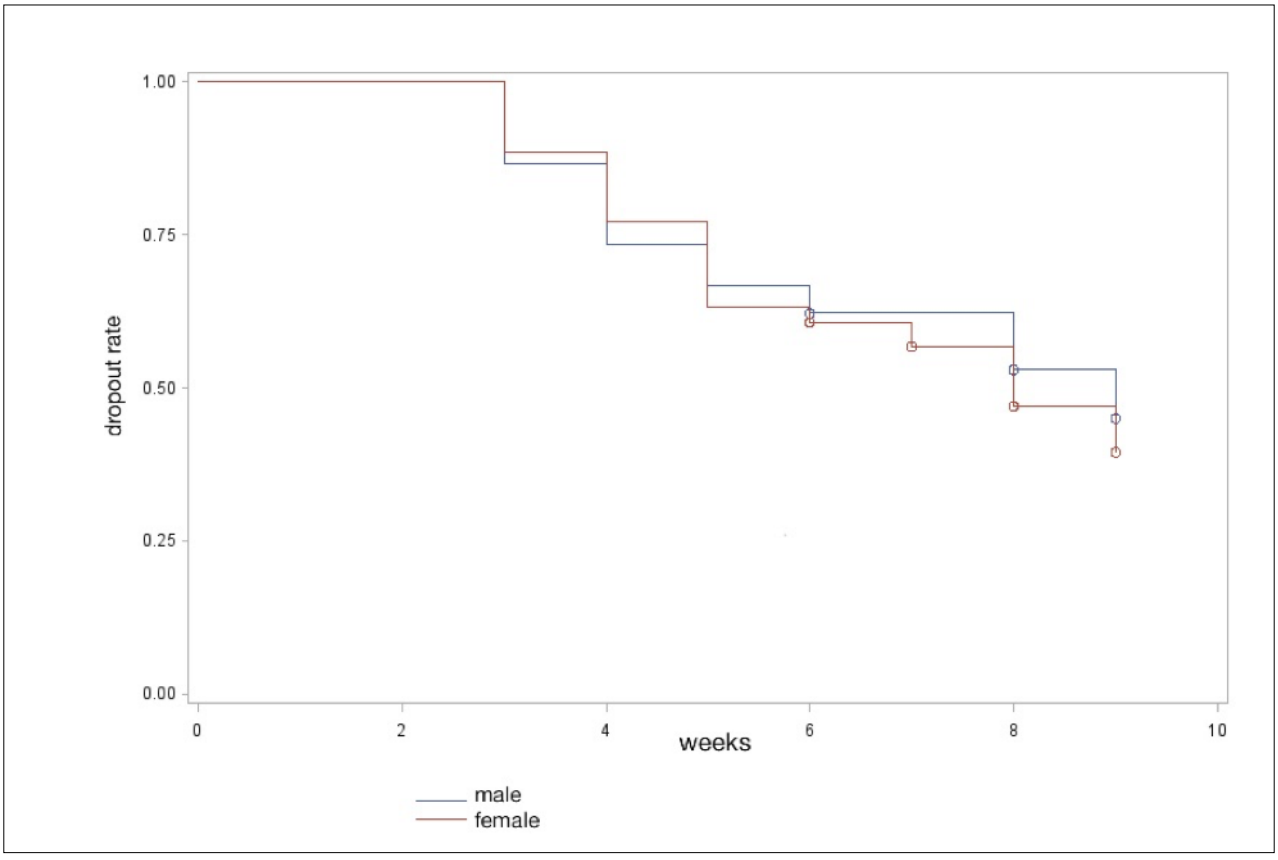
The present study aimed to investigate possible differences in smoking cessation treatment outcomes between men and women in a middle-income country. No differences were found for abstinence in the short- and long-term outcomes in this Brazilian sample. Treatment retention was also similar. However, women significantly presented more side effects than men, especially in the second half of the treatment. Increased dream activity, reduced duration of sleep, constipation, and weight loss were the most notable side effects for this outcome.

Women tend to have worse results than men when trying to quit smoking[19]. Despite having higher UKU side effects scores than men, women presented similar retention and quitting rates than men in the present study. This finding is consistent with results observed in large clinical trials[20]. The similar results among men and women - contrary to the idea that conventional smoking cessation programs are less effective in women[21] - observed in our study raise the hypotheses that varenicline may attenuate those genders treatment differences, despite the more frequent and intense side effects among women. Similarly, a meta-analysis[5] showed that men and women had similar nicotine abstinence rates when treated with varenicline. As women have worse outcomes than men when they are included in the placebo arms of trials, varenicline may compensate for the expected lower quitting rates among women[5]. Considering that women tend to seek treatment at higher rates than men in Brasil, as in many other countries, it would be essential to inform practitioners in smoking cessation services about varenicline's potential benefits for this gender[22].

Retention rates did not differ between genders and were similar to those found in previous randomized controlled smoking cessation trials[4] and naturalistic studies[23,24].

In another study that focused on gender differences, women also reported more side effects when using varenicline. Similarly, such differences did not impact dropout rates[9]. Interestingly, side effects were classified as mild for both genders. This fact may explain why gender differences regarding side effects did not influence dropout rates and abstinence in the short- and long-term in this sample. Our study found a higher prevalence of side effects compared to others[5]. This may be due to the use of the UKU in

FIGURE 1. TREATMENT DROPOUT RATES BY GENDER



our study compared to the self-assessment reports or questionnaires with a list of varenicline's commonly referred side effects in the others. UKU appears to be more sensitive than self-reports and other scales.

CONCLUSIONS

In summary, although women reported more side effects than men when using varenicline, this difference did not influence the treatment success rates. On the contrary, varenicline may even compensate for the expected lower success rates in quitting smoking among women, which have been observed in other studies. A limitation of this study was its small sample size. Larger sample sizes are needed to replicate our findings.

Authors Contributions

VC designed the study, edited and critically reviewed the manuscript, and approved its final version. PDG designed the study, edited and critically reviewed the manuscript, and approved its final version. JMCM designed the study, edited and critically reviewed the manuscript, and approved its final version. AM designed the study, edited and critically reviewed the manuscript, and approved its final version.

RESUMO

A vareniclina é uma opção farmacológica útil para a cessação do tabagismo. Infelizmente, há uma ausência de estudos sobre a eficácia, retenção e efeitos colaterais para este medicamento em países de baixa e média renda. O presente estudo teve como objetivo investigar diferenças entre gênero em relação a esses desfechos em uma amostra clínica brasileira (n = 124). O protocolo de tratamento de 12 semanas incluiu seis consultas com um psiquiatra e seis sessões de psicoterapia cognitivo-comportamental. Todos os indivíduos receberam vareniclina na primeira avaliação, seguindo a posologia padrão por 12 semanas e instrução para parar de fumar a partir da segunda semana de tratamento. Tanto o Mini-International Neuropsychiatric Interview (MINI) Plus quanto o Teste de Fagerstrom para Dependência de Nicotina foram aplicados no início do estudo. A escala de efeitos colaterais (UKU-Side Effects Rating Scale) foi aplicada nas semanas 3, 7 e 11, e o Questionário Breve de Fissura (Questionnaire of Smoking Urges-Brief) nas semanas 1, 5 e 9 para investigar os efeitos colaterais da medicação e fissura, respectivamente. No final do tratamento de 12 semanas, a abstinência foi avaliada bioquimicamente. Aos 6 e 12 meses após o tratamento, foram realizadas entrevistas telefônicas de acompanhamento para acessar a abstinência de nicotina. As taxas de abstinência e retenção de curto e longo prazo não diferiram entre gêneros. No entanto, as mulheres apresentaram mais efeitos colaterais do que os homens, principalmente na segunda metade do tratamento. Aumento da atividade dos sonhos, redução da duração do sono, constipação e perda de peso foram os efeitos colaterais mais notáveis. Apesar de as mulheres relatarem mais efeitos colaterais que os homens, essa diferença não influenciou as taxas de sucesso do tratamento.

PALAVRAS CHAVE: *Fumar. Vareniclina. Feminino. Mulheres.*

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ORIGINAL ARTICLE

The course of alterations in ureteral jet dynamics following kidney transplantation: a prospective observational cohort study

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SUMMARY

OBJECTIVES: *To prospectively investigate the alterations and normal ranges of ureteral jet dynamics after double-J-stent (DJS) removal in patients who underwent renal transplantation (RTx).*

METHODS: *Patients who underwent RTx were prospectively evaluated between November 2017 and June 2018. After RTx, Doppler ultrasonography (D-US) was performed on all patients after DJS removal. Renal artery resistive index (RA-Ri), renal pelvis anterior-posterior diameter (RP-APD), pelvicalyceal system dilation (PCSD), and ureteral jet flow dynamics (maximum and average velocity; JETmax and JETave) were measured by D-US. Also, patients' demographics, estimated glomerular filtration rate (eGFR) levels, and acute rejection were investigated in the study. Patients were assessed two different times by D-US, about 6 and 12 weeks after DJS removal, and the two different measurements were compared with the Wilcoxon test and Chi-square test.*

RESULTS: *A total of 25 patients were evaluated in the study. Nonobstructive PCSD rate (12% vs 8%), JETave (18.8 vs 12.9 cm/sec), and JETmax (29.2 vs 20 cm/sec) levels were significantly decreased (p values are 0.01, 0.010 and 0.014, respectively). In addition, monophasic and square pattern rates were significantly observed to increase over time (p=0.035); however, ureteral jet patterns were correlated between the two different D-US measurements (R=0.225, p=0.032).*

CONCLUSION: *After RTx, dilation rate and ureteral jet flow velocities were significantly decreased, and monophasic and square JETpattern rates were significantly increased over time. Ureteral jet dynamics can provide useful information about the follow-up of peristaltic activity in the pelvic-ureteric system.*

KEYWORDS: *Kidney transplantation. Urination/physiology. Urodynamics/physiology.*

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INTRODUCTION

Chronic renal failure (CRF) prevalence is in the range of 11-13%.[1] After routine follow-up with or without hemodialysis (HD), the standard management of CRF/end-stage kidney disease (EKD) is renal transplantation (RTx).[2] However, many complications can cause significant morbidity after RTx in these patients.[3] Therefore, close follow-up with urine amount, ultrasonography (US), and estimated glomerular filtration rate (eGFR) or serum creatinine level is needed.

Doppler US (D-US), which is a non-invasive and easy examination method to assess ureteral jet phenomenon showing peristaltic activity in the pelvic-ureteric system, can be used for the evaluation of RTx patients.[4] In the evaluation of the ureteral jet phenomenon with D-US, ureteral jet dynamics of maximum rate (JETmax), average rate (JETave), resistive index (JET-Ri), and wave form (JETpattern) of ureteral jet flow are measured in RTx patients.[4] A study found that JETmax and JETave were significantly low and the monophasic JETpattern was more common (66.1%) in RTx kidneys compared to healthy kidneys.[5] However, the course of these alterations was not clear after RTx. Therefore, we aimed to prospectively investigate the alterations and normal ranges for ureteral jet dynamics over time after double J stent (DJS) removal in a patient cohort who underwent RTx.

METHODS

After ethical approval was obtained from the local committee and informed consent forms were obtained from patients, patients who underwent RTx due to CRF/EKD in our tertiary hospital between November 2017 and June 2018 were prospectively evaluated. Patients' demographics (age, gender, and body mass index (BMI)), HD time, and basal eGFR level of patients were noted before RTx. Also, donor type (cadaver/live), RTx side (right/left iliac fossa), and operation time were noted. The Lich-Gregoir ureterovesical anastomosis technique was performed for all cases. Also, end-to-side anastomosis of donor-renal-artery to recipient external iliac artery or end-to-end anastomosis to recipient internal iliac artery was performed for arterial anastomosis. About four weeks after RTx, all DJS were removed with 17 fr flexible cystoscopy under local anesthesia. After DJS removal, the eGFR level and pelvicalyceal system dilation (PCSD) presence in the RTx kidney were also evaluated with renal US (R-US).

In the follow-up, two D-US examinations were performed to measure renal pelvis anterior-posterior diameter (RP-APD), renal artery resistive index (RA-Ri), and ureteral jet dynamics (JETmax, JETave, JET-Ri, and JETpattern) at different times.[6-9] First, D-US examination was performed about 6 weeks after DJS removal, and the second D-US examination was performed about 12 weeks after DJS removal. PCSD presence was also checked during the examinations by D-US, US, and magnetic resonance urography (MR-U). D-US was performed by an experienced radiologist (TA) who performed blind examination using 3-5 MHz convex probe (Aplio 500; Toshiba Medical System Corporation, Tokyo, Japan) (Figure 1). Patients were placed in the supine position with full bladder after oral hydration with 500-750 ml water. All D-US data were measured with angle correction. During D-US measurements, the insonation of a renal artery and ureteral jet at a Doppler angle equal or above 60° was strictly avoided. In addition, acute rejection presence was noted during the follow-up. For the determination of alterations in the ureteral jet dynamics, the D-US measurements were divided into two groups, i.e., first D-US examination and second D-US examination. Measurements were compared between the groups.

Statistical analysis

Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, Ill) software program was used for data analysis. To compare the paired data from the D-US examinations, the Wilcoxon signed ranks test, Chi-square test, and Pearson R correlation analysis were used. Data are given as mean ± SD. However, p-values are given according to the medians. The statistical significance was defined as $p<0.05$.

RESULTS

In the study, a total of 31 patients were investigated. Six of them were excluded for various reasons (two patients had missing data, one patient did not have any ureteral jet observed, and three patients had a postrenal obstruction and underwent DJS placement after the first D-US removed).

For the 25 patients included, the mean age was 41.5±13.3 (22-63) years, mean BMI was 23±3.9 (17.5-33.8) kg/m^2, 18 were male, and 7 were female. Mean HD time was 41.9±41.1 (0-144) months, and eGFR level was 12.6±6.1 (5.3-27.8) ml/min/1.73m^2 before RTx.

TABLE 1. DEMOGRAPHIC DATA OF THE PATIENTS

		Patients (n=25)
Age (year), mean±SD (min-max)		41.5±13.3 (22-63)
Gender, n (%)	Female	7 (28)
	Male	18 (72)
BMI (kg/m2), mean±SD (min-max)		23±3.9 (17.5-33.8)
Preoperative eGFR (ml/min/1.73m2), mean±SD (min-max)		12.6±6.1 (5.3-27.8)
HD positivity, n (%)		23 (92)
HD time (months), mean±SD (min-max)		41.9±41.1 (0-144)
RTx operation time (minutes), mean±SD (min-max)		204.4±18.3 (180-240)
RTx side, n (%)	Right	18 (72)
	Left	7 (28)
Donor, n (%)	Cadaver	14 (56)
	Live	11 (44)
Acute Rejection, n (%)	Negative	16 (64)
	Borderline changes	4 (16)
	Positive	5 (20)
eGFR after DJS removal (ml/min/1.73m2), mean±SD (min-max)		58±13.3 (20-77)

Abbreviations: BMI = body mass index, HD = hemodialysis, eGFR = estimated glomerular filtration rate

The types of donor were cadaver and live for 14 and 11 cases, respectively. The side of RTx was right and left iliac fossa for 18 and 7 patients, respectively. The mean RTx operation time was 204.4±18.3 (180-240) minutes. The mean eGFR level found was 58±13.3 (20-77) ml/min/1.73m^2 and 53.7±14.7 (23-80) ml/min/1.73m^2 for 6 and 12 weeks after DJS removal, respectively. Prednisolone and mycophenolate mofetil, or mycophenolic acid and everolimus, or tacrolimus were given as immunosuppressive and immunomodulatory drugs to all patients to protect against kidney rejection. Nevertheless, acute kidney rejection developed in five cases (T-cell-mediated rejection in three and antibody-mediated rejection in two patients), and they are still in follow-up.

The first D-US examination was performed about 6 weeks after DJS removal (mean time was 42.6±9.4 days). Nonobstructive PCSD rate, RP-APD, RA-Ri, JET-Ri, JETave, JETmax, and JETpattern are presented in Table 2. Three patients had nonobstructive dilation (obstruction excluded by D-US, urine amount, creatinine level and MR-U), JETave, and JETmax were 18.8±12 (7.1-58.1) cm/s and 29.2±18.7 (8-85.6) cm/s, respectively. Monophasic, square, and continuous patterns were present in 6 (24%), 7 (28%), and 7 (28%) patients, respectively.

The second D-US examination was performed about 6 weeks after the first D-US evaluation (mean time was 42.6±16.8 days). Nonobstructive PCSD rate, RP-APD, RA-Ri, and ureteral jet dynamics are also presented in Table 2. Two patients had nonobstructive dilation, JETave and JETmax were 12.9±7.4 (2.7-29.6) cm/s and 20±10.9 (4.4-40.2) cm/s, respectively. Monophasic, square, and continuous patterns were present in 9 (36%), 8 (32%), and 5 (20%) patients, respectively. In the follow-up, all nonobstructive dilations had regressed by the six-month US evaluation.

The course of the alterations in the ureteral jet dynamics and the comparison of the results are presented in Table 2. In addition, the first and second D-US measurements of patients are shown in figure 2. Nonobstructive PCSD rate, JETave, and JETmax levels were significantly decreased (p-values are 0.01, 0.010, and 0.014, respectively). In addition, monophasic (24% vs 36%), biphasic (12% vs 12%), triphasic (8% vs 0%), polyphasic (0% vs 0%), square (28% vs 32%), and continuous (28% vs 20%) patterns significantly differed over time (p=0.035). Also, monophasic and square pattern rates were significantly observed to increase over time (p=0.035); however, ureteral jet patterns were correlated between the two different D-US measurements (R=0.225, p=0.032).

DISCUSSION

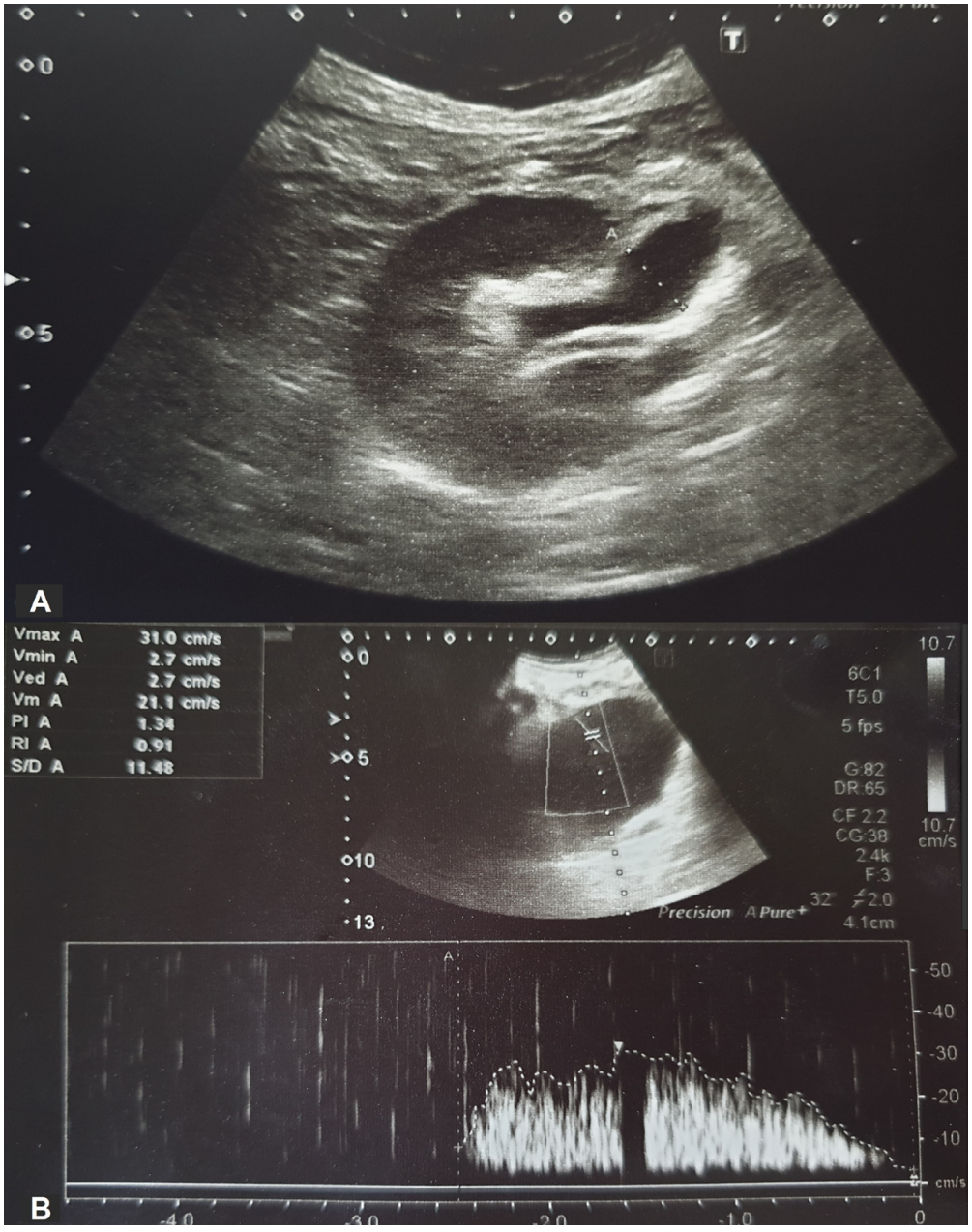
Ureteral jet dynamics measured by D-US were recently evaluated for upper urinary tract stone disease to predict ureteral obstruction and stone formation.[6,7,10,11] However, only a few studies have been reported so far about ureteral jet dynamics in RTx patients. A study reported that ureteral jets can be assessed noninvasively and easily using D-US for RTx kidneys.[4] Therefore, D-US imaging of the ureteral jet dynamics can be used to follow-up or exclude ureteral

obstruction in RTx kidneys.[4] In a recent study, the velocity and frequency of ureteral jets were found to be significantly lower in RTx kidneys compared with healthy kidneys.[5] In the literature, peak (JETmax) and mean (JETave) jet flow velocities varied from 16 to 150 cm/sec for healthy kidneys.[8,12,13] In this study, JETave and JETmax were 18.8±12 (7.1-58.1) cm/s and 29.2±18.7 (8-85.6) cm/s in the first D-US; and 12.9±7.4 (2.7-29.6) cm/s and 20±10.9 (4.4-40.2) cm/s in the second D-US examination, respectively. Although the ureteral jet frequency is a part of ureteral jet dynamics, we did not measure it in our RTx patients. Documenting ureteral jet frequency requires at least 30 minutes of D-US examination.[12] However, D-US was evaluated in about 5–10 minutes in most studies.[10,11,14,15] Therefore, we did not measure ureteral jet frequency because of the 10-minute continuous rapid examination.

JETpattern distribution rates differ from healthy native kidneys and RTx kidneys. In RTx kidneys, JET-patterns are present as 66.1%, 23.2%,3.6%, 0%, 5.4%, and 1.8% for monophasic, biphasic, triphasic, polyphasic, square and continuous patterns, respectively.[5] The monophasic wave pattern is more common in RTx kidneys (66.1%) than in healthy kidneys (2.6%).[5] In our

FIGURE 1. DATA MEASURED IN D-US EXAMINATION.

A) Renal pelvis anterior-posterior diameter (RP-APD); B) Ureteral jet dynamics as JETmax, JETave, and JETpattern.



series, monophasic, square, and continuous patterns were 24%, 28%, and 28% during the first D-US evaluation, and 36%, 32%, and 20% during the second D-US evaluation, respectively.

When we evaluated the course of the alterations in ureteral jet dynamics and comparison results, nonobstructive PCSD presence (3 vs. 2, p=0.01), mean JETave (18.8 vs. 12.9, p=0.010), and mean JETmax (29.2 vs. 20, p=0.014) levels significantly decreased over time. In addition, monophasic and square JETpattern rates were observed to increase over time significantly; however, ureteral jet patterns were correlated between the two different D-US measurements (R=0.225, p=0.032).

In light of all results, ureteral jet flow rates normally decrease after RTx. However, the most important finding of the study is that, during the second D-US examination, these flow rates also decrease further than the levels at the first D-US examination

FIGURE 2. IMAGING DATA OF FIRST AND SECOND D-US EXAMINATIONS IN A PATIENT

A) In the first examination, the patient had 6.5mm renal pelvis anterior-posterior diameter (RP-APD); B) 0.78 renal artery resistive index (RA-Ri); C) There were 34.5cm/s JETmax, 19.4cm/s JETave, and biphasic JETpattern in the evaluation of ureteral jet dynamics; D) In the second examination, the patient had 5mm RP-APD; E) 0.88 RA-Ri; F) There were 17.1cm/s JETmax, 11.4cm/s JETave, and continuous JETpattern in the patient as ureteral jet dynamics.

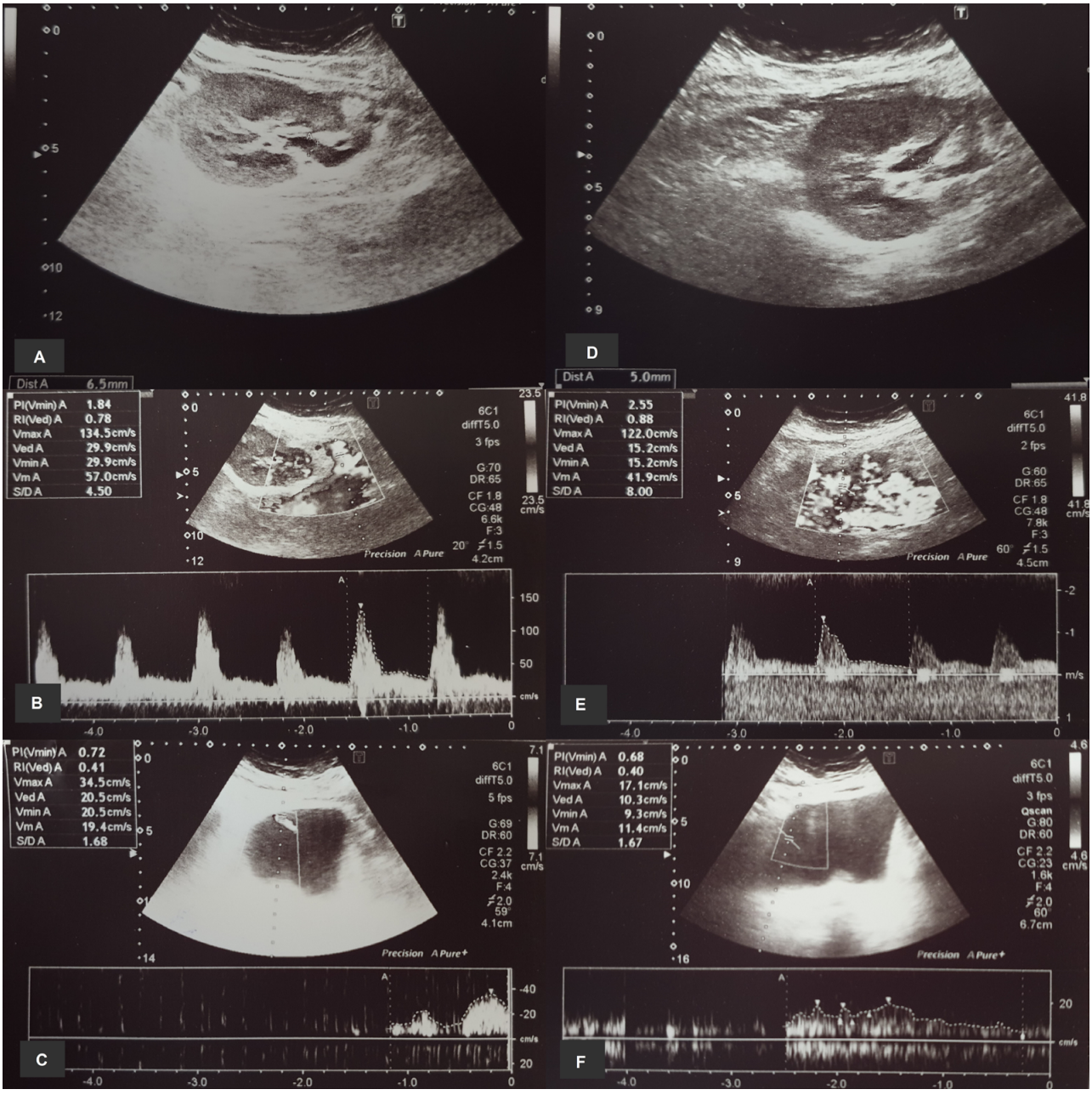


TABLE 2. D-US DATA AND COMPARISON OF THE RESULTS BETWEEN 6 WEEKS AND 12 WEEKS AFTER DJS REMOVAL IN PATIENTS WHO UNDERWENT RTx

		First D-US examination 6 weeks after DJS removal	Second D-US examination 12 weeks after DJS removal	p
Nonobstructive PCSD presence, n (%)		3 (12)	2 (8)	0.01#
RP-APD (mm), mean±SD (min-max)		6.8±4.3 (2.8-23)	6.4±3.2 (3.2-14.7)	0.509
RA-Ri, mean±SD (min-max)		0.76±0.08 (0.61-0.92)	0.76±0.08 (0.61-0.88)	0.948
JET-Ri, mean±SD (min-max)		0.66±0.18 (0.19-0.92)	0.66±0.23 (0.26-1.02)	0.765
JETave (cm/s), mean±SD (min-max)		18.8±12 (7.1-58.1)	12.9±7.4 (2.7-29.6)	0.01
JETmax (cm/s), mean±SD (min-max)		29.2±18.7 (8-85.6)	20±10.9 (4.4-40.2)	0.014
JETpattern, n (%)	Monophasic	6 (24)	9 (36)	0.035#
	Biphasic	3 (12)	3 (12)	
	Triphasic	2 (8)	0 (0)	
	Polyphasic	0 (0)	0 (0)	
	Square	7 (28)	8 (32)	R=0.225* p=0.032*
	Continuous	7 (28)	5 (20)	

Abbreviations: PCSD = pelvicaliceal system dilation, RP-APD = renal pelvis anterior-posterior diameter, RA-Ri = renal arterial resistive index, JET-Ri = resistive index of ureteral jet flow, JETave = average ureteral jet flow, JETmax = maximum ureteral jet flow, JETpattern = ureteral jet flow wave form pattern. P was calculated by Wilcoxon signed ranks test between paired data. #Chi-square analysis. *Pearson'R correlation analysis

for RTx kidneys. Therefore, D-US evaluation of ureteral jet dynamics performed at regular intervals and JETave, JETmax, and JETpattern measured after DJS removal may provide important data about reno-ureteral physiology. Our study continues, and measurements will be received at regular intervals. At the end of the study, all of these data will provide normal ranges for reno-ureteral unit dynamics in healthy RTx kidney, similarly to this preliminary paper. After receiving and knowing the normal ranges for the dynamics, alterations can be investigated and interpreted for complicated RTx patients compared to healthy RTx patients.

There are some limitations to our study. The study included a small number of patients and did not include measurement of ureteral jet frequency. Moreover, the cohort did not include any control group. However, it included short-term preliminary results of an observational cohort, and it is part of an ongoing study with a large cohort investigating all D-US measurements.

CONCLUSIONS

In conclusion, nonobstructive PCSD rate and ureteral jet flow velocities significantly decreased over time after RTx and DJS removal. Also, monophasic and square JETpattern rates were observed to increase over time significantly. Ureteral jet dynamics measured by D-US can provide useful information about the follow-up of peristaltic activity in the pelvic-ureteric system in relation to the health of RTx kidneys after DJS removal.

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RESUMO

OBJETIVOS: *Investigar prospectivamente as alterações e as variações normais da dinâmica do jato ureteral após a remoção do J-stent duplo (DJS) em pacientes submetidos a transplante renal (RTx).*

MÉTODOS: *Pacientes submetidos a RTx foram avaliados prospectivamente entre novembro de 2017 e junho de 2018. Após o RTx, o D-US foi realizado em todos os pacientes após a remoção do DJS. Índice de resistência da artéria renal (RA-Ri), diâmetro ântero-posterior da pelve renal (AP-DPR), dilatação do sistema pelvicaliceal (PCSD) e dinâmica do jato ureteral (velocidade máxima e média; JETmax e JETave) foram medidos por D-US. Além disso, a demografia dos pacientes, os níveis estimados de taxa de filtração glomerular (eGFR) e a rejeição aguda foram investigados no estudo. Os pacientes foram avaliados em dois momentos diferentes pelo D-US, cerca de 6 e 12 semanas após a remoção do DJS, e as duas medidas diferentes foram comparadas com o teste de Wilcoxon e o teste do qui-quadrado.*

RESULTADOS: *Um total de 25 pacientes foi avaliado no estudo. Taxa de PCSD não obstrutiva (12% vs. 8%), JETave (18,8 vs. 12,9 cm/seg) e JETmax (29,2 vs. 20 cm/seg), os níveis foram significativamente diminuídos (valores de p são 0,01, 0,010 e 0,014, respectivamente). Além disso, as taxas de padrão monofásico e quadrado foram significativamente observadas para aumentar ao longo do tempo (p=0,035); no entanto, padrões de jato ureteral foram correlacionados entre as duas diferentes medidas D-US (R=0,225, p=0,032).*

CONCLUSÃO: *Após o RTx, a velocidade de dilatação e as velocidades de fluxo do jato ureteral foram significativamente diminuídas e as taxas de JET padrão monofásico e quadrado foram significativamente aumentadas ao longo do tempo. A dinâmica do jato ureteral pode fornecer informações úteis sobre o acompanhamento da atividade peristáltica no sistema pélvico-ureteral.*

PALAVRAS-CHAVE: *Transplante de rim. Micção/fisiologia. Urodinâmica/fisiologia.*

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ORIGINAL ARTICLE

Association between Hemogram Parameters and Coronary Collateral Development in Subjects with Non-ST-Elevation Myocardial Infarction

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SUMMARY

OBJECTIVE: *Coronary collateral development (CCD) predicts the severity of coronary heart disease. Hemogram parameters, such as mean platelet volume (MPV), eosinophil, red cell distribution width, and platelet distribution width (PDW), are supposed novel inflammatory markers. We aimed to compare hemogram parameter values in patients presenting with non-ST-elevation myocardial infarction (NSTEMI) with adequate or inadequate CCD.*

METHODS: *A total of 177 patients with NSTEMI undergoing coronary arteriography were enrolled and divided into two groups based on the development of CCD: one group with adequate CCD (n=88) and the other with impaired CCD (n=89).*

RESULTS: *Baseline demographics and clinical risk factors were similar between the groups. Hemogram parameters were not significantly different between the two groups. However, compared to the inadequate CCD group, the median PDW was significantly higher in the adequate CCD group, 17.6 (1.4) vs. 17.8 (1.6) p=0.004. In a multivariate analysis, PDW (p=0.001, 95% CI for OR: 0.489(0,319-0,750) was found to be significantly different in the adequate CCD group compared to the inadequate CCD group. Pearson's correlation analysis revealed that PDW was significantly correlated with the Rentrop score (r=0.26, p<0.001).*

CONCLUSIONS: *We suggest that since PDW is an index that is inexpensive and easy to assess, it could serve as a marker of CCD in patients with NSTEMI.*

KEYWORDS: *Coronary collateral development, Non-ST-elevation myocardial infarction, Hematological parameters, Platelet distribution.*

INTRODUCTION

Coronary collateral circulation predicts the severity of coronary heart disease. The development of coronary collateral circulation can be a herald of the prognosis of patients with coronary disease. In fact, coronary collateral development (CCD) is a response to vascular occlusion injury in coronary arteries. The establishment of CCD has been proposed to be associated with inflammatory markers, such as high-sensitivity C-reactive protein, paraoxonase activity, and asymmetric dimethylarginine.[1-3] Hemogram

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parameters, such as mean platelet volume, neutrophil to lymphocyte ratio, eosinophil, red cell distribution width, and platelet distribution width (PDW), are supposed novel inflammatory markers. An increase or decrease in their levels in hemogram assays are proposed to be related to various inflammatory conditions. Moreover, elevated PDW has been shown to be a predictor of platelet activation.[4] Likewise, PDW was higher in patients with acute myocardial infarction compared to those in patients with stable coronary disease.[5]

In this retrospective study, we aimed to compare hemogram parameter values in patients presenting with non-ST-elevation myocardial infarction (NSTEMI) with adequate or inadequate coronary collateral development.

METHODS

Patients with coronary heart disease admitted to cardiology clinics of our institution with a diagnosis of non-ST-elevation myocardial infarction (NSTEMI) between April 2015 and June 2018 were consecutively evaluated for the presence of coronary collateral development. To reach about 95% power and 20% variability, the minimum number of participants required in the power analysis was calculated to be 80 subjects. To protect from possible losses during the study, 10% more subjects were included in the study. Therefore, a total of 88 patients were included in study, and 89 in the control group. The diagnosis of NSTEMI was established by the combination of the following; ischemic chest pain, a troponin-I level of >0.01ng/ml, and absence of ST-segment elevation on 12-lead chest electrocardiography (ECG). Patients who had at least 95% luminal narrowing in at least one major epicardial coronary artery were included. Clinical and laboratory findings were obtained from a review of the patients' files. Hypertension was defined as blood pressure >140/90mmHg or taking antihypertensive medication. Diabetes mellitus was defined as having a fasting glucose level ≥126 mg/dl or using antidiabetic agents. The presence of total cholesterol >200 mg/dl or triglyceride >150 mg/dl was accepted as hyperlipidemia.

The exclusion criteria were as follows: malignancy, idiopathic dilated or hypertrophic cardiomyopathy, history of blood transfusion within 3 months, leukemia, thrombocytopenia, congestive heart failure, moderate to severe renal failure, history of acute myocardial infarction with ST-segment elevation, coronary artery stenting or bypass operation, coronary artery stenosis < 95%, chronic obstructive pulmonary disease, severe hepatic dysfunction, atrial fibrillation, severe valvular disease, recent infection, and systemic inflammatory diseases. The study was approved by the institutional board.

Coronary angiographies were performed through the radial or femoral artery. Patients with coronary artery stenosis of ≥ 95%, by visual evaluation, were included. Coronary collateral circulation was graded according to the Rentrop classification,6 i.e., lack of filling in collateral vessels classified as Grade 0; filling in side branches via collateral channels without visualization of the epicardial artery classified as Grade 1; partial filling in the epicardial major coronary artery via collateral circulation classified as Grade 2; and complete filling in the epicardial major coronary artery classified as Grade 3. Two interventional cardiologists who were blinded to the study evaluated the angiographic results. The highest Rentrop grade was used for analysis in subjects with more than one coronary collateral circulation. Patients with Rentrop 0 and 1 were classified as inadequate CCD, whereas patients with Rentrop 2 and 3 were classified as adequate CCD.

Age, sex, and laboratory parameters of the participants were obtained from the database of the institution. Serum glucose, creatinine, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), baseline troponin, and baseline CK-MB were measured using an automatic biochemical analyzer (Architect C8000, USA). Hemogram parameters; hemoglobin, red cell distribution width(RDW), PDW, mean platelet volume(MPV), platelet count, neutrophil, monocyte and eosinophil counts were recorded (Cell Dyn 3700; Abbott Diagnostics, Lake Forest, Illinois, USA).

Statistical analysis was conducted using SPSS software (SPSS 15.0 for Windows, IBM Co, Chicago, IL, USA). The distribution of the variables in the study groups was analyzed by the Kolmogorov-Smirnov test. The normally distributed variables were compared by the t-test and expressed as mean ± standard deviation. Variables that did not present normal distribution were compared using the Mann Whitney U test and expressed as median (interquartile range). The chi-square test was used to compare nonparametric variables between study groups. Pearson correlation analyses were used to assess the correlations of MPV, PDW, RDW, monocyte, and eosinophil with the

Rentrop grade. Multivariate linear regression analysis was used to analyze the value of different baseline characteristics as independent predictors of inadequate CCD. To reveal the association of variables with adequate CCD, we performed univariate analysis. The multivariate logistic regression model was used for variables found significant in the univariate analysis in order to determine the independent prognostic factors of adequate CCD. A p-value lower than 0.05 was considered statistically significant.

RESULTS

The study population included 177 NSTEMI patients, of whom 88 had adequate CCD and 89 had inadequate CCD. Out of the 177 patients, 56 (32%) had Rentrop grade 0, 33 cases (18%) had grade 1, 47 cases (27%) had grade 2, and 41 cases (23%) had grade 3. Baseline demographics and clinical risk factors were similar between the groups, except that the median age was significantly lower in the poor CCD group [66 (35-88) vs. 71 (39-90), p=0.015]. Previous medications were also comparable between the two groups (Table 1). Serum biochemistry of the subjects in the inadequate and adequate CCD groups was not statistically different; however, compared to the inadequate CCD group, creatinine (p=0.070) tended to be higher in the adequate CCD group (Table-2). The platelet, RDW, MPV, monocytes, eosinophil, Neutrophil, and WBC counts were not significantly different between the two groups. However, compared to the inadequate CCD group, the median PDW was significantly higher in the adequate CCD 17.6 (1.4) vs. 17.8 (1.6) p=0.004 (Table 2). In the multivariate analysis, PDW (p=0.001, 95% CI for OR: 0.489 (0,319-0,750) were found to be significantly different in the adequate CCD group compared to the inadequate CCD group (Table 3). Pearson's correlation analysis revealed that PDW was significantly correlated with the Rentrop score (r=0.26, p<0.001).

DISCUSSION

The main finding of the present study is that an elevated PDW count is a possible predictor of adequate CCD in patients with NSTEMI. In the case of occlusion in a coronary vessel, myocardium may alternatively get blood flow from CCD. The development of coronary collateral circulation is affected by proximal location of stenosis, degree and severity of the stenosis, and duration of angina pectoris growth is usually categorized into angiogenesis and arteriogenesis[7]. New capillary development from existing capillaries is named angiogenesis, and collateral arterial development from existing arteries is named arteriogenesis.[8] These vessels are thin-walled microvascular conduits, which are composed of an endothelial lining, an internal elastic lamina, and one or two layers of smooth muscle cells.[8] Survival of the brain and heart mainly depend on arteriogenesis.[9] Passive dilation of existing channels, rupture of the elastic lamina, and extravasation of platelets and white blood cells, including monocytes, initiates

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY GROUPS

Baseline characteristics	Inadequate CCD (n=89)	Adequate CCD (n=88)	p
	MEDIAN (IQR)		
Age (years)	66 (21)	71 (22)	0.015
Body mass index (kg/m^2)	27.3 (7.6)	27.7 (6.3)	0.394
	X^2 test		
Male/female	51/38	61/27	0.097
Hypertension (%)	55(62%)	60(68%)	0.373
Smoking	28(31%)	28(32%)	0.959
Family history	10(11%)	9(10%)	0.828
Diabetes mellitus	34(38%)	33(37%)	0.923
Acetyl salysilate	24(27%)	35(43%)	0.071
Clopidogrel	13(15%)	10(11%)	0.521
Statin	33(37%)	44(50%)	0.083
Calcium channel blocker	21(24%)	21(24%)	0.967
ACE inhibitor	14(16%)	20(23%)	0.237
ARB	14(16%)	16(18%)	0.664
B-blocker	47(53%)	35(40%)	0.082

Note: CCD: coronary collateral development, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker.

arteriogenesis[9]. The production and release of angiogenic factors also play an important role in angiogenesis. Remodeling and proliferation of smooth muscle cells and endothelium are also needed for CCD.[10,11]

Platelet distribution width is a simple, easy to assess, and cheap marker of thrombocyte activation.[4] It indicates varied size platelets in circulation. PDW is the size variation of platelets and a marker of platelet activation. It is more specific than MPV.[4] Platelet swelling is not a fact that affects PDW.[4] Elevated PDW predicts circulating mature and immature thrombocytes in the bloodstream. Abnormal thrombocytosis[4] or heterogeneous demarcation of megakaryocytes[12] might be responsible for elevated PDW.

Literature includes many studies on PDW in various diseases and conditions. While some authors stated the elevation of PDW was associated with the severity of preeclampsia and eclampsia,[13] others emphasized the diagnostic role of PDW in pancreatic cancer.[14] Type 2 diabetes mellitus is associated with higher PDW values compared to the healthy population.[15] Moreover, authors have speculated that increased PDW levels could be a marker of activation in systemic lupus erythematosus.[16] Authors have studied PDW in cardiac conditions, too. It has been proposed that it may enhance the prediction of the development of heart failure in subjects with acute coronary syndrome.[17] In another study, PDW

TABLE 2. LABORATORY DATA OF THE STUDY GROUPS

	Inadequate CCD (n=89)	Adequate CCD (n=88)	p
	MEDIAN (IQR)		
Creatinine (mg/dl)	0.93 (0.37)	0.97 (0.39)	0.070
Fasting plasma glucose (mg/dl)	128 (82)	120 (62)	0.985
HDL-cholesterol (mg/dl)	42 (17)	42 (12)	0.376
Triglyceride (mg/dl)	114 (85)	129 (99)	0.323
Total cholesterol (mg/dl)	190 (63)	180 (81)	0.821
Baseline troponin [pg/ml]	10000 (7400)	9800 (6400)	0.628
Baseline CK-MB [IU/L]	78 (43)	73 (42)	0.211
Hemoglobin (gr/dl)	13 (3.7)	13.5 (2.6)	0.845
Hematocrit (%)	39 (10)	41 (8)	0.857
PDW (%)	17.6 (1.4)	17.8(1.6)	0.004
RDW (%)	16 (2)	17 (2)	0.071
MPV,fL	7.6 (1.7)	7.5(1.3)	0.586
WBC, (u/mm^3)	9.4 (4.2)	9.9(4.9)	0.714
Eosinophil, (u/mm^3)	0.110 (0.214)	0.076(0.146)	0.064
Neutrophil, (u/mm^3)	6.3 (4.2)	7.1(4.7)	0.266
Monocyte, (u/mm^3)	0.61 (0.30)	0.60(0.43)	0.738
	MEAN±SD		
Platelet counts (k/mm^3)	243±65	234±74	0.423
LDL-cholesterol (mg/dl)	117±44	116 ±42	0.853

PDW: platelet distribution width; RDW: Red cel distribution width; MPV: Mean platelet volume; HDL : high-density lipoprotein; LDL :low-density lipoprotein; WBC: White blood count

TABLE 3. MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF VARIABLES RELATED TO CCD

Category	B	SE	WALD	OR (95% CI)	P-value
RDW	-0.53	0.099	0.282	0.949(0.781-1.153)	0.595
PDW	-0.715	0.218	10.740	0.489(0.319-750)	0.001
MPV	0.290	0.164	3.138	1.337(1843-970)	0.076
Monocyte	-0.022	0.535	0.002	0.978(0.343-2.790)	0.967
WBC	0.140	0.194	0.520	1.150(0.787-1.681)	0.471
Neutrophil	-0.157	0.193	0.660	0.855(0.585-1.249)	0.417
Eosinophil	1.577	1.469	1.153	4.838(0.272-86.041	0.283
Age	-0.021	0.015	1.992	0.979(0.950-1.008)	0.158

Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval.

was introduced as a marker of the severity of the acute coronary syndrome along with a significant correlation with a high Gensini score.[18] Subjects with ST-segment-elevation myocardial infarction have greater PDW values compared to subjects with stable coronary disease;19 it is a very useful prognostic factor in long-term mortality following acute myocardial infarction[20].

Inflammation is involved in atherosclerosis, atherosclerotic plaque development, and clot formation on the surface of the plaque.[21] Kowara et al.[17] reported that the initial PDW value on admission predicts left ventricular functions in patients with acute coronary syndrome. In another study, it has been stated that PDW could be an independent marker of ST-segment-elevation myocardial infarction.[22] What causes an elevation in PDW value in patients with NSTEMI that have adequate CCD? PDW is a marker of platelet activation. Platelets activate in inflammatory conditions. Therefore, elevated PDW reflects the inflammatory burden in diseases characterized by inflammation. While coronary ischemia stimulates the development of collateral vessels, it also produces an inflammatory microenvironment. Since inflammatory cytokines stimulate angiogenesis,[23] the elevated PDW in the adequate CCD group compared to the inadequate group is not surprising. Thus, elevated PDW was noticed in this subset of patients.

The association between cardiac conditions and elevated PDW is clear; however, at this point, questions arise on the measurement of PDW. EDTA in hemogram tubes might cause swelling of the thrombocytes; thus, cause also an elevation in measured PDW value. [24] On the other hand, statins may have elevated PDW levels.[25] All hemogram assays are done within 5 minutes from drawing blood into hemogram tubes in our institution in order to prevent platelet swelling. Moreover, the statin use in the study groups in the present report was similar. The retrospective design and relatively small study population are among other limitations of the present study. However, to the best of our knowledge, this is the first study in the literature that reported increased PDW in NSTEMI patients with adequate CCD.

CONCLUSION

We suggest that since PDW is an index that is inexpensive and easy to assess, it could serve as a marker of collateral vessel development in patients with NSTEMI.

Conflict of interest
None

Sources of Funding
None

Author Contribution
IS, GA, AKM designed the study; IS, MZK, YG, AKM collected the data; MZK, GA, YG performed the literature review; IS, GA, YG performed the statistical analysis; IS, GA, AKM, MZK wrote the paper; MZK, YG, GA performed the critical review of the manuscript.

RESUMO

OBJETIVO: *O desenvolvimento colateral coronariano (CCD) prediz a gravidade da doença coronariana. Parâmetros de hemograma como volume plaquetário médio (VPM), eosinófilo, largura de distribuição de glóbulos vermelhos e largura de distribuição de plaquetas (PDW) são supostos novos marcadores inflamatórios. Nosso objetivo foi comparar os valores do parâmetro hemograma em pacientes com infarto do miocárdio sem supradesnivelamento do segmento ST (IAMSSST) com DCC adequado ou inadequado.*

MÉTODOS: *Um total de 177 pacientes com NSTEMI submetidos à arteriografia coronariana foram incluídos e divididos, com base no desenvolvimento de CCD, em dois grupos: grupo com CCD adequado (n = 88) e grupo com CCD alterado (n = 89).*

RESULTADOS: *Os dados demográficos e os fatores de risco clínicos basais foram semelhantes entre os grupos. Os parâmetros do hemograma não foram significativamente diferentes entre os dois grupos. Mas, em comparação com a mediana inadequada do grupo CCD, o PDW foi significativamente maior em CCD adequado de 17,6 (1,4) vs. 17,8 (1,6) p = 0,004. Na análise multivariada, PDW (p = 0,001, IC 95% para OR: 0,489 (0,319-0,750) foi significativamente diferente no grupo CCD adequado em comparação com o grupo CCD inadequado. A análise de correlação de Pearson revelou que PDW foi significativamente correlacionado com escore de aluguel (r = 0,26, p <0,001).*

CONCLUSÃO: *Sugerimos que, uma vez que a PDW é um índice barato e de fácil avaliação, pode servir como um marcador de DCC em pacientes com IAMSSST.*

PALAVRAS-CHAVE: *Desenvolvimento colateral coronário. Infarto do miocárdio sem supradesnivelamento do segmento ST. Parâmetros hematológicos. Distribuição plaquetária.*

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ORIGINAL ARTICLE

A significant relationship between personality traits and adhesive capsulitis

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SUMMARY

OBJECTIVE: *We investigated the associations between adhesive capsulitis (AC) and a specific psychological profile.*

METHODS: *We assessed 72 patients with phase-II AC. In our study, 36 patients were affected by primary disease and 36 by secondary disease. The inclusion criteria were as follows: unilateral AC and pain in the shoulder for at least two months. The exclusion criteria were: psychiatric and neurological manifestations with a previous diagnosis and inability to comprehend the instruments. Outcomes were determined at 52 weeks. Shoulder pain severity was assessed with the Visual Analog Scale. We also measured the range of motion with a universal goniometer and the strength with the Medical Research Council. We assessed the personality traits of our patients with the Cloninger's Temperament and Character Inventory and the Multidimensional Perfectionism Scale.*

RESULTS: *Patients with primary AC needed more time to improve the symptomatology compared to the group with the secondary disease ($p<0.01$). Patients with primary AC complained of severe and lasting pain more frequently than patients with the secondary disease ($p< 0.01$). In patients with primary disease, the prevalence of perfectionism, low levels of novelty seeking, and high levels of harm avoidance were 88.2 and 86.2%, and 80.4, respectively, and below 20 percent in patients with secondary AC disease.*

CONCLUSION: *We found a significant correlation between primary AC and particular personality traits, indicating an interaction between psychological and somatic factors.*

KEYWORDS: *Range of motion, articular. Shoulder pain. Bursitis. Personality. Somatoform disorders.*

INTRODUCTION

Adhesive capsulitis: a disease not to be underestimated

Adhesive capsulitis (AC) is characterized by adhesion and fibrosis in the shoulder capsule, decreased volume of the glenoid capsule and progressive loss of range of motion (ROM), shoulder pain and stiffness, causing severe disability.

The prevalence of shoulder pain has been reported to be between 4% and 26%[1]. Primary AC reportedly affects 2% to 5.3% of the general population. The prevalence of secondary AC, related to diabetes mellitus and thyroid disease, is reported to be between 4.3% and 38%. Prolonged immobilization, myocardial infarction, trauma, and autoimmune disease are other risk factors associated with secondary AC. AC is more

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prevalent in individuals who are 40 to 65 years of age, female, with a previous episode of AC in the contralateral arm[2]. It is still unclear whether there is any association of psychological traits comorbid with AC.

The condition usually lasts 2–3 years, but in about 40% of patients, it is not self-limiting; stiffness and disability persist beyond three years[3]. It has three phases: the painful stage that lasts one to two months, the frozen stage with loss of motion and decreased capsular volume lasting several months to a year or longer, and the thawing stage characterized by gradual improvement of range of motion over several months to years. ROM deficits may continue for years.

In most cases, conservative treatment improves symptoms and restores shoulder motion. In refractory cases, an arthroscopic capsular release is indicated. However, conservative and surgical management of AC is often prolonged and painful[4].

The diagnosis of AC is determined from the history and physical examination. Patients typically present with a gradual and progressive onset of pain, likely sleep-disturbing night pain, and pain at end ranges of movements. Patients also present with painful and restricted active and passive ROM of at least 60% in total active ROM in the affected shoulder compared with the unaffected contralateral shoulder; with the scapula stabilized, in both elevation and rotation that occurs for at least one month and has either reached a plateau or worsened[2].

Relationship between personality traits and AC

Behavioral observations and key features of the physical examination have greatly helped clinicians to identify both the presence and severity of diseases, as well as of AC[5].

We assessed the relationship between personality traits, using the Cloninger's Multidimensional Perfectionism Scale (MPS)[6] and Temperament and Character Inventory (TCI)[7], and the clinical characteristics of patients with AC (pain, ROM, time to recover, ROM, straight).

Purpose

The purpose of this study was to determine whether psychological factors could influence the results of the rehabilitative treatment of AC. We hypothesized that psychosomatic factors would be correlated with greater pain and delay in the recovery of ROM. We verified the effectiveness of a rehabilitation program for the management of AC associated with psychosomatic disorders. In addition, we performed a review of the literature on psychosocial factors related to AC.

METHODS

All procedures performed in our study involving human participants were in accordance with the ethical standards of the institution and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

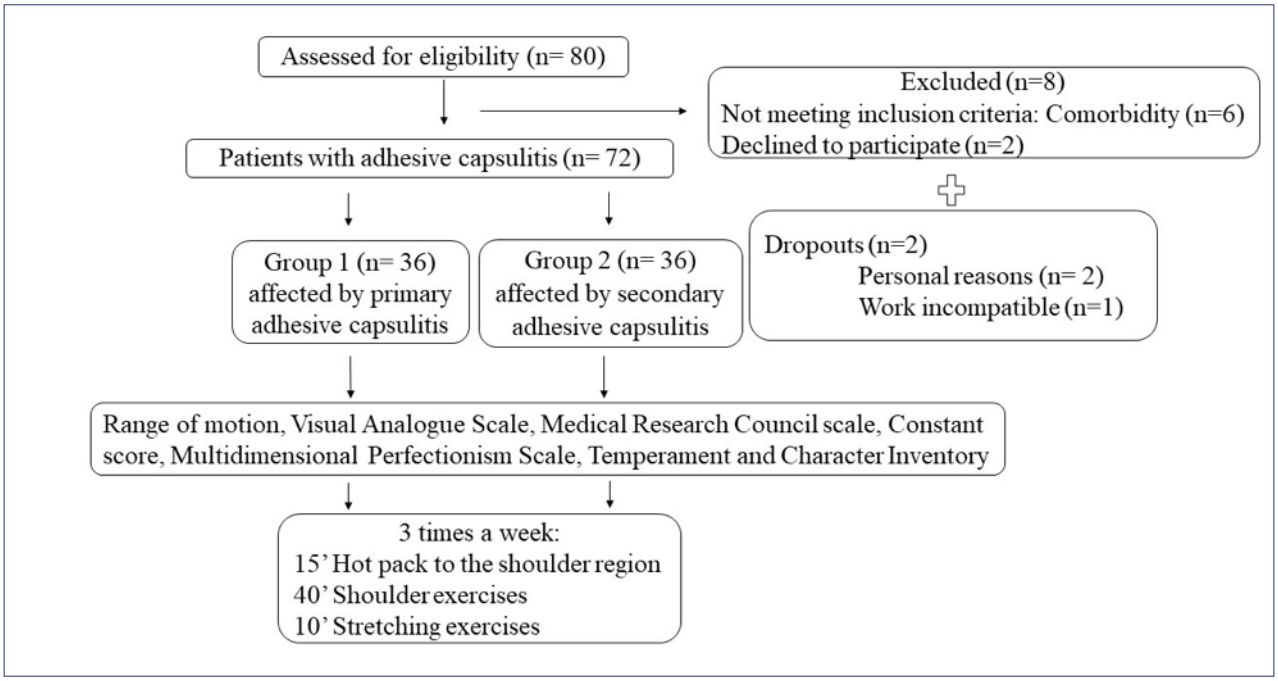
Study Design and Data Collection

We conducted a retrospective study at the Pain Medicine Department of our University from October 2017 to May 2018. A total of 80 potentially eligible outpatients were screened, 72 of whom met the inclusion criteria. The inclusion criteria were as follows: clinical diagnose of unilateral idiopathic or secondary AC (restricted active ROM of at least 60 % in total active ROM in the affected shoulder compared with the unaffected contralateral shoulder), patients with phase-II AC, pain in the shoulder for at least two months (mean VAS score of 9), who completed one year of physical therapy. Endocrinology disease, immobilization after surgical rotator cuff repair, or trauma were considered for the secondary AC group. Exclusion criteria were as follows: psychiatric and neurological manifestations with a previous diagnosis, history of inflammatory rheumatic disease, musculoskeletal deformities, peripheral neuropathy, major medical conditions such as unstable hypertension, severe cardiac and respiratory problems. Patients with an inability to comprehend the instruments were also excluded (Fig. 1).

Procedures

We assessed patients with phase-II AC; 36 patients (30 men and 6 women) were affected by primary AC and 36 by secondary AC (28 men and 8 women). The mean age was 55 years (range, 32-71 years). The mean duration of symptoms was 8.9 months (ranged from 8 weeks to 24 months). A questionnaire was used to collect sociodemographic data, including age, marital status, educational level, employment status, and total family income. The groups were homogeneous for relevant sociodemographic and general clinical features. Recorded clinical characteristics included the

FIGURE 1. FLOW DIAGRAM OF THE PATIENTS



length of history of AC symptomatology and current pharmacological treatment (steroid and nonsteroidal anti-inflammatory drugs) (Table 1).

Outcome measures

The patients of both groups were evaluated before the training program and 12 months after the suspension of the treatment.

Shoulder pain severity and subsequent sleep disturbances were assessed with a numeric range from 0 to 10 centimeters on the Visual Analog Scale (VAS). We also measured the range of motion (ROM: flexion, abduction, and external rotation) and the strength with the Medical Research Council scale (MRC) and Constant score (CMS)[8]. MRC grades muscle power on a scale of 0 to 5. CMS is a 100-points scale composed of a number of parameters that define the level of pain, strength, ROM, and the ability to carry out normal daily activities of the patient. The same Orthopedic and Physiatrist MD evaluated all the patients. Active shoulder flexion and abduction ROM were assessed with a universal goniometer.

During the first evaluation, patients were approached and interviewed in person during a psychiatric consultation. The personality traits of our patients were determined by the validated Italian version of the TCI[7] and the validated Italian MPS[9]. The TCI assessed personality by describing aspects of temperament and character. Several studies considered temperament as the more heritable personality component. It was stable throughout life and responsible for adaptive emotional responses and behavioral reactions to life experiences. The TCI was used to assess the tendency to depression and anxiety through four dimensions: harm avoidance (HA), novelty seeking (NS), reward dependence (RD), and persistence (P). In contrast, character was considered the more learned personality component. It matured throughout adulthood. It was assessed through three dimensions: self-directedness (SD), cooperativeness (C), and self-transcendence (ST)[6]. The MPS was used to measure perfectionism[7]. In the MPS, there was not a clinical cut-off score, but higher scores correlated with perfectionistic attitudes, especially after a significant stressful event[7].

TABLE 1. PATIENTS' DEMOGRAPHIC DETAILS AT BASELINE

Baseline parameters	Group 1 (n = 36) Primary AC	Group 2 (n = 36) Secondary AC
Age, years, yr, mean ± SD	51.5 ± 21.7	50.1 ± 21.0
Males	30	28
Females	6	8
Mean duration of symptoms (months)	8.9 ± 13.0	8.1 ± 12.4
Years of education yr, mean ± SD	13.1 ± 5.01	16.6±1.9
Occupation: Leave of absence, %, mean ± SD	30.0 ± 0.8	32.2 ± 0.4
Occupation: Working from home	35.8 ± 0.8	36.0 ± 0.4
Occupation: Others	31.3 ± 1.2	31.8 ± 0.9

Abbreviations: AC, adhesive capsulitis; SD, standard deviation; yr, years.

The Rehabilitation Program

The same therapists applied the rehabilitation program every time (3 times/week for one year), with progressive exercises. Patients underwent a rehabilitation program, performing standardized shoulder exercises 40 minutes daily, three times a week, for three months. Before physiotherapy exercises, the patients applied a hot pack to the shoulder region for 15 minutes. Then, stretching exercises included wand and Codman pendulum exercises (4 repetitions for each exercise), scapular elevation, adduction, and scapular stabilization exercises (20 repetitions for each exercise). At the end of every sitting, the patients applied an ice pack to the shoulder for 15 minutes. Over the year, the progression of the rehabilitation program provided warm-water exercise, aerobic training, progressive strength exercises, coordination and stretching exercises (Fig. 2). Each week, during physiotherapy sessions, patients were evaluated by physiotherapists, and by physicians each month. Outcomes were determined at 52 weeks.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS, Version 18.0 for Windows; SPSS Inc., Chicago, IL) was used for data analysis. Quantitative data were compared using the t-test, and qualitative data were compared using χ^2 test; $p < 0.05$ was considered with statistical significance.

RESULTS

At the baseline evaluation, the mean active anterior elevation was 90°, -10° of external rotation, and internal rotation level reached ipsilateral gluteus in both groups. After a year, the group with primary AC improved the ROM; the mean active elevation was 150°, 30° of external rotation, and internal rotation at level T12. Thus, the average gains were statistically significant ($p < 0.001$): 60° in forward active elevation, 40° in external rotation, 50° in internal rotation. Similar improvements in shoulder ROM in the group with secondary AC were obtained after 12 months, without a significant difference between groups 1 and 2 ($p > 0.05$) (Table 2).

The results showed that 12 months after the end of the training, the days required for the recovery of patients with primary AC were about 358, whereas for secondary AC they were 228. These values were statistically significant ($p < 0.001$) in the comparison between the two groups.

The mean results of CMS are described in table 2. In both groups, there was a significant difference in the partial and total scores of CMS 12 months after the end of the training ($p < 0.001$). There was a significant difference in the partial and total score of CMS in group 1 compared to group 2 ($p < 0.001$), except in the ROM ($p > 0.05$).

Compared to secondary AC, patients with primary AC were characterized by the tendency to be perfectionist, but also fearful, apprehensive, discouraged,

FIGURE 2. DESCRIPTION OF EXERCISES PERFORMED BY BOTH GROUPS DURING A STANDARD SESSION OF TRAINING

Exercises	Time	Description
40 minutes daily, 3 times a week		
WARM-UP		
Application of hot pack	5'	
Shoulder stability and fluidity	20 repetitions for each exercise for 5'	- Scapular elevation, - Adduction and scapular - Stabilization exercises
AEROBIC EXERCISES		
Warm-water exercise and Coordination training	10'	Overall mobility using water as resistance
ANAEROBIC EXERCISES		
Progressive strength exercises with elastic band	10'	- Anterior elevation - External rotation - Internal rotation
STRETCHING		
Flexibility exercises	4 repetitions for each exercise for 10'	- Wand exercises - Codman pendulum exercises

TABLE 2. ROM AT BASELINE AND AT THE FOLLOW UP IN BOTH GROUPS, OUTCOMES AFTER TREATMENT. CONSTANT SCORE 12 MONTHS AFTER THE END OF TRAINING — A PREVALENCE OF PSYCHOLOGICAL TRAITS IN PATIENTS WITH PRIMARY ADHESIVE CAPSULITIS.

ROM	Primary AC AB	Primary AC FU	DS	Paired t-test	P-value	Secondary AC AB	Secondary AC FU	DS	Paired t-test	P-value
Anterior elevation	89.8°	149.9°	5.3	67.4	p<0.001	90.1°	150.2°	6.0	50.9	p<0.001
External rotation	-9.4°	30.9°	2.5	93.6	p<0.001	-10.1°	31.2°	3.1	70.9	p<0.001
Internal rotation	30.6°	80.0°	4.1	48.0	p<0.001	31.8°	82.0°	9.8	48.2	p<0.001
ROM	Primary AC AB	Secondary AC AB	DS	Unpaired t-test	P-value	Primary AC FU	Secondary AC FU	DS	Unpaired t-test	P-value
Anterior elevation	89.8°	90.1°	3.6	1.3	p>0.05	149.9°	150.2°	2.9	2.7	p>0.05
External rotation	-9.4°	-10.1°	2.8	2.0	p>0.05	30.9°	31.2°	1.8	3.0	p>0.05
Internal rotation	30.6°	31.8°	3.1	1.9	p>0.05	80.0°	82.0°	2.4	1.8	p>0.05

CMS	Primary AC AB	Secondary AC AB	DS	Unpaired t-test	P-value	Primary AC FU	Secondary AC FU	DS	Unaired t-test	P-value
Pain	11.4/15	14.8/15	2.5	19.1	p>0.05	8.1/15	13.2/15	1.5	9.1	p<0.001
ADL	16.1/20	19.3/20	3.1	22.9	p>0.05	12.3/20	18.5/20	3.4	14.8	p<0.001
ROM	32.4/40	38.1/40	4.0	28.1	p>0.05	27.7/40	37.8/40	6.4	2.4	P>0.05
Strength	16.8/25	21.7/25	2.1	18.2	p>0.05	12.2/25	19.3/25	4.0	15.9	p<0.001
Total	77.2/100	93.1/100	8.1	32.1	p>0.05	59.8/100	87.6/100	10.2	12.4	p<0.001
CMS	Primary AC AB	Primary AC FU	DS	Paired t-test	P-value	Secondary AC AB	Secondary AC FU	DS	Paired t-test	P-value
Pain	11.4/15	8.1/15	3.1	10.2	p<0.001	14.8/15	13.2/15	1.2	11.2	p<0.001
ADL	16.1/20	12.3/20	4.0	14.1	p<0.001	19.3/20	18.5/20	1.8	15.5	p<0.001
ROM	32.4/40	27.7/40	5.1	11.4	p<0.001	38.1/40	37.8/40	1.3	12.7	p<0.001
Strength	16.8/25	12.2/25	3.8	15.2	p<0.001	21.7/25	19.3/25	2.1	16.1	p<0.001
Total	77.2/100	59.8/100	14.0	12.5	p<0.001	93.1/100	87.6/100	6.5	14.8	p<0.001

Psychological traits	Primary AC AB	Secondary AC AB	DS	$\chi 2$ test	P-value	Primary AC FU	Secondary AC FU	DS	$\chi 2$ test	P-value
Perfectionism	90.2 %	17.4%	22.5	91.1	p<0.001	88.2 %	14.4%	22.5	79.1	p<001
NS	89.2 %	19.2%	33.1	81.9	p<0.001	86.2 %	16.2%	23.1	82.9	p<001
HA	85.4 %	16.3%	24.0	82.1	p<0.001	80.4 %	18.3%	24.0	78.1	p<001
Psychological traits	Primary AC AB	Primary AC FU	DS	$\chi 2$ test	P-value	Secondary AC AB	Secondary AC FU	DS	$\chi 2$ test	P-value
Perfectionism	90.2 %	88.2 %	3.7	3.8	p>0.05	17.4%	14.4%		2.5	p>0.05
NS	89.2 %	86.2 %	4.9	2.10	p>0.05	19.2%	16.2%		1.8	p>0.05
HA	85.4 %	80.4 %	2.9	3.5	p>0.05	16.3%	18.3%		3.0	p>0.05

Abbreviations: AC, adhesive capsulitis; DS, standard deviation; ROM, range of motion; AB, at baseline; FU, follow up; CMS, Constant score; ADL, Activity daily living; HA, harm avoidance; NS, novelty-seeking

insecure, negativistic (due to their higher levels on HA), slow tempered, slow to engage, and unenthusiastic (due to their lower levels on NS). Between the two groups, after 12 months, in patients with primary AC, the prevalence of perfectionism, decreased NS, and increased HA were 88.2, 86.2%, and 80.4, respectively, and below 20 percent in patients with secondary AC (p<001) (Table 2). No other tendency for depression and anxiety was found. The results confirmed that perfectionism, high levels of HA, and low levels of NS characterized the subgroup of patients with AC primary. The rehabilitation treatment did not modify the psychological traits of the groups (p>0.05) (Table 2).

There was a significant association between pain intensity and personality traits. At the beginning of the study, the mean pain intensity was similar between the two groups: 4.1 in the primary AC group, 3.9 in the secondary AC group, without a significant difference between them (P>0.05). After eight months, in the primary AC group, the mean VAS score was 7, whereas in the secondary AC group, it was 4 , with a significant difference between the two groups (p<0.001). After 12 months, the mean

VAS score in the primary AC group was 5, and in the secondary AC group, it was 3. So, there was a statistically significant ($p < 0.001$) improvement in the VAS score in the group with secondary AC compared to the group with primary AC. Results indicated that patients with primary AC complained more of severe and lasting pain than patients with secondary AC, as of their psychological disorders (Fig. 3).

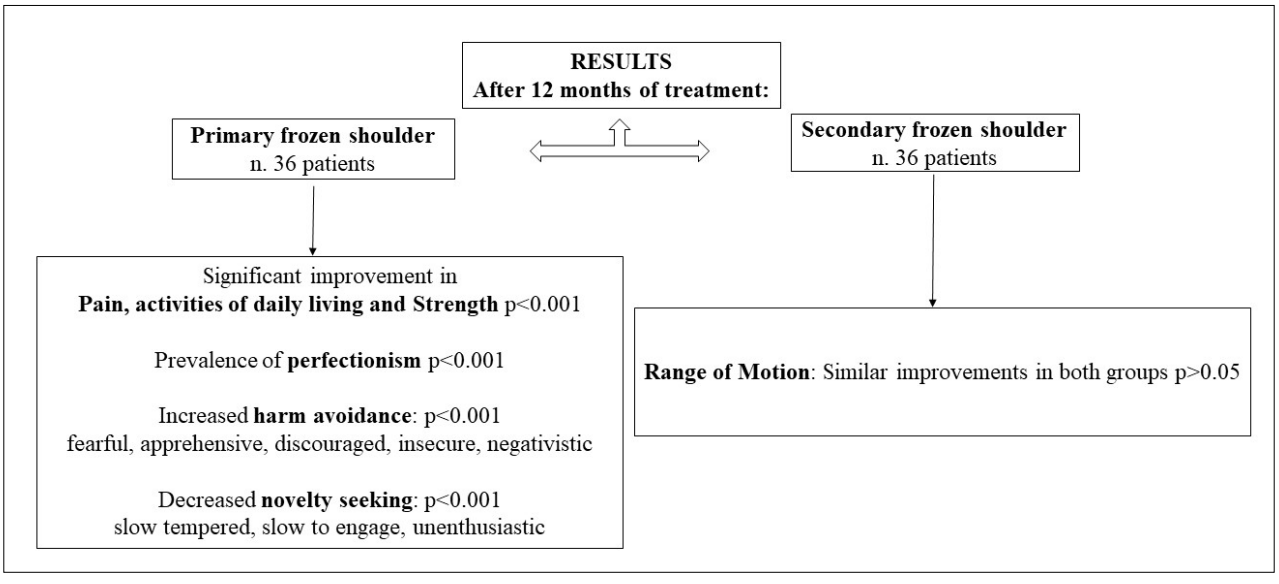
DISCUSSION

The study evaluated personality traits in patients with primary and secondary AC. We found a significant correlation between primary adhesive capsulitis and particular personality traits, indicating an interaction between psychological and somatic factors. According to the psychological assessment, patients with primary AC tended to be characterized by psycho-somatic disorder more often than patients with secondary AC. The results confirmed that perfectionism, high levels of HA, and low levels of NS, characterized the subgroup of patients with primary AC. In addition, there was a significant association between pain intensity and personality traits.

The relationship between AC and psychological disorders has been meagerly investigated. A few studies have suggested the existence of a "periarthritic personality" in patients with frozen shoulder[10,11]. An interesting piece of research indicates that depression and anxiety may coexist with AC[10]. Behavioral observations and key features of the physical examination have greatly helped clinicians to identify both the presence and severity of diseases, as well as of AC. Patients who had syndromes with ill-defined pathologic mechanisms, such as irritable bowel syndrome and fibromyalgia, had significantly high rates of anxiety and depressive disorders[5]. So, in addition to severe medical conditions and musculoskeletal shoulder disorder, we suggest screening for the presence of psychosocial issues that affected prognostication and treatment decision-making for rehabilitation. Stressful life events, psychological distress, depressive and anxiety disorders were associated with a range of medical symptoms without identified pathology, with increased health care utilization and increased costs[12]. Kelley et al.[2] underlined that a longer recovery, chronic symptoms, and work loss in patients with shoulder pain were correlated with elevated scores on the Tampa Scale of Kinesiophobia and the Fear-Avoidance Beliefs Questionnaire.

According to our results, whatever was the cause of AC, a person's susceptibility to pain could cause a psychological discomfort and encourage the onset of AC. On the basis of AC pathogenesis, multifactorial elements of personality, stress, or depression, amplified by the neurophysiologic effects of disturbed sleep, also due to pain, produced a neurochemical disturbance in CNS function. This perturbation included a reduction or impairment of function involving the pain-modulation pathways[13]. Another piece of research described neuroticism, conscientiousness, openness, and agreeableness

FIGURE 3. FLOW DIAGRAM OF THE RESULTS



as significant predictors of pain anxiety[14], and two other studies investigated the relationship between depression and anxiety symptoms in patients with idiopathic AC[15,16]. According to Ebrahimzadeh et al.[15] depression is present in 77% of these patients, anxiety in 27%, and both symptoms in 27% of their sample. Internal and external rotations were more restricted among patients who had symptoms of depression than elevation and abduction. VAS scores were higher in patients with symptoms of depression[15]. Toprak et al. [16] did not differentiate between patients with primary and secondary AC; they compared them with a healthy control group and demonstrated a higher prevalence of pain, anxiety, and sleep disturbance in patients with AC, rather than depression symptoms[16]. Both studies[15,16] confirmed that a psychiatric evaluation could be beneficial in AC patients to formulate the treatment.

Our observations about the peculiar personality traits of patients with AC corroborated findings of previous studies, identifying cognitive-behavioral tendencies. So, the physiatrist could employ specific educational strategies to optimize patient outcomes and potentially provide indications for referring the patient for consultation with a mental health practitioner[2,17-19].

CONCLUSIONS

AC is a debilitating condition. Psychological factors can have an effect on pain and shoulder dysfunction. The substantial functional impairment, distress, and costs associated with medical symptoms suggest a better understanding of the biopsychosocial cause of AC.

Patients with primary AC were characterized by the tendency to be perfectionist, but also fearful, apprehensive, discouraged, insecure, negativistic, slow tempered, slow to engage, unenthusiastic. Compared to secondary AC, they complained more about severe and lasting pain than patients with secondary AC.

This analysis should help to provide a specific diagnosis and an eventual additional therapeutic approach to patients with primary AC, evaluating psychological factors. We suggest that personality traits, such as high levels of HA and low levels of NS and perfectionism, may have different responses to treatment. These findings can help to identify patients who potentially require a longer treatment course or those whose outcomes are less satisfactory. According to these findings, personality assessment could be useful in the diagnostic process and in the therapeutic interventions.

RESUMO

OBJETIVO: *Investigar as associações entre a capsulite adesiva (CA) e um perfil psicológico específico.*

METODOLOGIA: *Foram avaliados 72 pacientes com CA fase II. Em nosso estudo, 36 pacientes foram afetados pela doença primária e 36 pela secundária. Os critérios de inclusão foram os seguintes: CA unilateral e dor no ombro durante por pelo menos dois meses. Os critérios de exclusão foram: manifestações neurológicas e psiquiátricas com um diagnóstico prévio e incapacidade de compreender os instrumentos de medição utilizados. Os resultados foram determinados após 52 semanas. A intensidade da dor no ombro foi avaliada usando a Escala Visual Analógica. Também medimos a amplitude de movimento com um goniômetro universal e a força com a escala do Conselho de Pesquisa Médica. Avaliamos os traços da personalidade dos nossos pacientes através do Inventário de Temperamento e Caráter de Cloninger e da Escala Multidimensional de Perfeccionismo.*

RESULTADOS: *Pacientes com CA primária precisaram de mais tempo para melhorar a sintomatologia quando comparados ao grupo secundário (p<0,01). Pacientes com CA primária apresentaram mais queixas de dor intensa e duradoura do que pacientes secundários (p< 0,01). Em pacientes com a doença primária, a prevalência de perfeccionismo, baixos níveis de procura por novidade, e altos níveis de prevenção de danos foram 88,2, 86,2% e 80,4, respectivamente, e abaixo de 20% em pacientes secundários.*

CONCLUSÃO: *Encontramos uma correlação significativa entre CA primária e traços de personalidade específicos, indicando uma interação entre fatores psicológicos e somáticos.*

PALAVRAS CHAVE: *Amplitude de movimento articular. Dor de ombro. Bursite. Personalidade. Transtornos somatoformes.*

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ORIGINAL ARTICLE

The influence of phytoestrogens or estrogens on the proliferation of the rat endocervical mucosa

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SUMMARY

INTRODUCTION: *Although estrogen therapy is widely used against post-menopausal symptoms, it can present adverse effects, including endometrial cancer. Soy isoflavones are considered a possible alternative to estrogen therapy. However, there are still concerns whether isoflavones exert trophic effects on the uterine cervix.*

OBJECTIVES: *To evaluate the histomorphometric and immunohistochemical alterations in the uterine cervix of ovariectomized rats treated with soy isoflavones (Iso).*

METHODS: *Fifteen adult Wistar rats were ovariectomized (Ovx) and divided into three groups: Group I (Ovx), administered with vehicle solution; Group II (OVX-Iso), administered with concentrated extract of Iso (150 mg/kg) by gavage; and Group III (OVX-E2), treated with 17β-estradiol (10 µg/kg), subcutaneously. After 30 days of treatments, the uterine cervix was fixed in 10% formaldehyde and processed for paraffin-embedding. Sections were stained with Hematoxylin and eosin for morphological and morphometric studies or subjected to immunohistochemistry for detections of Ki-67 and vascular endothelial growth factor-A (Vegf-A). The data obtained were subjected to statistical analysis ($p \leq 0.05$).*

RESULTS: *We noted an atrophic uterine cervix in GI, whereas it was more voluminous in GII and even more voluminous in GIII. The thickness of the cervical mucosa was significantly higher in GIII, as compared to GI and GII. The cell proliferation (Ki-67) was significantly elevated in the estradiol and isoflavones treated groups, whereas Vegf-A immunoexpression was significantly higher in GIII, as compared to groups GII and GI.*

CONCLUSIONS: *Soy isoflavones cause less trophic and proliferative effects in the uterine cervix of rats as compared to estrogen.*

KEYWORDS: *Cervix uteri. Isoflavones. Estrogens. Ovariectomy. Rats.*

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INTRODUCTION

Postmenopause is characterized by a pronounced reduction in estrogen levels, which leads to vasomotor symptoms such as hot flashes and night sweats. These conditions can worsen the overall quality of life and, in particular, the quality of sleep by increasing sleep disturbances. Many women may concurrently become depressed, suffer from vaginal dryness, and lose bone mass. All of these changes may have a negative effect on women. In order to mitigate the negative outcomes of hypoestrogenism, hormone therapy is indicated.[1-3] However, there are other negative consequences, including cardiovascular diseases, which appear upon late-onset hormone therapy (over 10 years after menopause) or after 60 years of age.[4] Another concern is the breast cancer risk, which may increase with combined estrogen-progestin therapy.[4,5] Hence, there is a demand for alternative therapies against postmenopausal symptoms.

Soy isoflavones have a chemical structure similar to that of estrogens, making it possible for the former to bind to estrogen receptors, especially the β receptor. Such binding produces biological effects similar to those of estrogens but with less impact on the cardiovascular system and on breast tissue.[6-8] Studies of the genital system have reported that isoflavones may increase the proliferation of both the vaginal[9] and endometrial epithelia.[10] Meanwhile, little is known about the effects of isoflavones on the cervical epithelium.

The cervical epithelium is divided into ectocervical and endocervical epithelia, with a transition region between them.[11] It has been hypothesized that this transition region may be susceptible to develop cervical neoplasia due to human papillomavirus (HPV) infection.[12] Thus, it is important to investigate substances that could show trophic effects in this region. Ford et al.[13] reported trophic effects on the cervix following treatment with genistein, a soy isoflavone, and suggested an estrogenic action by these compounds. Nevertheless, there are still few data on cervical epithelial behavior, mainly on the endocervical epithelium. Thus, in this study, we assessed the effects of a concentrated soy isoflavone extract on the endocervix of ovariectomized rats.

METHODS

Animals and study design

This was a randomized controlled experimental study, evaluated and approved by the Research Ethics Committee of the Universidade Federal de São Paulo – Escola Paulista de Medicina (UNIFESP/EPM); project Number: 0136-12. All the experimental procedures were conducted following national and international guidelines of animal use and care.

The experiment was carried out on 15 EPM-1 Wistar (*Rattus norvegicus albinus*) female rats aged 95 days and weighing ±227g each, provided by the Centro de Desenvolvimento de Modelos Experimentais em Medicina e Biologia-CEDEME, UNIFESP/EPM. The rats were transported to the animal facility of the laboratory of Histology and Structural Biology of UNIFESP/EPM. The animals were kept in cages (45x30x15cm) in an environment with controlled lighting and temperature (constant light/dark cycle of 12/12 hours and room temperature at 22°C) and were given food and water *ad libitum*.

After one week of the adaptation period, vaginal smear tests for five consecutive days were performed in all rats to monitor their estrous cycle. Only rats with regular estrous cycles remained in the experiment. Thereafter, the animals were anesthetized (ketamine, 0.08 mL/100g of body weight; xylazine, 0.04 mL/100g of body weight) and subsequently subjected to bilateral ovariectomy (Ovx).

In order to recover from Ovx surgery and ensure estrogen depletion, a period of twenty-one days after Ovx was adopted; the animals were then randomly divided into three groups, as follows: Group I (Ovx) – administered with 0.1 mL of a vehicle solution of propylene glycol (Sinthy® - São Paulo, Brasil) by gavage; Group II (Ovx-Iso) – administered with 150 mg/kg of a concentrated extract of soy isoflavones, diluted in 0.1 mL of propylene glycol, by gavage. Group III (Ovx-E2) -subcutaneous administration of 10 µg/kg of 17β-estradiol (Sigma-Aldrich Chemicals, Oakville, ON, Canada) diluted in corn oil.

The soy extract used in this study was concentrated and enriched with isoflavones (Novasoy®, Archer Daniels Midland, Decatur, IL, EUA) with the following proportions: 40% of total isoflavones (genistein, daidzein, and glycitein at a ratio of 1.3:1:0.3, respectively), 7% to 12% of proteins, 4% of ashes, 6% of humidity, and the remaining 41% was made up of soy phytocomponents. Treatments lasted for 30 consecutive days. No deaths were reported throughout the experimental period.

Material collection and histological processing

After treatment, the animals were anesthetized with ketamine (0.08 mL/100g of body weight) and

xylazine (0.04/100g of body weight) and euthanized by decapitation using an appropriate rodent guillotine. Subsequently, a laparotomy was performed to visualize the genital organs and collect the cervices. The cervices were then were fixed in 10% formaldehyde (phosphate buffer, pH 7.2, 0.1M) for 24 hours and subsequently processed for paraffin embedding, which was carried out in such a way to obtain longitudinal sections of the cervical region (Figure 1).

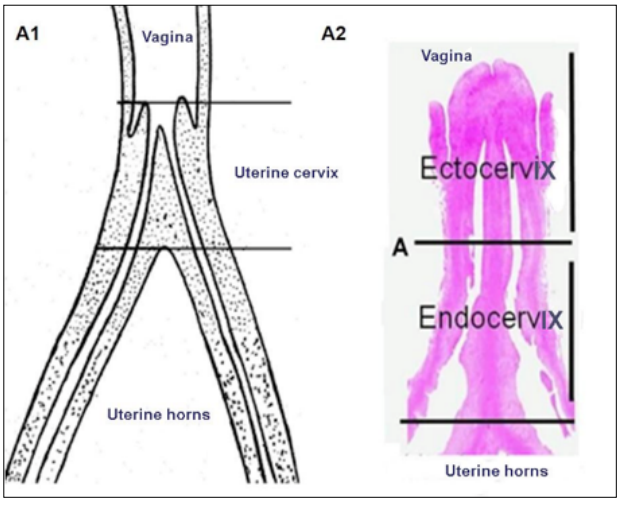
After histological processing, the 4-µm longitudinal sections (Minot Leica® RM2035 microtome) were stained with hematoxylin and eosin (H.E) for morphological and histomorphometric analyses or subjected to immunohistochemistry. Photomicrographs of the cervical sections were documented using a light microscope (Axiolab Standard 20, Carl Zeiss) coupled with a high-resolution video camera (AxionCam, Carl Zeiss) and subsequently underwent histomorphometric analysis.

Histomorphometry of the cervix

The histomorphometric evaluation was carried out with an image capture system, and the resulting images were analyzed using the Axiovision 4.8 REL (Carl Zeiss) software. Measurements were performed with 2.5x objective lenses for assessment of cervical thickness and 10x objective lenses for evaluation of the glandular area.

For each animal, five sections with 80-µm intervals between them were made in order to analyze the following parameters: a) epithelial thickness, measurements were taken from the upper margin (luminal border) of the cervical epithelium outward in eight distinct regions of the endocervix, which were averaged to obtain a mean expressed in µm; b) glandular area, the space occupied by the "glands" was outlined to cover an area up to 500 mm² of the cervix in two semi-serial sections. The areas were added up and expressed in mm².

FIGURE 1. DIAGRAM (A1) AND A LONGITUDINAL HISTOLOGICAL SECTION STAINED WITH H.E (A2) SHOWING THE REGION OF INTEREST. NOTE THAT THE REGION OF INTEREST WAS ESTABLISHED IN THE MID-REGION OF THE UTERINE CERVIX NAMED AS "A".



Immunohistochemical method

After the sections were deparaffinized and hydrated, endogenous peroxidase activity was blocked with 3% H2O2 for 10 minutes. Next, the sections underwent antigen retrieval in a steam cooker with 10-mM sodium citrate buffer solution (pH6.0) for 1 hour. After cooling, the sections were washed with phosphate-buffered saline (PBS) and incubated with 4% bovine serum albumin (BSA) for 20 minutes to block unspecified protein sites. Subsequently, the sections were incubated overnight with rabbit monoclonal primary antibodies (anti-Ki-67 or anti-Vegf-A, Spring, Bioscience, CA, USA) diluted at 1/200 and 1/300, respectively, in a humidity chamber at 4°C. For negative controls, the primary antibodies were replaced by *non-immune serum*. Afterward, the sections were washed in PBS and incubated in a streptavidin-biotinylated secondary antibody (Dako, Denmark) for 30 minutes. After the washes, reactions were revealed with 3,3-diaminobenzidine (DAB) (Dako, Denmark), counterstained with Harris hematoxylin, and mounted using a permanent mounting medium (Entellan®). A brownish color in the nuclei for Ki-67 and the cell border region or the cell cytoplasm for Vegf-A was adopted as a standard of positivity.

The slides were examined under a light microscope with a 10x objective lens (Axiolab Standard 20, Carl Zeiss) coupled with a high-resolution video camera (AxioCam, Carl Zeiss). The percentage of Ki67-positive cells was calculated in each section; at least 500 cells per animal were counted, and a mean was calculated for each rat in every experimental group. Meanwhile, the immunoreactivity for Vegf-A was assessed through a semiquantitative scoring system (H) as previously reported[6] and validated in our laboratory by Carbonel et al.[7]. Accordingly, a score of 0 was considered negative immunoreactivity, whereas positive immunoreactivities received scores from 1 to 3 according to the intensity of immunoreactivity.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). A comparison of the groups was made

with the Kruskal-Wallis test followed by the Dunn test. The level of significance for the rejection of the null hypothesis was $p \leq 0.05$. Graph Pad Prism 5.0 (San Diego, CA, USA) software was used for every test.

RESULTS

Morphological analysis

The endocervix from GI (Ovx) was covered by cuboidal epithelium with a reduction in crypts lined with the same type of epithelium as in the other two groups. The endocervix from GIII (Ovx-E2) displayed a considerably thicker epithelium of the cervical mucosa than the other two groups. The epithelium itself showed signs of proliferation attested by mitosis and stratification, whereas the lamina propria had abundant crypts. The endocervix of all rats from GII (Ovx-Iso) exhibited greater signs of proliferation than that from GI, and columnar epithelium with a few areas of stratification was noticed. The histological pattern of one rat from GII was similar to that of GI. (Table 1, Figure 2).

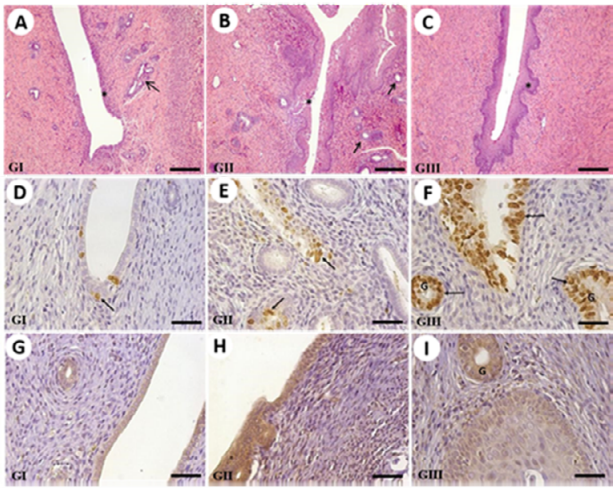
Histomorphometry of the cervical mucosa

The endocervical mucosa was significantly thicker in GIII (Ovx-E2) than in GI (Ovx) and GII. Meanwhile, the glandular area in GIII (Ovx-E2) was considerably smaller than that in GI (Ovx) and GII (Ovx-Iso). Moreover, the endocervical mucosa in GI and GII presented a similar size. (Table 1, Figure 2).

Immunohistochemical detection of Ki-67 and Vegf-A

The percentage of Ki-67-positive cells was significantly higher in GII (Ovx-Iso) and GIII (Ovx-E2) than in GI. However, this percentage was more noticeable in GIII. Furthermore, Vegf-A immunoreactivity was significantly greater in GIII (Ovx-E2) than in GII (Ovx-Iso) and GI (Ovx). (Table 1, Figure 2).

FIGURE 2. PHOTOMICROGRAPHS OF HISTOLOGICAL SECTIONS OF UTERINE CERVIX PORTIONS OF UTERI OF RATS STAINED WITH H.E (A - C), OR SUBJECTED TO IMMUNOHISTOCHEMISTRY AND COUNTERSTAINED WITH HARRIS' HEMATOXYLIN FOR THE DETECTION OF KI-67 (D - F) AND VEGF-A (G - I). NOTE GREATER THICKNESS OF THE EPITHELIUM OF THE ENDOCERVICAL MUCOSA (ASTERISKS) IN THE GII GROUP (OVX-ISO) (B), WHICH IS MORE EVIDENT IN THE GIII (OVX-E2) (C) AS COMPARED TO GI (OVX) (A). LARGER AREA OCCUPIED BY "GLANDS" (ARROWS) CAN ALSO BE SEEN IN GI (A) AND GII (B) AS COMPARED TO GIII (C). A HIGHER IMMUNOREACTIVITY (ARROWS) TO KI-67 (F) AND VEGF-A (I) CAN ALSO BE NOTICED IN THE GIII GROUP (OVX-E2), WHEN COMPARED TO GI (OVX) (D, G) AND GII (E, H). BARS: 50μM



DISCUSSION

The concern about hormone therapy was the consequence of the study published by WHI (Women's Health Initiative) in 2002, in which they reported that combined hormone therapy using estrogens

TABLE 1. DATA EXPRESSED AS MEAN ± STANDARD DEVIATION. ABBREVIATIONS: TEM = THICKNESS OF THE ENDOCERVICAL MUCOSA; GA = "GLANDULAR" AREA; CE = CERVICAL EPITHELIUM; GE = "GLANDULAR" EPITHELIUM.

	GROUPS		
Parameters	GI (Ovx)	GII (Ovx-Iso)	GIII (Ovx-E2)
TEM	360.30 ± 11.40	400.40 ± 23.90*	584.70 ± 33.80**
GA	28.60 ± 2.40	24.40 ± 2.50	17.80 ± 6.40*
Ki67/CE (%)	1.40 ± 0.640	4.60 ± 1.60*	14.80 ± 1.40**
Ki67/GE (%)	2.46 ± 1.60	6.12 ± 2.80*	12.18 ± 1.16**
Vegf-A (Scores)	1.80 ± 0.37	2.01 ± 0.04	2.90 ± 0.15*

TEM - GIII>GII>GI, *p<0.05; GIII>GI, **p <0.01; GA - GII=GI>GIII, *p<0.05; Ki67/CE/GE - GIII>GII>GI, *p<0.05; GIII>GI, **p <0.01. Vegf-A - GIII>GII=GI,*p<0.05.

and progestogens correlated with breast cancer[4]. Therefore, some investigators started looking for options to ameliorate the symptoms of menopause. In fact, isoflavones became an alternative therapy for treating postmenopausal women with symptoms of hypoestrogenism.

Isoflavones are structurally similar to endogenous estrogens because they exhibit a phenolic ring with a hydroxyl radical in carbon[3]. Such a structure grants them a selective binding ability showing a strong affinity for alpha and beta estrogen receptors.[13,14] These compounds have been found to have the same beneficial effects as estrogen therapy against menopausal symptoms but without the side effects[15]. Although the effects of isoflavones are reported to be weaker than those of endogenous estrogens, the impact of isoflavones on the cervix has not been explored so far[16]. In our results, soy isoflavones administration exhibited some trophic effects on the uterine cervical, but it was weaker when compared to estrogen, indicating that isoflavones produced less proliferative effects on the cervix of rats than estrogens.

In fact, we observed a thicker cervical mucosa epithelium in the groups treated with estrogens and isoflavones in contrast to the animals treated with the vehicle. Nonetheless, besides exhibiting the thickest epithelium, the estrogen-treated group showed the highest percentage of Ki-67-positive cells. Ki-67 is a nuclear marker of cell proliferation, and it is expressed in all of the cell cycle phases, except in GO.[7,16-20] Therefore, our results indicate that the thicker cervical mucosa seen in GIII was the result, at least in part, of the increased rate of cell proliferation. On the other hand, it is worth emphasizing that although isoflavones exhibited a trophic effect on the thickness of the cervical mucosa, this effect was significantly smaller than that exerted by estrogens. This suggests that the estrogenic activity of isoflavones is weaker in the endocervical region when compared to estrogens. In fact, isoflavone activity is 500 to 1000 times less potent than that of endogenous estrogens.[21]

Our data suggest that isoflavones exert a proliferative effect on the ectocervix, similar to what has been previously reported about the vaginal epithelium and endocervix. These descriptions are, in turn, similar to what occurs in the cervix.[22] Thus, patients with chronic diseases such as HPV must receive special care to prevent their reactivation, which could be triggered by isoflavone-induced tissue proliferation.[23-25]

CONCLUSION

In conclusion, our results show that isoflavones have trophic and proliferative effects on rat endocervix; however, these effects are less potent than those produced by estrogens.

Conflict of interest

The authors declare no conflict of interest in relation to this paper.

Author Contributions

Conceptualization, [1]P.C.F, [1]R.S.S., [1]A.A.F.C., [2]E.C.B., and [1]M.J.S; J.M.S.J Funding acquisition, M.J.S., A.A.F.C., E.C.B., Resources, M.J.S., Execution of experiment, P.C.F., [1]R.F-S., G.R.S.S.; A.A.F.C; Data collection, P.C.F., R.F-S., G.R.S.S.; A.A.F.C.; Data analysis, [1]P.C.F, [1]R.S.S., [1]A.A.F.C., G.R.S.S., R.F-S [2]E.C.B, and [1]M.J.S.; Writing-original draft, P.C.F., A.A.F.C., R.F-S., Writing-review & editing, R.S.S., A.A.F.C., M.J.S., R.F-S., E.S.C. and [2]J.M.S.J; Supervision, M.J.S; Project Administration, P.C.F., and M.J.S.

RESUMO

INTRODUÇÃO: *Embora a terapia estrogênica seja amplamente utilizada contra sintomas pós-menopausais, ela pode apresentar efeitos adversos, incluindo câncer de mama e endometrial. Assim, as isoflavonas da soja são consideradas uma alternativa possível à terapia estrogênica. No entanto, ainda há controvérsias se estes compostos exercem efeitos tróficos significativos no colo do útero.*

OBJETIVOS: *Avaliar as alterações histomorfométricas e imuno-histoquímicas no colo do útero de ratas ovariectomizadas tratadas com isoflavonas da soja (iso).*

MÉTODOS: *Quinze ratas Wistar adultas foram ovariectomizadas bilateralmente (Ovx) e separadas em três grupos: Grupo I (Ovx) - veículo (propilenoglicol); Grupo II (Ovx-Iso) - receberam extrato concentrado de Iso (150 mg/kg) e Grupo III (Ovx-E2) - tratado com 17β-estradiol (10 µg/kg); as soluções foram administradas via gavagem por 30 dias consecutivos. Posteriormente, os colos uterinos foram retirados, fixados em formaldeído a 10% tamponado e processados para inclusão em parafina. Cortes (4 µm) foram coradas com hematoxilina e eosina para estudo morfológico e morfométricos, enquanto outros foram submetidos à imuno-histoquímica para detecção de Ki-67 e do fator de crescimento endotelial vascular-A (Vegf-A). Os dados obtidos foram submetidos à análise estatística (p≤0,05).*

RESULTADOS: *Observamos a presença de colo uterino atrófico no GI (Ovx), sendo este mais volumoso no GII (Ovx+Iso) e ainda mais volumoso no GIII (Ovx+E2). A espessura da mucosa cervical foi significativamente maior no GIII (Ovx-E2), em comparação ao GI (Ovx) e ao GII (Ovx-Iso). A proliferação celular (Ki-67) foi significativamente mais elevada nos grupos tratados com estradiol e isoflavonas, enquanto a imunoexpressão de Vegf-A foi significativamente maior no GIII (Ovx-E2), em comparação ao GII (Ovx-Iso) e ao GI (Ovx-E2).*

CONCLUSÕES: *As isoflavonas da soja causam menos efeitos tróficos e proliferativos no colo do útero de ratas em comparação ao estrogênio.*

PALAVRAS-CHAVE: *Colo do útero. Isoflavonas. Estrogênios. Ovariectomia. Ratos.*

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ORIGINAL ARTICLE

Body composition among long distance runners

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SUMMARY

OBJECTIVE: *The current study aimed to examine the body composition of adult male ultra-trail runners (UTR) according to their level of participation (regional UTR-R, vs. national UTR-N).*

METHODS: *The sample was composed of 44 adult male UTR (aged 36.5±7.2 years; UTR-R: n=25; UTR-N: n=19). Body composition was assessed by air displacement plethysmography, bioelectrical impedance, and dual-energy X-ray absorptiometry. In addition, the Food Frequency Questionnaire (FFQ) was applied. A comparison between the groups was performed using independent samples t-test.*

RESULTS: *Significant differences between groups contrasting in the competitive level were found for chronological age (in years; UTR-R: 38.8±8.2 vs. UTR-N: 33.5±4.1); body density (in L.kg-1; UTR-R: 1.062±0.015 vs. UTR-N: 1.074±0.009); and fat mass (in kg; UTR-R: 12.7±6.8 vs. UTR-N: 7.6±2.7).*

CONCLUSION: *UTR-N were younger, presented higher values for body density, and had less fat mass, although no significant differences were found for fat-free mass. The current study evidenced the profile of long-distance runners and the need for weight management programs to regulate body composition.*

KEYWORDS: *Plethysmography. Electric impedance. Absorptiometry, photon.*

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INTRODUCTION

Ultra-trail running is gaining social popularity, and the number of participants is continuously increasing with competitions ranging between 42-99 km. The sport comprises intermittent intensities of effort (i.e., walking and running in a broad spectrum of positive and negative slopes) at different contexts (type of ground, wind)[1]. As for many other sports, body composition has been considered a determinant factor, particularly because it requires the displacement of the whole body corresponding to inertia for running, cycling, and swimming[2,3]. A substantial inter-variability in morphology and body composition has been noted in the literature[4]. Previous studies[5,6] compared competitive runners with recreational runners, or runners with athletes from other sports. The literature consistently suggests that long-distance runners are characterized by small body size, including body mass and its components[7,8]. Fat mass corresponds to the biological form of energy storage, and a minimum level is recommended for athletes of long events[9]. The optimal combination of body size, muscularity, and fat should be viewed as sport-specific.

Cross-sectional studies on long-distance runners corroborated the negative role of fat mass (FM) in running performance[1,4]. Although the two-component model has been reported as the most common option to examine body composition, it should be recognized that fat-free mass (FFM) is constituted of several other components, such as water, protein, mineral, and glycogen[10]. An accurate assessment of body composition requires concurrent technologies to inform about fat mass, lean body mass, bone mineral content (BMC), body water (total, intra-cellular, extra-cellular) for the whole body and regions of interests (trunk, appendicular). Other models, termed 3-compartment and 4-compartment, allow a better estimation of body composition, although assumptions are required[10]. Information obtained from concurrent technologies (bioimpedance analysis, air displacement plethysmography, dual-energy x-ray absorptiometry, respectively BIA, ADP, DXA) is often combined to produce more robust estimates[3,11,12].

Prolonged episodes of exercise require specific demands of nutrients and hydration. The diet, in parallel to training, has a relevant impact on body composition and the ability to overcome fatigue[13-15]. By inference, the contribution of carbohydrate, protein, fat intake, and micronutrients should be considered as part of the training of long-distance runners. It is consensual that the ingestion of carbohydrates before and during prolonged exercises would delay fatigue, saving the hepatic and muscular glycogen by providing glucose directly to the active muscles. More recently, recommendations have emerged for improving performance by including lipid supplementation through the ingestion of medium-chain triglycerides during exercise or a high-fat diet during the days before competition[15]. In the meantime, although body composition and diet are often recognized as crucial for long-distance runners, the literature devoted to the concurrent assessment of body composition in long-distance runners is still lacking, particularly at competitive levels. The present study aimed to examine the body composition of male adult ultra-trail runners (UTR) and, additionally, to compare participants by level of participation (regional versus national). Given the negative contribution of FM in long-distance anti-gravitational efforts, it was hypothesized that better athletes are characterized by lower levels of fat mass with fat-free mass adequately kept.

METHODS

Procedures and sample

The procedures of the current study fit the guidelines for research[16]. The project was previously approved by two Ethics Committees (University of Coimbra: CE/FCDEF-UC/00102014; University of Porto: CEFADE 17.2017). Participants were recruited by convenience. The sample corresponds to male runners who participated in official competitions in the Coimbra area and, after being contacted, demonstrated availability to visit the Coimbra University Stadium 2-4 weeks after the event for data collection. Their geographic origins covered seven districts. Participants individually signed an informed consent form prior to data collection. All measurements were obtained by experienced technicians. The sample was composed of 44 adult male runners. The inclusion criteria were: experience in UTR for two or more years; participation in regional or national competitions organized by the *Portuguese Trail Running Association;* having concluded a minimum of five competitions in the previous season. Additionally, the exclusion criterion was the presence of musculoskeletal injury, affecting training time during the previous two months. Runners were divided according to their level of practice: regional ultra trail runners *vs.* national ultra trail runners, respectively (UTR-R and UTR-N). The regional level

included runners without objectives for the nation-wide ranking, while the national group included those qualified for the nation-wide championship in addition to participation in international events during the past two seasons. The later athletes are systematically exposed to individual coaching with prescriptions for training, diet, and recovering methods.

Anthropometry

Stature was measured with a portable stadiometer (Harpenden stadiometer, model 98.603, Holtain, Crosswell, UK). Measurements were performed to 0.1cm accuracy. Body mass was quantified using a portable scale (SECA balance, model 770, Hanover, MD, USA) with a precision of 0.1kg.

Air-displaced plethysmography (ADP)

Body volume was assessed by air-displaced plethysmography (Bod Pod Body Composition System, model Bod Pod 2006, Life Measurement Instruments, Concord, CA, USA). The instrument was previously calibrated with a 50.255L cylinder following the procedures issued by the manufacturer. Participants were using lycra underwear and a swimming cap. Each individual repeated the test at least two times until a maximum variation of 150mL was obtained. The whole-body volume was adjusted for estimated thoracic gas volume. Afterward, body density was calculated by dividing the body mass (kg) by the body volume (L). The percentage of fat mass was estimated from body density using the equation proposed for normal weight adults[17].

Bioelectrical impedance analysis (BIA)

Total body water was measured using an electric bioimpedance analyzer (Akern, model BIA101, Akern Srl, Florence, Italy) and the specific software recommended by the manufacturer (Bodygram - version 1.3 Akern Srl, Florence, Italy). Participants were lying in the dorsal position, and electrodes were placed on the hand and feet, passing an electric current with very low intensity (800µA) and with a constant frequency (50kHz).

Dual-energy x-ray absorptiometry (DXA)

DXA was used to estimate the body composition of the whole body and lower limbs. The above-mentioned data were obtained using LUNAR (Lunar DPX-MD+, Software: enCORE version 4.00.145, GE Lunar Corporation, Madison, WI, USA) with participants placed on the table of the equipment in dorsal decubitus position following the recommendations of the manufacturer. Data acquisition and analysis were performed by an experienced technician in a certified laboratory.

Food frequency questionnaire (FFQ)

A self-administered questionnaire (FFQ) was applied to obtain seasonality, frequency, and portion volume for 86 food items. The questionnaire was adapted for Portuguese[18] and informed about the habitual consumption using a scale of nine options (from "*never or less than once a month*" to "*6 or more times per day*"). The final values summarize the number of calories and macronutrients.

Analyses

Descriptive statistic was calculated for the total sample (range, mean, standard error of the mean, 95% confidence interval of the mean and standard deviation). Normality was examined. For the comparison between the groups, an independent samples t-test was used. The magnitude of the effects was interpreted as follows[19]: <0.20 (trivial); 0.20 to 0.59 (small); 0.60 to 1.19 (moderate); 1.20 to 1.99 (large); 2.00 to 3.99 (very large); ≥4.00 (extremely large). The significance level was established at 5%. Statistical analyses were performed using the Statistical Package for the Social Sciences - SPSS, version 25 for Windows (SPSS Inc., IBM Company, Armonk, NY, USA).

RESULTS

Table 1 summarizes descriptive statistics for the total sample. Comparisons of UTR according to the competitive level are presented in Table 2. UTR-N were younger ($t= 2.808$; $p<0.01$; $d= 0.80$), with higher values for body density ($t= –3.369$; $p<0.01$; $d= –0.96$), lower values for body volume ($t= 2.135$; $p<0.05$; $d= 0.67$) and, consequently, for fat mass ($t= 3.425$; $p<0.01$; $d= 0.96$). No differences were found for fat-free mass by ADP, total body water by BIA, or lean soft tissue from DXA. For all the above-mentioned significant differences, the magnitude of the differences was moderate ($0.6<d<1.2$). Fat mass (in kg) was similar by two concurrent protocols, as shown in Figure 1.

DISCUSSION

The current study examined inter-variability according to competitive level among male Portuguese

TABLE 1. DESCRIPTIVE STATISTICS FOR THE TOTAL SAMPLE OF ADULT MALE LONG-DISTANCE RUNNERS (N=44) AND NORMALITY TEST FOR CHRONOLOGICAL AGE, TRAINING EXPERIENCE, BODY SIZE GIVEN BY STATURE AND BODY MASS, AND INDICATORS OF BODY COMPOSITION.

Variable	units	Range		Mean			Standard deviation	Normality	
		Minimum	Maximum	Value	Standard error	(95%CI)		K-S value	p
Chronological age	years	23.3	53.2	36.5	1.1	(34.3 to 38.7)	7.2	0.081	0.20
Training experience	years	2	17	4.0	0.4	(3.10 to 4.8)	2.8	0.314	<0.01
Stature	cm	161.8	189.7	174.4	1.0	(172.3 to 176.5)	6.9	0.109	0.20
Body mass	kg	58.5	100.5	73.0	1.5	(70.1 to 76.0)	9.6	0.142	0.03
ADP									
Body volume	L	54.382	98.103	68.508	1.455	(65.574 to 71.441)	9.649	0.137	0.04
Body density	kg/L	1.025	1.088	1.067	0.002	(1.063 to 1.071)	0.014	0.232	<0.01
Fat mass	%	4.8	33.2	13.9	0.9	(12.0 to 15.8)	6.2	0.229	<0.01
	kg	3.0	33.4	10.5	0.9	(8.6 to 12.3))	6.0	0.186	<0.01
Fat free mass	kg	51.5	77.3	62.6	1.0	(60.5 to 64.7)	6.9	0.093	0.20
BIA									
Total body water	L	34.8	58.6	45.0	0.8	(43.4 to 46.6)	5.4	0.092	0.20
DXA – whole body									
BMC	g	2413	4128	3219	68	(3082 to 3356)	451	0.095	0.20
BMD	g/cm2	1.103	1.450	1.268	0.014	(1.234 to 1.296)	0.093	0.098	0.20
Fat tissue	kg	4.2	33.6	10.8	0.9	(8.9 to 12.6)	6.2	0.172	<0.01
Lean soft tissue	kg	48.3	72.3	58.6	0.9	(56.8 to 60.4)	5.9	0.102	0.20
DXA – lower limbs									
BMC	g	932	1635	1250	26	(1198 to 1303)	174	0.112	0.20
BMD	g/cm2	1.172	1.620	1.423	0.017	(1.388 to 1.458)	0.114	0.098	0.20
Fat tissue	kg	1.2	9.5	3.2	0.3	(2.7 to 3.8)	1.7	0.174	<0.01
Lean soft tissue	kg	16.9	25.1	20.6	0.3	(20.0 to 21.3)	2.2	0.098	0.20

ADP (air displacement plethysmography); BIA (bioelectrical impedance analysis); DXA (dual-energy X-ray absorptiometry); BMC (bone mineral content); BMD (bone mineral density); 95% CI (95% confidence interval); K-S (Kolmogorov-Smirnov test); p (significance level)

ultra-trail runners characterized by low levels of fat mass. In addition, regional and national groups differed in body volume and, consequently, in body density with implications in the mean values of estimated fat mass. Finally, although concurrent methods for assessing body composition did not fully agree, both air displacement plethysmography and DXA technology evidenced that national runners have lower levels of fat mass.

The chronological age of the sample in the current study was similar to that calculated in other studies dealing with long-distance runners[5]. The results of the current study suggested that stature and body mass are similar to data obtained from long-distance athletes, such as marathon runners and 24-hour ultra-marathon runners[7]. The mean value of body fat percentage of the present study was substantially lower than 161-km ultra marathoners[20] and 65-km mountain ultra-marathon[4], despite the substantial inter-individual variability for fatness. Runners contrasting in competitive level demonstrated distinct values for body composition. As expected, UTR-N exhibited lower levels of fat mass, which was reasonably reported by different protocols: DXA and air displacement plethysmography, suggesting both as valid options for weight management.

Estimates for body composition using ADP, BIA, and DXA were within normal variation for athletes of similar sport events[21]. Both groups of UTR presented the same amount of fat-free tissue, but UTR-R significantly carried lower values of body density (higher amount of fat). It is intuitively established that if a long-distance runner, such as a marathon runner, exceeds 15% of FM, he will probably perform at a lower running pace[9]. Another important aspect of body composition refers to bone tissue (content, density). UTR are exposed to repetitive mechanical impacts believed to be positively associated with bone health parameters, although in the current study, the differences between the groups for BMC (whole body and lower limbs) appeared negligible (see Figure 1). This suggests that the main benefits are probably observed between non-athletes and the trivial to small variability among athletes could be explained by the intensity

TABLE 2. MEAN AND STANDARD DEVIATION BY COMPETITIVE GROUP (REGIONAL VS NATIONAL) AND COMPARISONS BETWEEN THE GROUPS, INCLUDING MAGNITUDE EFFECTS OF THE MEAN DIFFERENCES ON CHRONOLOGICAL AGE, TRAINING EXPERIENCE, BODY SIZE GIVEN BY STATURE AND BODY MASS, AND INDICATORS OF BODY COMPOSITION PLUS DIET NUTRIENTS (OBTAINED FROM FOOD FREQUENCY QUESTIONNAIRE).

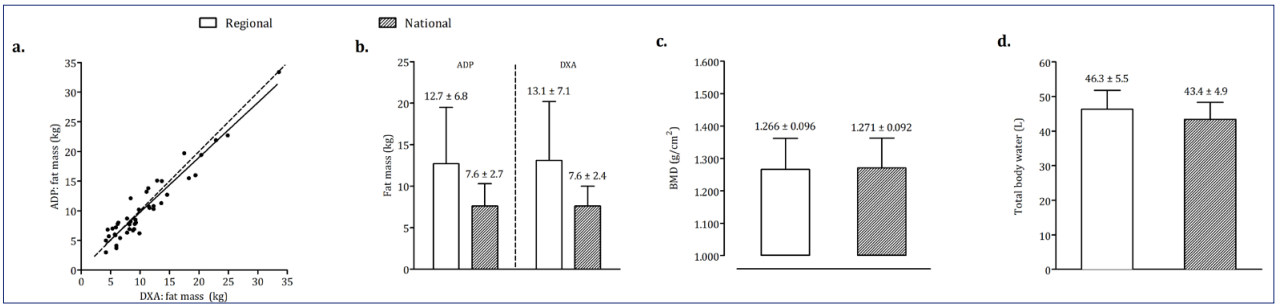
Yi: Dependent variable		X: Independent variable		Comparison				
		Regional (n=25)	National (n=19)	Difference of means (95%CI)	t-student		Magnitude of effects	
					t-value	p	d	(qualitative)
Chronological age	years	38.8±8.2	33.5±4.1	5.3 (1.5 to 9.1)	2.808	<0.01	0.80	(moderate)
Training experience	years	3.9±3.0	4.1±2.6	0.2 (-1.9 to 1.6)	-0.199	0.84	-0.07	(trivial)
Stature	cm	174.2±6.5	174.7±7.4	0.5 (-4.8 to 3.7)	-0.243	0.81	-0.03	(trivial)
Body mass	kg	75.4±10.5	69.9±7.6	5.5 (-0.2 to 11.2)	1.931	0.06	0.60	(moderate)
ADP: Body volume	L	71.110±10.611	65.085±7.106	6.025 (0.330 to 11.721)	2.135	0.04	0.67	(moderate)
ADP: Body density	L/kg	1.062±0.015	1.074±0.009	-0.012 (-0.020 to -0.005)	-3.369	<0.01	-0.96	(moderate)
ADP: Fat mass	%	16.2±6.8	10.8±3.7	5.4 (2.2 to 8.6)	3.388	<0.01	0.97	(moderate)
	kg	12.7±6.8	7.6±2.7	5.1 (2.1 to 8.1)	3.425	<0.01	0.96	(moderate)
ADP: Fat free mass	kg	62.8±6.7	62.3±7.4	0.4 (-3.8 to 4.7)	0.210	0.84	0.07	(trivial)
BIA: Total body water	L	46.3±5.5	43.4±4.9	3.0 (0.3 to 6.1)	1.829	0.07	0.57	(small)
DXA – whole-body:BMC	g	3196±435	3250±482	-54 (-336 to 226)	-0.387	0.70	-0.12	(trivial)
DXA – whole-body:BMD	g/cm^2	1.266±0.096	1.271±0.092	-0.005 (-0.063 to 0.052)	-0.188	0.85	-0.05	(trivial)
DXA – whole-body:FT	kg	13.1±7.1	7.6±2.4	5.5 (2.4 to 8.6)	3.648	<0.01	1.01	(moderate)
DXA – whole-body:LST	kg	58.5±6.0	58.7±6.0	-0.2 (-3.9 to 3.5)	-0.112	0.91	-0.03	(trivial)
DXA – lower limbs: BMC	g	1246±176	1257±176	-11 (-119 to 97)	-0.206	0.84	-0.06	(trivial)
DXA – lower limbs: BMD	g/cm^2	1.405±0.114	1.446±0.115	-0.040 (-0.110 to 0.030)	-1.158	0.25	-0.37	(small)
DXA – lower limbs: FT	kg	3.9±1.9	2.4±0.8	1.6 (0.6 to 2.5)	3.686	<0.01	1.04	(moderate)
DXA – lower limbs: LST	kg	20.7±2.4	20.6±2.1	0.2 (-1.2 to 1.5)	0.243	0.81	0.04	(trivial)
FFQ: Calories	kcal	2629±856	2323±755	306 (194 to 806)	1.234	0.22	0.38	(small)
FFQ: Proteins	%	21.2±4.8	21.7±3.5	-0.5 (-3.1 to 2.1)	-0.404	0.69	-0.12	(trivial)
FFQ: Carbohydrates	%	46.4±7.6	49.0±8.7	-2.5 (-7.5 to 2.5)	-1.016	0.32	-0.33	(small)
FFQ: Total fat	%	33.7±4.9	30.8±5.5	2.8 (-0.4 to 6.0)	1.796	0.08	0.57	(small)
FFQ: Saturated fat	%	8.8±1.9	8.5±2.8	0.3 (-1.1 to 1.7)	0.448	0.66	0.13	(trivial)
FFQ: Monounsaturated fat	%	15.0±3.3	13.3±3.0	1.7 (-0.2 to 3.6)	1.762	0.09	0.55	(small)
FFQ: Polyunsaturated fat	%	5.4±0.9	4.6±1.2	0.9 (0.2 to 1.5)	2.680	<0.01	0.79	(moderate)
FFQ: Cholesterol	mg	528±167	441±143	88 (-8 to 184)	1.841	0.07	0.57	(small)
FFQ: Fibres	g	37.0±16.6	56.5±97.8	-19.5 (-59.6 to 20.6)	0.981	0.33	-0.31	(small)
FFQ: Ethanol	g	8.9±6.3	8.8±8.8	0.1 (-4.5 to 6.5)	0.040	0.97	0.01	(trivial)
FFQ: calcium	mg	1149±562	1110±642	39 (-328 to 406)	0.215	0.83	0.07	(trivial)

ADP (air displacement plethysmography); BIA (bioelectrical impedance analysis); DXA (dual energy X-ray absorptiometry); BMC (bone mineral content); BMD (bone mineral density); FT (fat tissue); LST (lean soft tissue); FFQ (food frequency questionnaire); 95% CI (95% confidence interval); t (t-student test value); p (significance); d (d- Cohen value).

of their participation. However, low values of bone mineral density were reported among long-distance runners[22], which may be a consequence of stress, including hormonal changes, overtraining, unbalanced diet, and excessively low levels of fat mass. At an ultra-endurance event, such as an ultra-marathon, the runner could experience an average daily expenditure >8.600Kcal, and at the end of the 5-day running event, the energetic cost approach 59.079Kcal[15]. Future studies need to examine the diet of professional and amateur UTR participants by using interviews to understand the complex system of erroneous prescription and intuitive beliefs (including supplements). It is possible that the questionnaire (FFQ) used in the present study does not capture all facts for athletes, an aspect that can be considered a weakness of this study and should be the focus of future studies, probably covering a complete season. However, UTR athletes are supposed to have minimum knowledge about nutrition and hydration[15]. BIA was used in the current study and offered an estimate of total body water. Future studies may consider phase angle as an indicator of tissue integrity in relation to performance and fatigue.

The limitations of the present study must be recognized. The sample is not representative of Portuguese ultra-trail runners. Future research also needs to approach the diet using a multi-protocol approach (questionnaire, interview, diary reports). Nevertheless,

FIGURE 1



this study has strengths. It combined methodologies to assess body composition and compared the athletes by competitive level. It was possible to profile long-distance runners and to identify the need for adequate weight management, particularly body composition. Altogether, the study shows the need for more specific and effective training supervision, including nutrition.

CONCLUSION

In summary, ultra-trail runners were characterized by low levels of body fat, demanding accurate regulation of body weight, including adequate maintenance of fat-free mass. These goals require an appropriate diet regarding total calories and nutrients to maintain the integrity of bone and muscle tissues. Bioimpedance is more popularized than absorptiometry and plethysmography and emerged, in the current study, as a reasonable option for assessing body composition.

Conflict of interest

The authors do not have any conflict of interest.

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RESUMO

OBJETIVO: *O presente estudo objetivou examinar a composição corporal dos corredores de ultra-trail (UTR) e, adicionalmente, comparar dois grupos de acordo com o nível de participação (Regional vs. Nacional, respectivamente UTR-R e UTR-N).*

MÉTODOS: *A amostra foi composta por 44 corredores adultos masculinos (36,5±7,2 anos de idade; UTR-R: n=25; UTR-N: n=19). A composição corporal foi avaliada recorrendo à pletismografia de ar deslocado, bioimpedância elétrica e absorciometria de raios X de dupla energia. Adicionalmente, foi utilizado o Questionário de Frequência Alimentar. A comparação entre grupos foi realizada com base na prova t-student para amostras independentes.*

RESULTADOS: *Foram encontradas diferenças significativas por nível de competição para as seguintes variáveis dependentes: idade cronológica (em anos; UTR-R: 38,8±8,2 vs. UTR-N: 33,5±4,1); densidade corporal (em kg/L; UTR-R: 1,062±0,015 L/kg vs. UTR-N: 1,074±0,009); massa gorda (em kg; UTR-R: 12,7±6,8 kg vs. UTR-N: 7,6±2,7).*

CONCLUSÃO: *Os UTR-N tendem a ser mais jovens e apresentam valores superiores de densidade corporal e, consequentemente, valores menores de massa gorda, sendo a massa isenta de gordura semelhante entre os grupos. O presente estudo determinou o perfil dos corredores adultos masculinos de longa distância (ultra-trail), realçando a importância de uma cuidadosa regulação da massa corporal.*

PALAVRAS-CHAVE: *Pletismografia. Impedância elétrica. Absorciometria de fóton.*

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ORIGINAL ARTICLE

Conditions of vulnerability to the inadequate treatment of bronchiolitis

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SUMMARY

OBJECTIVE: *To analyze clinical and demographic variables possibly associated with the prescriptions of non-recommended but routinely used therapies for infants with acute viral bronchiolitis.*

METHODS: *A cross-sectional study included hospitalized infants with bronchiolitis caused by the respiratory syncytial virus. Those with other associated infections and/or morbidities were excluded. The data were collected from medical records.*

RESULTS: *Among 120 cases, 90% used inhaled beta-agonists, 72.5% corticosteroids, 40% antibiotics, and 66.7% inhaled hypertonic saline solution. The use of bronchodilators did not present an independent association with another variable. More frequent use of corticosteroids was associated with low oximetry, longer hospitalization time, and age>3 months. Antibiotic therapy was associated with the presence of fever, longer hospitalization, and age>3 months. Inhaled hypertonic saline solution was associated with longer hospitalization time.*

CONCLUSIONS: *Non-recommended prescriptions were frequent. Corticosteroid and antibiotic therapy were associated with signs of severity, as expected, but interestingly, they were more frequently used in infants above 3m, which suggested less safety in the diagnosis of viral bronchiolitis in these patients. The use of bronchodilators was even more worrying since they were indiscriminately used, without association with another variable related to the severity or characteristics of the host. The use of the inhaled hypertonic solution, although not associated with severity, seems to have implied a longer hospitalization time. The identification of these conditions of greater vulnerability to the prescription of inappropriate therapies contributes to the implantation of protocols for the bronchiolitis treatment, for continuing education and for analysis of the effectiveness of the strategies employed.*

DESCRIPTORS: *Respiratory syncytial viruses. Bronchiolitis. Drug therapy.*

INTRODUCTION

Acute viral bronchiolitis (AVB) has been known for over 50 years as a viral infection that affects infants and whose main etiologic agent is the respiratory syncytial virus (RSV). AVB is considered the main cause of hospitalization among infants[1,2]. It affects about 60 million infants yearly worldwide and causes over 3.4 million hospitalizations and about 160,000 deaths, but various aspects of its etiopathogenesis remain unknown, and there have been few therapeutic advances[1-3].

Currently, the recommended treatment for bronchiolitis consists of implementing patient support measures through oxygen and hydration[4-6]. However, its clinical similarity with an asthma crisis leads to

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frequent prescriptions of bronchodilators, corticosteroids, and antibiotics worldwide. Seeking to improve the management of these patients and avoid inappropriate therapies, national and international guidelines were created based on scientific evidence, which do not recommend the routine use of these medications. Some countries have instituted the use of guidelines and follow up under continuing education of the health professionals responsible for such therapies and obtained an improvement in the adequacy of AVB treatment[7-10].

On the other hand, different patterns of AVB have been described, including phenotypes and characteristics such as gene expression profile and transcriptomes associated with the etiologic agent[11,12].

In the present study, we investigated the associations between demographic and clinical variables and the therapies used despite not being recommended for routine treatment. The identification of conditions under greater vulnerability to the prescription of inadequate therapy seeks to contribute to the implementation of protocols for the treatment of AVB, to continuing education, and further comparisons and analysis of the effectiveness of the strategies employed.

METHODS

A descriptive and analytic cross-sectional study was carried out on a convenience sample of infants aged between 0 and 2 years treated in the Pediatrics Emergency Service of the Hospital do Servidor Público Estadual of São Paulo (IAMSPE) and subsequently admitted to this service, between November 2012 and October 2014, and who underwent etiological investigation for the respiratory syncytial virus during the first episode of wheezing. This hospital provides high-complexity assistance for 1.3 million state employees and their dependants.

We included all infants in their first episode of wheezing (defined by the presence of wheezing during pulmonary auscultation) and submitted nasopharyngeal aspirate collection for RSV investigation. We excluded patients diagnosed with or suspected of other associated infections at admission or during hospitalization, as well as those with other comorbidities. The data were collected from medical records and via telephone by means of standardized protocols. We analyzed the sociodemographic characteristics, signs, and symptoms on admission, medications prescribed during hospitalization, the need for mechanical ventilation and supplemental oxygen. The medication prescriptions were evaluated according to the age of the patient, the hospitalization day of the week, presence of fever (on admission), oximetry value (on admission), hospitalization in the intensive care unit (ICU), and length of hospitalization. In accordance with the service protocol during the period studied, oxygen therapy was indicated for maintaining the levels of oxygen saturation above 95%, according to pulse oximetry. The criteria for hospitalization were signs of tachydyspnea and/or maintenance of oxygen saturation below 95% after a reassessment of the initial service in the Emergency Room.

The collection of nasopharyngeal secretion was performed by the nursing staff routinely in all infants with a first episode of wheezing, and the samples were immediately submitted to the hospital laboratory. The rapid immunochromatographic test used was the RSV BinaxNOW® by Alere, which can identify subgroups A and B of RSV, with results available in 15 minutes.

The parents or legal guardians of the children were informed about the study and signed the informed consent form. This study was approved by the Research Ethics Committee of the Faculty of Medicine of USP (40796314.4.0000.0065).

Statistical analysis

We used the chi-square test for categorical variables and the Mann-Whitney test for continuous ones. The associations (variables/outcomes) were studied by univariate and multivariate logistic regression analysis. The variables whose associations were significant in the univariate analysis were included in the multivariate analysis. P values < 0.05 was considered statistically significant. We used Stata 10.0®.

RESULTS

A total of 321 tests were conducted to detect RSV in the pediatrics emergency service of IAMSPE in infants with a first episode of wheezing. There were 192 samples with negative results, which were not selected. Among the 129 (40.2%) samples with positive results for RSV, seven were excluded from the study because the patients had comorbidities: pneumonia (four cases), heart disease (one case), and two patients were excluded because they were infected with RSV during their hospitalization due to another pathology. Two medical charts were not located. There was, therefore, a total of 1.5% losses and 5.4% exclusions.

The number of cases of bronchiolitis due to RSV was higher in the months of March, April, May, and June of the years 2013 and 2014. Their sociodemographic characteristics are presented in Table 1. The most frequent signs and symptoms at the time of admission were: cough (96.7%), oxygen saturation of <95% (76.5%), respiratory discomfort (67.2%), wheezing (61.5%), and fever (35%). The therapeutic interventions are presented in Table 2. Admission to the ICU was necessary in 15% of cases, and 7.5% were submitted to invasive ventilation.

TABLE 1. SOCIODEMOGRAPHIC CHARACTERISTICS OF INFANTS WITH RESPIRATORY SYNCYTIAL VIRUS INFECTION

SEX	N (%)
Female	62 (51.6%)
Male	58 (48.3%)
COLOR*	N (%)
White	54 (58.1%)
Brown	21 (22.6%)
Black	18 (19.3%)
Yellow	0 (0%)
Indigenous	0 (0%)
TYPE OF DELIVERY	
C-section	72 (62.6%)
Vaginal	43 (37.4%)
Prematurity (GA<37 weeks)	N (%)
Yes	15 (13.1%)
No	100 (86.9%)
BIRTH WEIGHT	N (%)
<1,500 g	4 (3.5%)
1,500 – 2,000 g	6 (5.2%)
2,001 – 2,500 g	6 (5.2%)
2,501 – 3,000 g	21 (18.3%)
<3,000 g	78 (67.8%)
BREASTFEEDING	N (%)
Yes	83 (84.7%)
No	15 (15.3%)
BREASTFEEDING DURATION (months)	MD (DP)
Exclusive	3.7 (4.12)
Mixed	5.9 (8.01)
SIBLINGS	N (%)
Yes	73 (78.5%)
No	20 (21.5%)
NUMBER OF SIBLINGS	N (%)
1 Sibling	42 (58.3%)
2 Siblings	23 (31.9%)
3 Siblings	3 (4.2%)
4 Siblings	2 (2.8%)
>4 Siblings	2 (2.8%)
NURSERY	N (%)
Yes	69 (76.7%)
No	21 (23.3%)
SMOKING DURING PREGNANCY	N (%)
Yes	05 (5.6%)
No	85 (94.4%)
SMOKERS IN THE HOUSEHOLD	N (%)
Yes	11 (11.9%)
AGE AT ADMISSION (months)	MDI (IQ)
	6.8 (2.7-12.4)
DAY OF ADMISSION	N (%)
Weekday	102 (85%)
Weekend	18 (15%)

The univariate analyses of the associations between the therapy used and the variables "age", "presence of fever", and "pulse oximetry", with the variables "hospitalization during weekends," "hospitalization in Intensive Care Unit" and "hospitalization length" and the multivariate analyses of the therapy associations used with an adjustment for variables that were significant in the univariate analyses are presented in Table 3.

DISCUSSION

The study examined the associations between the approaches used in infants with bronchiolitis caused by RSV and clinical and demographic factors.

TABLE 2. THERAPY INTERVENTIONS PRESCRIBED TO INFANTS ADMITTED WITH RESPIRATORY SYNCYTIAL VIRUS INFECTION

THERAPY	N (%)
Admission to ICU	18 (15%)
Invasive ventilation	09 (7.5%)
Corticosteroids	87 (72.5%)
Intravenous fluids	84 (70%)
Inhaled epinephrine	12 (10%)
Inhaled hypertonic saline solution	80 (66.7%)
Inhaled bronchodilator	108 (90%)
Oxygen therapy	97 (80.8%)
Antibiotics	48 (40%)
Clarithromycin	16 (33.3%)
Penicillin	6 (12.5%)
Azithromycin	3 (6.2%)
Ampicillin	2 (4.2%)
Others	2 (4.2%)
DURATION OF THERAPY (days)	MD (DP)
Invasive ventilation	5.7 (3.38)
Corticosteroids	4.7 (3.25)
Intravenous fluids	3.2 (2.1)
Inhaled epinephrine	1.9 (1.3)
Inhaled hypertonic saline solution	4.4 (2.9)
Inhaled bronchodilator	5.2 (3.8)
Oxygen therapy	4.7 (2.9)
Antibiotics	3.4 (2.97)

TABLE 3. UNIVARIATE AND MULTIVARIATE ANALYSES OF THE ASSOCIATIONS BETWEEN THE THERAPIES USED AND DEMOGRAPHIC AND CLINICAL VARIABLES

UNIVARIATE ANALYSES									
Therapy	<3 m			Fever			O_2<95%		
	N (%)	OR	p	N (%)	OR	p	N (%)	OR	p
ATB	08(24.2)	0.37	0.03	24(57.1)	3	0.006	37(42)	1	0.91
BD	29(87.9)	0.74	0.63	38(90.5)	1.08	0.89	86(97.7)	15.0	0.001
CS	15(45.5)	0.17	0.00	30(71.4)	0.92	0.85	71(80.7)	3.34	0.01
HSS	25(75.8)	1.82	0.19	28(66.7)	1.00	1.00	64(72.7)	2.13	0.09
Oxygen	29(87.9)	2.02	0.23	33(78.6)	0.80	0.64	79(89.8)	3.9	0.01
Total	33(100)	42(100)	88(100)						

	WKND*			ICU			HOSP.T**	
	N (%)	OR	p	N (%)	OR	p	OR	p
ATB	06(33.3)	0.7	0.53	12(66.7)	3.6	0.02	2.51	<0.001
BD	17(94.4)	2.05	0.50	16(88.9)	0.87	0.86	2.13	0.08
CS	13(72.2)	0.98	0.98	16(88.9)	3.49	0.11	2.24	0.005
HSS	14(77.8)	1.91	0.28	11(61.1)	0.75	0.59	3.58	<0.001
Oxygen	16(88.9)	2.07	0.35	18(100)	nsa	nsa	18	<0.001
Total	18(100)	18(100)						

MULTIVARIATE ANALYSIS		
USE OF ANTIBIOTICS		
	OR	p
Fever	3.0	0.013
Age <3 m	0.21	0.007
Length of hospital stay	2.53	0.003
Admission to ICU	3.44	0.07
USE OF CORTICOSTEROIDS		
	OR	p
Sat O_2<95%	3.17	0.037
Length of hospital stay	2.98	0.004
Age <3 m	0.67	<0.001
USE OF INHALED HYPERTONIC SALINE SOLUTION		
	OR	p
Length of hospital stay	3.07	<0.001
Sat O_2<95%	1.89	0.201
USE OF BRONCHODILATORS		
	OR	p
Age <3 m	0.734	0.634
OXYGEN THERAPY		
	OR	p
Sat O_2<95%	3.324	0.056
Length of hospital stay	12.9	<0.001

ATB = antibiotic therapy; BD = inhaled bronchodilators; CS = corticosteroids; HSS = inhaled hypertonic saline solution. *WKND = weekends, ICU = Intensive Care Unit** HOSP.T = duration of hospitalization. Time analyzed in three categories: <3 days, between 4 and 7 days, and >7 days. The OR refers to the chance of going from one category to the next. Logistic regression test

The prescriptions rates of bronchodilators, corticosteroids, and antibiotics were high, contrary to the recommendations of the main guidelines in force during the time of hospitalization of these patients[5,13]. The analyses of the conditions associated with such practices emphasize the value placed on clinical severity. One aspect not previously explored stands out: a younger age proved to be a protective factor, which suggests that the infants older than 3 months are more vulnerable to inappropriate therapies and, therefore, guidelines for health professionals should highlight recommendations for this age group.

The odds of corticosteroids prescription for young infants was approximately 50% lower than for those older than 3 months. It is interesting to note that we only included cases with confirmed etiologic identification of RSV, and, despite that, the use of corticosteroids was high. These results suggest that the RSV etiology in younger infants has been less associated with recurrent wheezing or asthma. It is possible that the inflammatory process involved in the pathogenesis of AVB and the clinical presentation similarity with an asthma crisis are the reason for this use, despite the contrary recommendations of the guidelines[4-6,13]. The prescription of corticosteroids was also independently associated with lower levels of oxygen saturation, which suggests the influence of the severity of the respiratory condition. It is worth noting that the level adopted for the institution of oxygen therapy was an oxygen saturation below 95%, which may have contributed to the assessment of severity. However, the excessive and erroneous use of corticotherapy in AVB is described worldwide, despite not being recommended by national and international consensus[4-6]. Several studies have failed to show the benefits of its systemic or inhaled use in reducing hospitalization time or rates[14].

Age less than 3 months was also a protector for

the use of antibiotics. Using the same reasoning previously applied to the association between corticosteroids and age, it is possible that the diagnosis of associated bacterial pneumonia was suspected more often in older children, while AVB as a single diagnosis was more often associated with young infants. The use of antibiotics is banned in all guidelines, except for severe cases with the need for mechanical ventilation or concomitant bacterial infection (confirmed or suspected)[4-6]. Against recommendations, antibiotics were administered to 40% of patients with RSV infection. The presence of fever at admission was a factor that favored the prescription of this drug; however, studies indicate that the low probability of fever being associated with a severe bacterial coinfection in BVA does not justify the introduction of an antimicrobial agent[15].

The use of inhaled bronchodilators was high. Pulse oximetry below 95% was associated with the prescription of this drug; however, this association was not independent after adjustment for confounding factors, which suggests that the indication was indiscriminate, regardless of other variables. The 2016 guidelines by the American Academy of Pediatrics allowed resorting to the use of this drug as a therapeutic trial and maintaining it in the event of a satisfactory clinical response[13]. A multicenter study that included 28 studies on beta-agonists and 1,912 patients showed that the drug does not reduce the hospitalization rate or time[16]. Current guidelines do not recommend the use of beta-agonists in children with bronchiolitis[4.6].

The inhaled epinephrine is also not recommended by the guidelines for the treatment of AVB[5.13]. Epinephrine was administered to 10% of patients for post-extubation laryngitis. A Cochrane systematic review showed no reduction in the time of hospitalization for bronchiolitis with the use of epinephrine or any other benefit[17].

The inhalation of 3% hypertonic saline was prescribed for most of the patients in the study. After adjustments for confounding factors, the prescription of this medication was not associated with low levels of oxygen saturation. As mentioned above, it is possible that the 95% threshold for saturation led to increased use of the hypertonic saline solution, due to the clinical severity. Another interesting aspect was the independent association of the use of the inhaled hypertonic saline solution, and longer hospitalization time, i.e., the therapy may have led to prolonged hospitalization, possibly due to a delay in its suspension. At the time, National Guideline advocated for the use of the hypertonic saline solution in association with bronchodilators in order to avoid reflex bronchospasm[5]. What supported this indication was the possibility of promoting an osmotic flow towards the mucus present in the airways and improving cell rhythm[18]. Although the inhaled hypertonic solution can promote benefits in patients with cystic fibrosis, the same thing is not observed in AVB[18]. The current 2017 guidelines by the Brazilian Society of Pediatrics do not recommend the routine use of inhaled hypertonic saline solution[5]. Other guidelines, such as the British, also do not recommend it[6].

The characteristics of the sample studied point to the possibility of applying these results in clinical practice. The identification of RSV present in respiratory secretions in 40.2% of AVB cases was compatible with data from the literature since the study included infants hospitalized for a period of two years, even outside of the seasonality period of RSV, which decreases the final positivity of the etiological investigation for this agent[19.20]. The sociodemographic characteristics and breastfeeding rates found are compatible with the population of infants born at term in the city of São Paulo[5.21]. The clinical presentation with a predominance of cough, hypoxemia, respiratory distress, and wheezing is characteristic of AVB caused by RSV with moderate to high severity. The need for hospitalization in the Intensive Care Unit and mechanical ventilation are in line with other Brazilian studies that reported rates of ICU hospitalization between 6% and 17%, with the need for invasive ventilation between 2.8% and 7%[22.23].

The application of the guidelines, with the training of the medical and nursing staff, seems to be the most effective measure to reduce inappropriate therapies. A multicenter study carried out in 21 hospitals showed a decrease of 68% in the prescription of corticosteroids, 29% in the use of bronchodilators, and 44% in the number of radiographs after interactive monthly seminars[11]. Another aspect that could contribute to the conduct is the availability of tests to identify the etiology of AVB. Although the guidelines do not state the need for etiologic confirmation of AVB in order to implement the recommended therapies, there is still controversy since some studies show an impact on the etiologic diagnosis[22.24]. A study conducted at the University Hospital of the Universidade de São

Paulo, in São Paulo, observed that, after the implementation of investigation for respiratory viruses, there was a reduction by 32.2% in the prescription of antibiotics for patients who tested positive a virus and 9.2% for those with negative results. This reduction was not observed regarding the use of corticosteroids and bronchodilators[22].

It is possible that a set of measures that include the identification of the viral agent in addition to treatment recommendations based on national and international guidelines implemented with continuing education support programs to the entire staff involved in patient care can promote proper patient support, minimizing unnecessary drug prescriptions.

The identification of demographic and clinical conditions associated with the increased use of inappropriate therapies can contribute to continuing education and the greater protection of patients more vulnerable to iatrogenesis.

CONCLUSIONS

Infants with more severe clinical conditions, especially those over the age of 3 months, were more vulnerable to the inadvertent use of corticosteroids and antibiotics, possibly by greater insecurity in the diagnosis of viral bronchiolitis, although the etiology by RSV was identified. Even more worrying was the indiscriminate use of bronchodilators, which was not associated with any variable. It is also noteworthy that inhaled hypertonic solution was indicated regardless of the severity of the case and seems to have caused a greater time of hospitalization.

Contribution of the authors

Kattia C. Naves - Project design, data collection, analysis and interpretation of the results, drafting of the manuscript. Sandra E. Vieira - Conception of the study, project design, analysis and interpretation of the results, production and revision of the manuscript.

RESUMO

OBJETIVOS: *Analisar variáveis clínicas e demográficas possivelmente associadas às prescrições de terapêuticas não recomendadas, porém rotineiramente utilizadas, para lactentes com bronquiolite viral aguda.*

MÉTODOS: *Estudo transversal incluiu lactentes hospitalizados com bronquiolite por vírus sincicial respiratório. Excluídos aqueles com outras infecções e/ou morbidades. Dados coletados de prontuários.*

RESULTADOS: *Analisados 120 casos, para os quais foram prescritos: beta-agonistas inalatórios a 90%; corticosteroides a 72,5%, antibióticos a 40% e solução salina hipertônica inalatória a 66,7%. O uso de broncodilatadores não apresentou associação independente com outra variável. Maior uso de corticosteroide associou-se à baixa oximetria, maior tempo de internação e idade >3 meses. Antibioticoterapia associou-se à presença de febre, maior tempo de internação e idade >3 meses. Solução salina hipertônica inalatória associou-se a maior tempo de internação.*

CONCLUSÕES: *A frequência das prescrições não recomendadas foi elevada. Corticosteroide e antibioticoterapia foram associados a sinais de gravidade, como esperado, porém, interessantemente, foram mais utilizados nos lactentes com idade acima de 3 meses, o que sugeriu menor segurança no diagnóstico de bronquiolite viral nesses pacientes. O uso de broncodilatadores foi ainda mais preocupante, uma vez que foram indiscriminadamente utilizados, sem associação com outra variável, seja relacionada à gravidade, seja a características do hospedeiro. O uso de solução hipertônica inalatória, apesar de não associado à gravidade, parece ter implicado maior tempo de internação. A identificação dessas condições de maior vulnerabilidade à prescrição de terapêuticas inadequadas contribui para a implantação de protocolos para o tratamento da BVA, para educação continuada e para posteriores comparações e análises de eficácia das estratégias empregadas.*

PALAVRAS-CHAVE: *Vírus sinciciais respiratórios. Bronquiolite. Tratamento farmacológico.*

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ORIGINAL ARTICLE

Medical schools in Brasil: population, economic and historical analysis

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SUMMARY

BACKGROUND: *To describe the current distribution and historical evolution of undergraduate courses in medicine in Brasil.*

METHODS: *Analytical cross-sectional study of secondary data. Through the Ministry of Education, the data of the medical courses were obtained, and through the Brazilian Institute of Geography and Statistics, the population and economic data of the Brazilian states were obtained.*

RESULTS: *In Brasil, there were 298 medical courses (1,42 courses / million inhabitants) in January 2018, totaling 31,126 vacancies per year, with 9,217 gratuitous vacancies (29.6%) and 17,963 vacancies in the hinterland (57, 7%). In Brazilian states, there are positive and statistically significant (p <0.001) correlations of the variables: "vacancies" and "population" (R 0.92); "vacancies" and "gross domestic product" ("GDP") (R 0.83); "percentage of vacancies in the hinterland" and "population in the hinterland" (R 0.71) and "percentage of vacancies in the hinterland" and "GDP" (R 0.64). There was a negative and statistically significant correlation between "gratuitous vacancy percentage" and "GDP" (R -0.54, p = 0.003). More paid courses than gratuitous courses and more courses in the hinterland than in the capitals have been created since 1964, in proportions that have remained similar since then, but in higher numbers since 2002.*

CONCLUSIONS: *The distribution of medical courses in Brasil correlates with the population and economical production of each state. The expansion of Brazilian medical education, which has been accelerated since 2002, is based mainly on paid courses in the hinterland, in the same pattern since 1964.*

KEYWORDS: *Medical Education; Medical Schools; Brasil; Population; Gross Domestic Product*

INTRODUCTION

The current concern of Brazilian society regarding medical education, its quality, number of job openings, and access to it by the population is well-known[1]. There is evidence that the concentration of human resources in health is associated with improved indicators of population health; thus, the evaluation of the distribution of these resources in health systems becomes imperative[2,3].

Programs to provide and institute medical services in the interior of Brasil date back to the deployment of the Rondon Project, in 1968[4]. Since then, various programs encouraging medical work in the countryside, some of them facilitating opening new medical schools, have been implemented, culminating, most recently, in the More Doctors Medical Program (*Programa Mais Médicos*), launched in 2013[5].

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Since then, the interest in describing the distribution of medical schools across the Brazilian territory has grown and was embodied by an interinstitutional study initiated in 2014 named "Medical Demography in Brasil"[6]. The publication, which is currently in its fourth update, presents the distribution of medical schools and their openings in Brasil, per state of the federation and region, according to their "public" or "private" nature and its location in "capitals" or the "hinterland". The data indicated a distribution of vacancies proportional to the population of Brazilian states, with a predominance of private schools (mainly in the South and Southeast regions) and located in hinterland cities[6].

In order to seek a better understanding of the mechanisms that determine the distribution of medical schools in Brasil, we questioned whether population, economic, or political pressures are its main determinants. To answer this question, we correlated the population and gross domestic product of each state with the number of vacancies in medical schools, as well as its characteristics of gratuitousness and hinterland location. Additionally, we sought to identify the influence of the political alignment of the country in the distribution and characteristics of schools, describing the growth in the number of programs and their behavior of gratuitousness and hinterland location throughout historical periods based on the administrations of the federal government.

METHODS

This is an analytical cross-sectional study based on secondary data obtained from public databases. We searched the E-MEC website of the Ministry of Education (http://emec.mec.gov.br/) and obtained data from undergraduate medical courses in Brasil by the 31st January 2018. By using the "advanced search" tool, selecting "graduate program", filling it out with the word "medicine", marking the item "exact search", and selecting each Brazilian state, we obtained the following information: name of the institution of higher education, free or paid access, year of beginning, number of annual vacancies, and municipality of location.

The data about the population of each Brazilian state, their Gross Domestic Product (GDP), and population residing in their capitals were obtained by consulting the results of the 2010 demographic census on the Brazilian Institute of Geography and Statistics (IBGE) website (https://censo2010.ibge.gov.br/). We considered the population residing in the hinterland of each state as the difference between the total state population and the population of the municipality corresponding to the capital of the state.

For the historical analysis, we considered the following periods: Colony and Empire (1808-1889), Old Republic (1890-1929); Vargas Period (1930-1944); Pre-Military Period (1945-1963); Military Period (1964-1984); Sarney Government (1985-1989); Collor-Itamar (1990-1993); Fernando Henrique (1994-2001); Lula (2002-2009); Dilma (2010-2015), and Temer (2016-2018).

We created a database in Excel™ 2010 (Microsoft®) and, from it, exported and analyzed the data using Statistica ™ 13.3 (Tibco®).

RESULTS

Table 1 presents the number of undergraduate medical programs and their annual vacancies in each Brazilian state, in addition to the number of gratuitous vacancies and vacancies in programs in cities located in the hinterland of the states. In Brasil, until January of 2018, there were 298 undergraduate medical programs, totaling 31,126 anual vacancies, 9,217 gratuitous (29.6% in relation to the national total), and 17,963 located in the hinterland of the country (57.7% in relation to the national total).

The Brazilian population, according to the 2010 Demographic Census, is 190,755,799, of which 145,289,754 (76.2%) reside outside the city corresponding to the capital. The Brazilian GDP in the same year was R$ 3,770,085 million. The largest population (21,262,199 inhabitants, 21.6% of the national total) and the largest GDP (R$ 1,247,596 million, 33.1% of the national GDP) are found in the state of São Paulo.

Figure 1a analyzes the correlation between the variables "annual vacancies in medical programs" and the "population" in Brazilian states and the Federal District, with a Spearman coefficient of 0.92 ($p<0.001$). Figure 1b presents the correlation between the variables "annual vacancies in medical programs" and "GDP" in Brazilian states and the Federal District, with a Spearman coefficient of 0.83 ($p<0.001$). Figure 1c correlates the variables "percentage of annual vacancies in medical programs located in the hinterland" and "percentage population residing in the hinterland" in Brazilian states, with a Spearman coefficient of 0.71 ($p<0.001$). Figure 1d, analyzes the correlation between

TABLE 1. ANNUAL NUMBER OF VACANCIES IN UNDERGRADUATE MEDICAL SCHOOLS IN BRAZILIAN STATES

Number of vacancies in medical schools in Brazilian states (absolute number and percentage in relation to the national total), free vacancies (absolute number and percentage in relation to the state total), vacancies located in the hinterland (absolute number and percentage in relation to the state total), gratuitous vacancies located in the hinterland (absolute number and percentage in relation to the number of vacancies in the hinterland of the state) and gratuitous vacancies located in the capital (absolute number and percentage in relation to the number of vacancies in the state capital)

State	Medical schools	Vacancies	Gratuitous Vacancies	Vacancies in the hinterland	Gratuitous vacancies in the hinterland	Gratuitous vacancies in the capital
SP	55	6,279 (20.1%)	756 (12.0%)	4,465 (71.1%)	575 (12.9%)	181 (10.0%)
MG	43	4,475 (14.3%)	1,405 (31.4%)	3,213 (71.8%)	1,085 (33.8%)	320 (25.4%)
RJ	20	2,821 (9.0%)	694 (24.6%)	1,655 (58.7%)	240 (14.5%)	454 (38.9%)
PR	19	1,894 (6.0%)	490 (25.9%)	1,135 (59.9%)	300 (26.4%)	190 (25.0%)
BA	19	1,798 (5.7%)	673 (37.4%)	838 (46.6%)	453 (54.1%)	220 (22.9%)
RS	19	1,762 (5.6%)	680 (38.6%)	1,402 (79.6%)	440 (31.4%)	240 (66.7%)
GO	12	12.82 (4.1%)	220 (17.2%)	1,048 (81.7%)	110 (10.5%)	110 (47.0%)
PE	10	1,110 (3.5%)	510 (45.9%)	340 (30.6%)	220 (64.7%)	290 (37.7%)
CE	8	1,036 (3.2%)	400 (38.6%)	400 (38.5%)	160 (40.0%)	240 (37.7%)
PB	9	1,026 (3.2%)	265 (25.8%)	544 (53.0%)	120 (22.1%)	145 (30.1%)
SC	12	910 (2.9%)	140 (15.5%)	810 (89.0%)	40 (4.9%)	100 (100%)
PA	6	610 (1.9%)	310 (50.8%)	60 (9.8%)	60 (100%)	250 (45.5%)
PI	7	601 (1.9%)	240 (39.9%)	190 (31.6%)	110 (57.9%)	130 (31.6%)
TO	6	600 (1.9%)	160 (26.7%)	420 (70.0%)	100 (23.8%)	60 (33.3%)
AM	5	585 (1.8%)	290 (49.6%)	48 (9.2%)	48 (100%)	242 (45.1%)
MA	6	579 (1.8%)	330 (57.0%)	330 (57%)	230 (69.7%)	100 (40.2%)
ES	5	570 (1.8%)	80 (14.0%)	270 (47.4%)	0 (0.0%)	80 (26.7%)
AL	5	495 (1.5%)	210 (42.4%)	60 (12.1%)	60 (100%)	150 (34.5%)
DF	5	476 (1.5%)	156 (32.8%)	-	-	-
RN	5	472 (1.5%)	280 (59.3%)	180 (38.1%)	180 (100%)	100 (34.2%)
MT	6	431 (1.3%)	240 (55.7%)	280 (65%)	160 (57.1%)	80 (53.0%)
MS	5	388 (1.2%)	268 (69.1%)	140 (36.1%)	140 (100%)	128 (51.6%)
ORA	4	325 (1.0%)	40 (12.3%)	75 (23.1%)	0 (0.0%)	40 (16.0%)
SE	3	300 (1.0%)	160 (53.3%)	60 (20.0%)	60 (100%)	100 (41.7%)
AC	2	161 (0.5%)	80 (49.7%)	0 (0.0%)	-	80 (49.7%)
RR	1	80 (0.2%)	80 (100%)	0 (0.0%)	-	80 (100%)
AP	1	60 (0.2%)	60 (100%)	0 (0.0%)	-	60 (100%)
Brasil	298	31,126	9,217 (29.6%)*	17,953.001 (57.7%)*	4,891 (27.2%)*	4,170 (32.9%)*

Source: E-mec, accessed on the 31st January 2018. * Percentage in relation to the national data

TABLE 2. IN EACH PERIOD OF BRAZILIAN HISTORY: UNDERGRADUATE MEDICAL SCHOOLS CREATED IN BRASIL

Created medical schools (absolute number and percentage in relation to the current total), gratuitous medical schools created (absolute number and percentage in relation to the total at the time), medical schools created in the hinterland (outside of municipality capitals, absolute number and percentage in relation to the total at the time), Brazilian population and schools/million inhabitants ratio

Historical period	Created medical schools	Gratuitous medical schools created in the period	Medical Schools created in the hinterland in the period	Brazilian population*	Medical school/million inhabitants
1808-1889	2 (0.7%)	2 (100%)	0 (0.0%)	14,333,915	0.14
1890-1929	8 (2.7%)	8 (100%)	1 (12.5%)	30,635,605	0.33
1930-1944	2 (0.7%)	2 (100%)	0 (0.0%)	41,236,315	0.29
1945-1963	25 (8.4%)	19 (76.0%)	9 (35.7%)	51,944,397	0.71
1964-1984	36 (12.1%)	13 (36.1%)	26 (72.2%)	121,150,573	0.60
1985-1989	3 (1.0%)	1 (33.3%)	3 (100%)	146,917,459	0.52
1990-1993	1 (0.3%)	0 (0.0%)	1 (100%)	146,917,459	0.52
1994-2001	30 (10.1%)	10 (33.3%)	20 (66.7%)	169,590,693	0.63
2002-2009	69 (23.2%)	17 (24.6%)	42 (60.9%)	190,755,799	0.92
2010-2015	85 (28.5%)	31 (36.5%)	66 (77.6%)	204,450,649†	1.28
2016-2018	37 (12.4%)	9 (24.3%)	34 (91.9%)	209,240,607‡	1.42
Total	298	112	203		

Sources: E-mec, acessed on the 31st January 2018. * IBGE, population estimates, 1890, 1920, 1940, 1960, 1980, 1991, 2000, and 2010.† IBGE, population estimate, 2015. ‡ IBGE population estimate, 2018.

the variables "percentage of annual vacancies in medical programs located in the hinterland" and the "GDP" in Brazilian states, with a Spearman coefficient of 0.64 ($p<0.001$). There are positive and statistically significant correlations between these variables. Figure 1e considers the correlation between the variables "percentage of gratuitous vacancies in medical schools" and "GDP" in Brazilian states and the Federal District, with a Spearman coefficient of -0.54 ($p= 0.003$). Note that there is a negative and statistically significant correlation between the variables. There was no significant correlation between the percentage of gratuitous vacancies in the hinterland and the percentage of the population residing in the hinterland or the GDP of the states.

Table 2 presents the number and characteristics of the undergraduate medical programs created in each period of Brazilian history, correlated to the Brazilian population growth. On the percentage of gratuitousness, the table shows a single inflection point: after 1964, the proportion of gratuitous courses is significantly lower than in the previous period ($p<0.001$, Fisher's exact test). The same year marks the inflection point for the percentage of hinterland location: after 1964, the proportion of courses in the hinterland is significantly higher than in the previous period ($p<0.001$, Fisher's exact test). These percentages have not changed significantly since then. As for the quantitative aspect, the year that marks a significant change is 2002: between 1964 and 2001, the course/million inhabitants ratio remained stable between 0.52 and 0.63. After 2002, it increased gradually, between 0.92 and 1.42. The difference between the averages of the index in the two periods (1964-2001 *vs.* 2002-2018) is significant ($p=0.04$, *t*-test).

DISCUSSION

In the present study, we observed correlations between the distribution of vacancies in medical schools in Brazilian states and their populations and economic production. The data correlation does not allow us to infer cause and effect relationships, because the opening of a medical school in a municipality, especially in medium-sized ones, can attract people and business to the region just as much as the population growth and economic development of a municipality can create a need and the conditions necessary for the opening of a medical school. Both relationships can be true and complementary.

There are also correlations between the percentage of the population residing in the hinterland of each state, the state's GDP, and the percentage of vacancies in medical schools in the hinterland. In the same way, we can consider a possible two-way relationship of cause and effect: a more populous and productive hinterland allows the emergence of medical schools, which in turn enriches the social and economic life of cities in the hinterland.

We also found a significant negative correlation between the state GDP and the percentage of gratuitous vacancies in medical schools. This finding reinforces the link between the growth of a region and the emergence of private medical schools. Thus, we can deduce that the expansion of medical education in Brasil has occurred at a more accelerated pace where there is more economical and population growth and that the private initiative is the main force behind this expansion.

The analysis of historical data (Table 2) shows that the proportion of gratuitous medical schools located in the capitals was predominant until 1964. From then on, paid programs and located in the hinterland of states became more predominant. Since 1964, the proportion of gratuitous medical schools created in each period has varied between 24% and 36%, with no significant difference between any of the subsequent governments, and the hinterland location has varied between 62% and 91%. The trend toward the expansion of medical education by means of paid programs seems to have started even before that, from the end of the Getúlio Vargas administration[1], but it was certainly consolidated during the military period and so remained ever since. In quantitative terms, it should be noted that the medical school/million inhabitants ratio, which remained stable between 0.52 and 0.63 from 1964 until 2001 (number of medical schools followed the population growth), jumped to 0.92 in the period from 2002 to 2009 and increased progressively until 2018, reaching 1.42 medical schools per million inhabitants (number of medical schools growing faster than the population).

We cannot say that all medical education located in the hinterland is predominantly offered through paid schools because the percentage of gratuitous vacancies in the hinterland of the states (27.2%) is not significantly different from the national average (29.6%), nor from the percentage of gratuitous vacancies in the state capitals (32.9%). The correlation between the percentage of gratuitous vacancies in state hinterlands

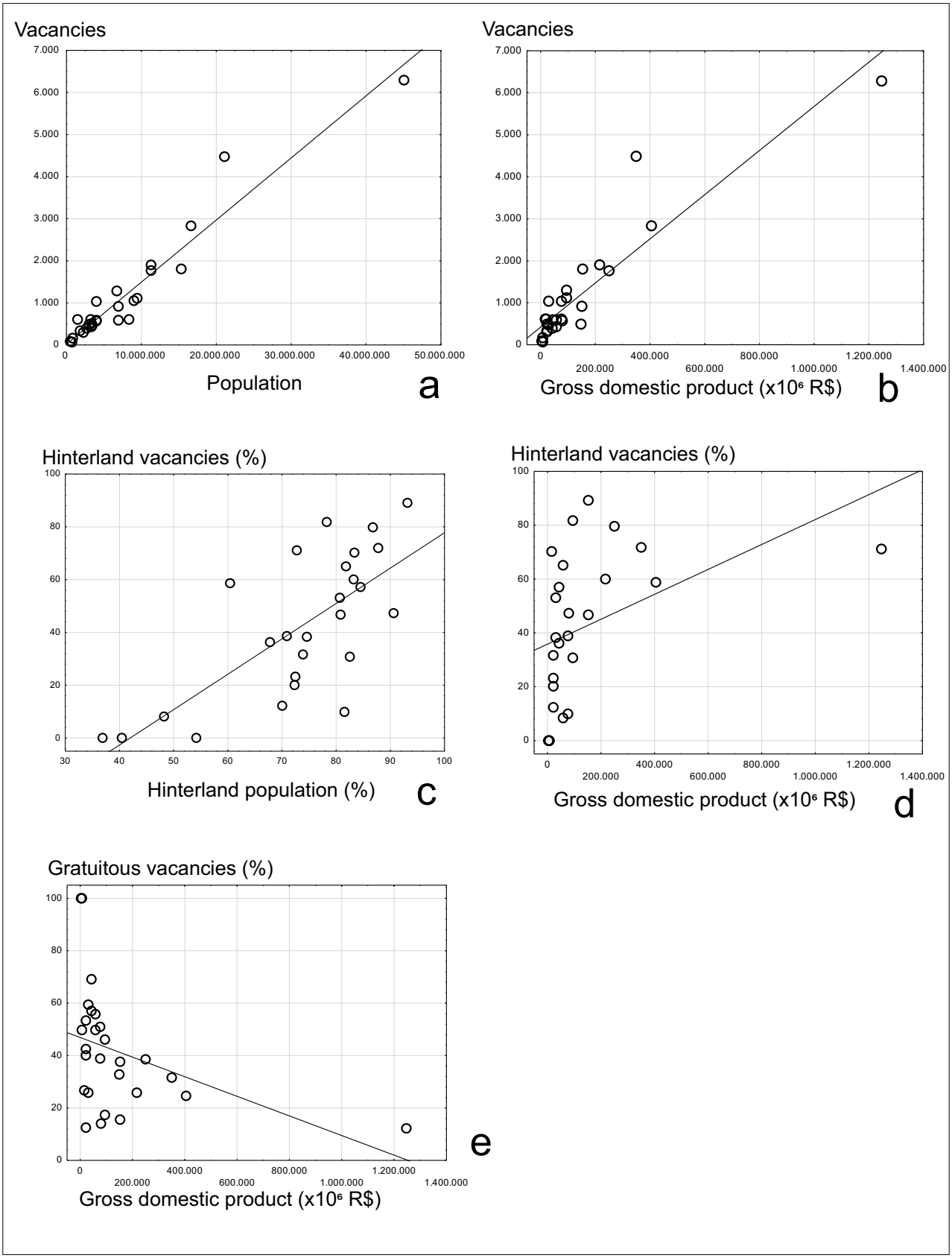

FIGURA 1. CORRELATIONS BETWEEN VACANCIES IN MEDICAL SCHOOLS AND POPULATION OR GROSS DOMESTIC PRODUCT IN BRAZILIAN STATES.

with the GDP or with the percentage of the population residing in the hinterland was not significant. What can be observed is the great variability between states in the distribution of gratuitous vacancies between the capital and the hinterland: some maintain a similar proportion between capital and hinterland (SP, 10.0% and 12.9%; CE, 37.7%, and 40.0%; MT, 53.0% and 57.1%); others have a greater proportion of gratuitous vacancies in the capitals (SC, 100% and 4.9%; RJ, 38.9% and 14.5%; RS, 66.7% and 31.4%); while others have a lower proportion in the capitals (BA, 22.9% and 54.1%; 37.7%, and 64.7% PA, 45.5%; and 100%). Three states have 100% of vacancies in the capitals gratuitous (SC, RR, and AP), and six states (PA, AM, AL, RN, MS, and SE) have 100% of their vacancies in the hinterland gratuitous. Therefore, in some states, the spread of medical schools in the hinterland occurred predominantly through paid schools, while in others, through gratuitous schools, whereas in most states, both cases occurred.

The scientific literature gives scant attention to the number and characteristics of medical schools worldwide and in Brasil. Particularly, there are no data on the number of vacancies in medical schools, only about the number of schools. For this reason, we can compare the data on the number of medical schools (and not vacancies) in Brasil with global data. It is important to highlight that this comparison has less reliability than that based on the number of vacancies: in some countries, medical schools offer a large number of annual vacancies (and, therefore, there is a small number of medical schools in the country), while in Brasil we have an average of around 100 annual vacancies per medical school.

In Brasil, there are currently 1.42 medical schools per million inhabitants. A study from 2014 found 0.20 medical schools per million inhabitants in the African continent; 0.81 in the Americas; 0.28 in Asia; 0.55 in Europe; and 0.81 in Oceania[7]. These data place Brasil above the global average and the average of the American continent in terms of the number of medical schools in relation to the population; we found that such accelerated growth took place from 2002.

In 2002, Eckhert[8] traced the number and distribution of medical schools using publicly available information from the yearbook of the World Health Organization (WHO): at that time, three countries had more than 100 medical schools: China (150), India (144), and the USA (144). In 2007, Boulet et al.[9] reported that over a third of medical schools were in four countries: India (219), the USA (147), China (130), and Brasil(84). In 2014, Duvivier et al. [7] identified the countries with the highest number of medical schools: India (304), Brasil(182), the USA (173), China (147), and Pakistan (86). From a perspective of the production of human resources, such concentration suggests that the distribution of medical schools is determined not only by the population need[7,9].

The qualitative characteristics of each school, such as their "free" or "paid" nature, could be more directly influenced by political factors[1,10,11]. Our study detected a single change in the proportion of "free" to "paid" programs over the analyzed historical periods, in which the proportion was reversed, with the predominance of paid schools after 1964. Since then, there has been no significant change in this pattern in any subsequent period. Regarding the investigation of the population access routes to medical education, this study has the limitation of not presenting data on public funding for access to paid programs. Since 2001, the Brazilian Ministry of Education offered the Student Financing Fund (FIES) program, which may indicate that, although the driving force behind the expansion of medical education from the second half of the 20th did not come from public investments, from the 21st century, public resources were invested in education differently (offering financial credit for low-income students to attend paid institutions)[12]. In addition, other factors may be listed as facilitators of access to higher education: when a school is established in hinterland cities, even "paid" ones, students can save on expenses regarding housing or moving to another city, all of which would be necessary in order to have access to a "gratuitous" institution in a more distant capital with a higher cost of living, for example.

Finally, it is worth emphasizing that our study did not explored the correlation between the distribution of medical schools and subsequent city of work of graduated doctors. The factors that influence this process are complex and include multiple variables of economic and social nature (local working conditions, regional conditions of diagnostic and therapeutic support, and the educational possibilities for the professionals and their relatives).

Despite the limitations, studies like this provide valuable data for a more thorough evaluation of the scenario of medical schools in the country and subsidize the development of strategies that promote a better distribution of physicians across the national territory.

CONCLUSION

This study showed that Brasil had, in January of 2018, 298 medical schools, totaling 31,126 annual vacancies, of which the minority were gratuitous (29.6% of the vacancies), and most were located in the hinterland of the country (57.7% of the vacancies). The distribution of vacancies across Brazilian states is directly correlated to the population and Gross Domestic Product of each state, and the proportion of vacancies in the hinterland of Brazilian states also correlates directly to the proportion of the population residing in the hinterland and the Gross Domestic Product of each state. However, the proportion of gratuitous vacancies in medical programs is inversely correlated to the Gross Domestic Product of each state. We also found that the expansion in number of Brazilian medical schools grew more than the population from 2002, predominantly due to paid schools located in the hinterland of states, a characteristic that has remained unchanged since 1964.

Author Contributions

Silva ACV: study design, data collection and analysis, drafting of the manuscript, and approval of the final version. Godoi DF: data interpretation, drafting and critical review of the manuscript, and approval of the final version. Neves FS: study design, data analysis and interpretation, drafting and critical review of the manuscript, and approval of the final version.

RESUMO

OBJETIVO: *Descrever a distribuição e evolução histórica das vagas em cursos de graduação em medicina no Brasil.*

MÉTODOS: *Estudo transversal analítico de dados secundários. No Ministério da Educação obtiveram-se dados dos cursos de medicina e no Instituto Brasileiro de Geografia e Estatística foram obtidos dados populacionais e econômicos dos estados.*

RESULTADOS: *Havia no Brasil, até janeiro de 2018, 298 cursos de medicina (1,42 curso/milhão de habitantes), totalizando 31.126 vagas anuais, com 9.217 vagas gratuitas (29,6%) e 17.963 vagas no interior do País (57,7%). Nos estados há correlações positivas e significativas (p<0,001) das variáveis: "vagas em medicina" e "população" (R 0,92); "vagas em medicina" e "produto interno bruto" ("PIB") (R 0,83); "percentual de vagas em medicina no interior" e "população no interior" (R 0,71) e "percentual de vagas em medicina no interior" e "PIB" (R 0,64). Há correlação negativa e significativa entre "percentual de vagas gratuitas" e "PIB" (R –0,54, p=0,003). Passaram a ser criados mais cursos pagos do que gratuitos e mais cursos no interior do que nas capitais a partir de 1964 (p <0,001), e a relação curso/milhão de habitantes aumentou a partir de 2002 (p<0,001).*

CONCLUSÕES: *A distribuição de vagas em cursos de medicina no Brasil correlaciona-se à população e à produção econômica de cada estado. A expansão do ensino médico brasileiro, acelerada além do crescimento populacional a partir de 2002, é baseada principalmente em cursos pagos no interior dos estados brasileiros, característica inalterada desde 1964.*

PALAVRAS-CHAVE: *Educação médica. Faculdades de medicina. Brasil. População. Produto Interno Bruto.*

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ORIGINAL ARTICLE

Is electrosurgery fulguration a better procedure for Bartholin's gland cyst?

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SUMMARY

OBJECTIVE: *To evaluate the effectiveness of electrosurgery fulguration as a treatment for Bartholin's gland cysts.*

DESIGN: *Retrospective study with a comparative control group performed on Hospital Brigadeiro and in the Disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo from February 2005 to March 2009. Patients: Patients with Bartholin's gland cyst were divided into three treatment groups: group 1 electrosurgery (n=169 cases); group 2 - gland excision with the conventional technique using a cold scalpel (n = 51 cases); group 3 - marsupialization (n=11 cases). We reviewed the clinical and surgical history, physical examination, description of the surgical technique, postoperative results (success and complications), and follow-up data.*

RESULTS: *There is no difference between groups in relation to intraoperative bleeding, hematoma, and complete healing in a single treatment session. However, electrosurgery shows the lower percentage of recurrences 18 (10,7%) compared to the Marsupialization technique (group 3, p=.031). Recurrences occurred in 18 (10,7%), 3 (5,9%), and 4 (36,4%) cases. After retreatment by the same technique, there was a complete cure rate of 90% (152/169) for group 1, and 98% (50/51) for group 2. The cost of group 1 was lower than that of other groups.*

CONCLUSION: *The fulguration with electrosurgery of the capsule of Bartholin's cyst is an effective method of treatment, andthe cost of this technique is lower than the conventional technique and marsupialization.*

KEYWORDS: *Bartholin's glands. Cysts/diagnosis/therapy. Electrosurgery.*

INTRODUCTION

The Bartholin's gland duct cyst (Bartholin cyst) is a relatively common condition, affecting about 2% of women during the reproductive ages[1-3]. The Bartholin's glands are two major vestibular glands, located deep in the perineum, between the bulb and the transverse muscle, with the function of making the vestibule lubrication for sexual intercourse, with drainage of secretions through ducts measuring about

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2 cm in length and which debouch at 4 and 8 hours in the vulvar vestibule, immediately lateral to the hymen[4-6]. The Bartholin cyst is a result of obstruction and cystic duct dilatation, resulting from some causes like local trauma, chronic inflammatory processes, even vaginal infections[4-8].

The therapeutic approach is varied, and frequent recurrences have been reported with the use of some non-excisional methods[2]. The therapeutic option should consider the desire and possibility to preserve the function of the gland. Patients should be advised to understand, evaluate treatment alternatives, and communicate their preferences. The clinical profile and recurrence are important parameters in the therapeutic option[9].

After recurrence or persistence, the classic, definitive treatment can be performed by excision of the cystic capsule, a technique that requires hospitalization and deep anesthesia (spinal or general) with high costs. This procedure may have some complications, such as massive bleeding intraoperatively, dyspareunia, slow healing, hematomas, infections of the suture, and recurrence[10,11]. For this reason, alternatives have been considered by some investigators[3,8].

Electrosurgery has proved applicable in various diseases of the lower genital tract as a method of excisional and destructive treatment. It has been used as first-line treatment of precursor lesions of cervical cancer, as well as in applications in the vagina and vulva, as a practical alternative, with low cost: outpatient, local anesthesia, low complication rates, and similar effectiveness[12-15]. Therefore, the aim of this study is to analyze the effectiveness of the treatment of the Bartholin cyst using a cystic capsule vaporization technique with a high-frequency electrosurgical unit compared to the classic excisional surgery and marsupialization.

METHODS

We performed a retrospective chart review of 169 women consecutively treated at the department of gynecology at the Hospital Brigadeiro for the treatment of Bartholin cyst. All procedures were performed in an outpatient clinic with local anesthesia, through drainage and cauterization capsuling using high-frequency electrosurgical unit (trademark Wavetronic 5000 – LLEP LOKTAL Master of Medical Electronics).

Patients had been treated in the period from February 2005 to March 2009. Inclusion criteria: patients with a diagnosis of Bartholin's gland cyst, unilateral or bilateral, where incision and drainage were performed, in addition to cauterization of the capsule, and who attended at least one follow-up for postoperative evaluation. Exclusion criteria: other types of cysts of the vulva and who did not attend any post-operative follow-up. We used the records of outpatient surgical procedures performed and analysis of medical records.

For the control group, we analyzed the medical records of 62 women who underwent Excision Conventional Cold Scalpel (n=51) or Marsupialization (n=11) for the treatment of Bartholin cyst, at the Disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia, Hospital das Clínicas, da Faculdade de Medicina da Universidade de São Paulo in the same period that the study group, and who attended at least one follow-up for postoperative evaluation. These procedures were performed in hospitalized patients, in the surgical center, with spinal anesthesia.

This study was approved by the Ethics Committee for Analysis of Research Projects of the Hospital das Clínicas on Jan 2010 (protocol nº 1306/09) and approved by the Research Ethics Committee of the University Hospital of USP on Dec 2011 (CEP-HU/USP: 1138/11), with the approval of the Informed Consent Form. Record– SISNEP CAAE (Nacional System of Research Ethics and Certified for Ethical Assessment): 1104.0.015.000-09.

Electrosurgical technique

The surgical technique usually consists in: infiltration anesthesia of the superficial mucosa of the vestibule with 2% lidocaine; linear incision of the mucosa and cystic capsule, 10 to 15 mm in length, using electrosurgical electrode needle, calibrated at the cutting function, power 45%; draining of the cystic content and internal cleaning of the capsule with saline and gauze; complementation of local anesthesia around the capsule (as an option, pudendal nerve block procedure can be performed); vaporization of cystic capsule using spherical electrode 3 to 5 mm in diameter calibrated in the function of vaporization at 50% power; hemostasis review, preferably using gel hemostatic ferric perchlorate 50%. In the presence of bilateral cysts, both sides were treated in the same session.

In cases without an inflammatory process prior to the procedure, one group received prophylactic antibiotics, and the other did not. Antibiotic therapy was given in cases of inflammation. In abscesses, generally, the procedure was performed at two steps: first

session only incision, drainage, and administration of a broad-spectrum antibiotic; in the second session, after 7 to 10 days, completion of capsular vaporization.

Postoperative follow-ups were scheduled usually after 7, 30, 90, and 180 days to ward off infections, evaluate healing, and possible relapse; annual controls were maintained to detect late relapses. Recurrence was defined as the occurrence of cyst or abscess in the region previously treated.

Gland excision, conventional cold scalpel technique

This procedure requires the excision of the Bartholin gland and surrounding tissue, with careful hemostasis and suturing. It should be performed only in the operating room to ensure appropriate anesthesia.

Marsupialization technique

This procedure consists of a vertical incision of the cystic mass in the vestibule and outside the hymenal ring. The incision should be about 1.5 to 3 cm and followed by suturing of the inner edge of the incision to the external mucosa.

STATISTICAL ANALYSIS

We analyzed the frequency of complete healing, intraoperative and postoperative complications, recurrence, and compared these data with the alternative surgical technique for the control group. The results were described using absolute frequencies and percentages for qualitative variables, average, standard deviation, and confidence intervals of 95% for quantitative variables (previously Kolmogoroy-Smimov test to adjust normal distribution). For the comparison groups, we employed the Chi-square or Fisher test, Yates continuity correction test, and Student's tests on qualitative and quantitative variables, respectively. We used a significance level of 0.05, and the software used was SPSS version 19.0.

RESULTS

Group 1

Fulguration of the cystic capsule with Electrosurgery: of the 169 patients who underwent Electrosurgery, 88 (52%) had involvement of the cyst on the right, 73 (43%) left, 8 (5%) the disease was bilateral. The mean age of patients was 36 ± 9,4 years (17-60 years). In relation to parity, 20 (14%) were nulliparous or had only one abortion, 36 (25%) had at least one cesarean birth and without vaginal delivery, and 88 (61%) at least one vaginal delivery. The mean diameter of the cysts was 3.1±1.5cm.

Intraoperative complications occurred in 3 (2%) cases, with severe bleeding that was controlled with hemostatic sutures, without hospitalization nor blood transfusion. The mean postoperative follow-up was 15±14,7 months. The time for complete healing was about 20-40 days.

Regarding the final result of the surgery, represented by complete healing, 87% (147/169) of patients had satisfactory healing, considered complete cure without late complications; 10,7% (18/169) had recurrence; and 2% (4/169) showed significant scarring complications (one case of endometriosis with vulvar pain, dyspareunia and associated vaginismus, which was reoperated and persists with pain; 1 case of persistent local pain, and reoperation performed excision of fibrous capsule, asymptomatic after surgery; 2 cases of scar retraction, but without significant symptoms, and without the need for surgical correction). After retreatment by the same technique, the cure rate was 90% (152/169).

The mean interval to recurrence was 9.3 months (2-40 months). Recurrence occurred in less than 6 months (2 and 5 months) in 3 cases (16.8%), over 6 months in 15 cases (83%; 5 cases occurred over 1 year).

Among the 18 cases of recurrence, 15 (83%) had a diagnosis of cyst and 3 (17%) of abscess, 15 (83%) had a history of Bartholin's gland pathology, 12 cases had previous drainage, 10 (56%) did not use and 4 (22%) used prophylactic antibiotics, in 4 (22%) the antibiotic regimen was therapeutic, with slightly higher index of postoperative infection (5/18, 28%). There was no statistical difference in the mean age and cyst diameter.

After recurrence, 4 patients underwent classical excision, and 8 underwent the same technique, 4 patients did not undergo a new procedure, and 2 were not reported. Of the 8 patients who underwent the same technique, 5 remained asymptomatic after an average of 6 months, 1 relapsed again and underwent classical excision, 2 patients did not return for control.

Group 2: Gland excision conventional cold scalpel

In the group that underwent excision of the cystic capsule with cold scapel (n=51), 20/47 (43%) had involvement of the gland on the right, 26 (55%) left, 1 (2%) case bilateral.

TABLE 1. ELECTROSURGERY FULGURATION OF THE CYST OF BARTHOLIN'S GLAND: A COMPARATIVE STUDY WITH CONVENTIONAL COLD KNIFE GLAND EXCISION AND MARSUPIALIZATION

Procedure study size	Age (years)	Affected side n (%) side	Duration of Bartholin pathology (month)	Previously treated or with recurrence for Bartholin pathology n/N (%)	No. Cysts/No. Abscesses (%)	Diameter (cm)	Anesthesia type/n
Electrosurgery N = 169	35.9±9.4 (17 - 60)	88(52.1%)R; 73(43.2%)L; 8(4.7%)Bil.	35.8±45.4 (1 - 252)	67/101 (66.3%)	163/6 (96.4%)	3.1±1.5 (1 - 10)	local 169
conventional cold knife gland excision N = 51	35.8±9.9 (18 - 61)	20/47(42.6%)R; 26/47(55.3%)L; 1/47(2.1%)Bil.	21.6±20.3 (1 - 84)	35/48 (72.9%)	47/4 (92.2%)	3.2±1.4 (2 - 10)	spinal 51
Marsupialization	30±9.3	4(36.4%)R;	31.8±31.8	10/11	8/3	3.6±2.1	
N = 11	(19 - 46)	6(54.5%)L; 1(9.1%)Bil.	(1 - 108)	(90.9%)	(72.7%)	(1,5 - 8)	spinal 11
p-value and CI95% Electrosurgery vs. conventional cold knife gland excision	.948 ζ [-2.90; 3.10]	.294ϕ	.002 ζ [5.32; 23.08]	.408ϕ [-.22; 0.09]	.365 Ψ [-.04; .12]	.672 ζ [-.57; .37]	
p-value and CI95% Electrosurgery vs Marsupialization	.046 ζ [.13; 11.67]	.551ϕ -	.774 ζ [-23.30; 31.50]	.184 Ψ [-.44; .04]	.012 Ψ [.01; .50]	.298 ζ [-1.45; .45]	

Age, Duration of Bartholin pathology and Diameter are presented as mean ± standard deviation (minimum and maximum) ϕ Chi Square test ζ Student test Ψ Yates's correction for continuity

TABLE 2. ELECTROSURGERY FULGURATION OF THE CYST OF BARTHOLIN'S GLAND: A COMPARATIVE STUDY WITH GLAND EXCISION CONVENTIONAL COLD KNIFE AND MARSUPIALIZATION

Procedure, study size	Intraoperative complication (bleeding) n/N (%)	Immediate postoperative complication (hematoma) n/N (%)	Follow-up after surgery (months)	Postoperative infection n/N (%)	Recurrences n/N (%)	Time at recurrence	Other complications n/N (%)
Electrosurgery N = 169	3/169 (1,8%)	0/169 (0%)	14.9±14.7 (1 - 54)	8/52 (15,4%)	18/169 (10,7%)	3/18 (16.7%) < 6m; 15/18 (83.3%) >6m	4/169 (2,4%)**
gland excision conventional cold knife N = 51	1/51 (2%)	3/51 (5,9%)	20.4±24.7 (1 - 84)	11/51 (21.6%)	3/51 (5,9%)	2/3 (66.7%)< 6m; 1/3 (33.3%)>6m	2/51 (3.9%)***
Marsupialization N = 11	0/11 (0%)	0/11 (0%)	24.2±26.3 (1 - 84)	1/11 (9.1%)	4/11 (36.4%)	1/4 (25%)< 6m; 3/4 (75%)>6m	0/11 (0%)
p-value and CI95% Electrosurgery vs. gland excision conventional cold knife	1.000 ή -	.012 ή [-.12; .00]	.136 ζ [-12.79; 1.78]	.419φ [-.21; .09]	.457 φ [-.03; .13]	.128 ή [-.99; .06]	.915 Ψ [-.07; .04]
p-value and CI95% Electrosurgery vs Marsupialization	1.000 ή -	1.000 ή -	.273 ζ [-27.15; 8.55]	.946 Ψ [-.13; .25]	.041 Ψ [-.55; -.09]	1.000 ή -	1.000 ή -

Follow-up after surgery is presented as mean ± standard deviation (minimum and maximum). *Patients only with prophylactic antibiotics. **Local endometriosis with severe pain (1), local pain (1), scar retraction extensive (2). ***Local pain (2). φ Chi-Square test ή Fisher Test ζ Student test ΨYates's correction for continuity

The mean age was 36 years-old (±10 years-old). In relation to parity, 21 (41%) of the women had a history of at least one vaginal delivery, but no description of possible episiotomy or perineal lacerations, 13 (26%) only had cesareans, 9 (18%) were nulliparous or had only had an abortion, and in 8 (15%) there was no description.

The mean time between the onset of symptoms and treatment was 22 months (1-84 months) in 41 women. There was a history of recurrence or

previous treatment for Bartholin's gland pathology in 35 women (73% of 48 reported cases), and a median of 3 (1-10) previous surgical drainage in 19 cases. The initial diagnosis was a cyst in 47 (92%) cases, and abscess in 4 (8%). The mean diameter of the cysts was 3.2 cm (2-10 cm) reported on 37 patients. Regarding intraoperative complications, 1 (2%) case of severe bleeding was reported. Complications in the immediate postoperative hematoma occurred in 3 (6%) cases, treated with local ice.

Patients were instructed to return for evaluation of healing in 7-14 days, and 30-60 days. After this period, they were discharged and returned to routine gynecological appointments between 6 months and annually. The mean follow-up was 20.4±24.7 months (1-84 months). Infection and/or dehiscence occurred in 11 (22%) cases; non-disabling local pain for 4 months in 2 (4%) cases. The time of complete healing was about 20-30 days. Complete healing was reported in 48 (94%) cases, and recurrence in 3 (6%) (2 cases occurred after 4 months of surgery, a new excision was performed, a new relapse occurred, marsupialization was performed, and the patient remains asymptomatic), and in 1 case relapse occurred after 2 years (drainage was performed, the deep cyst remains asymptomatic). There was a mean of 11 months (4-24 months) for recurrence. Considering the cases of retreatment by the same technique, the complete cure rate was 98% (50/51 cases). In cases of recurrence, the mean age was 35 years, the mean cyst diameter 2.8 cm, all had a history of previous treatment.

Group 3: Marsupialization

In patients who underwent Marsupialization (n=11), the mean age was 30±9,3 years-old. Six women (55%) had at least one vaginal delivery without episiotomy description, 2 (18%) had exclusively cesarean, 3 (27%) were nulliparous.

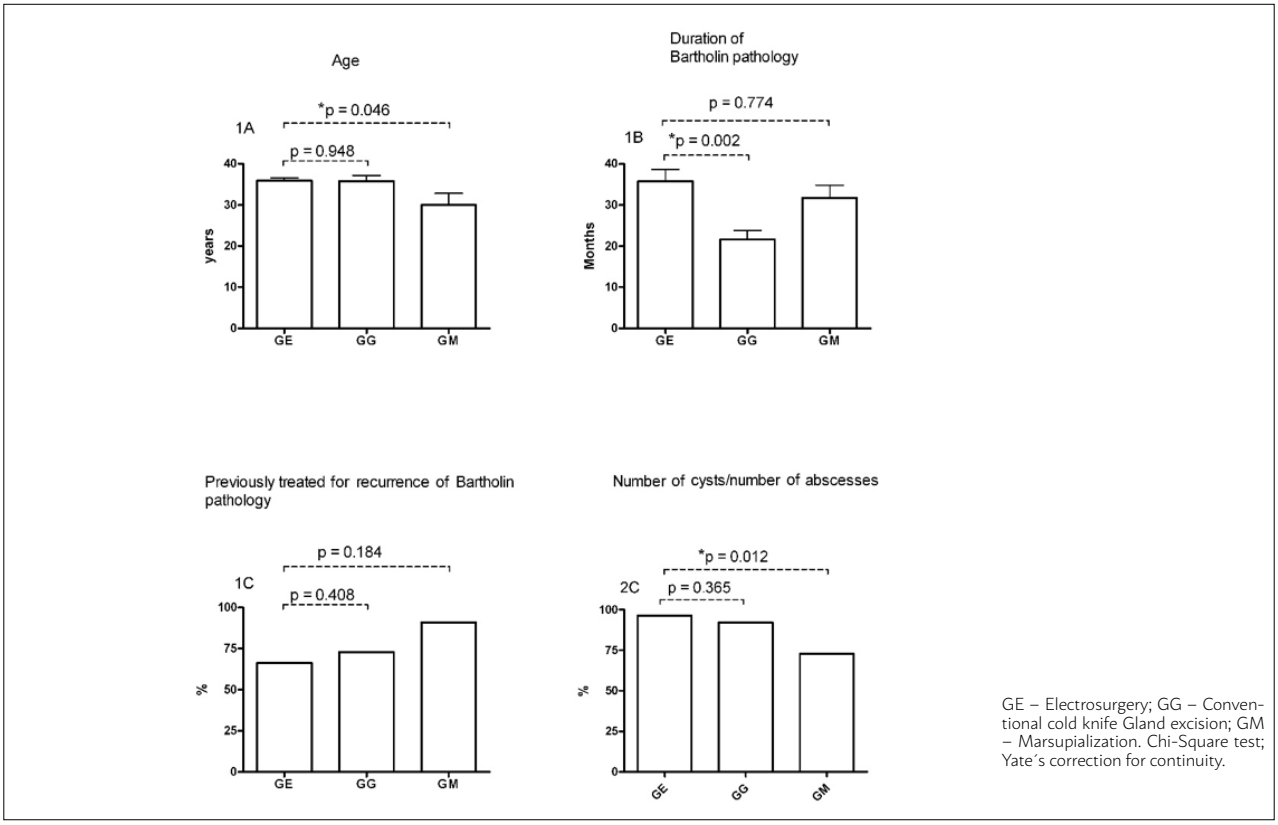
The mean time between the onset of symptoms and Marsupialization was 31,8 months (1-108 months). Ten patients (91%) had a history of Bartholin's disease, or prior treatment with an average of six episodes of recurrences (3-15) in 9 cases, and previous average drainage 3 (1-7). A cyst was diagnosed in 8 (73%) cases, and abscess in 3 (27%). Mean diameter cyst: 3.6±2.1 cm (1.5-8 cm) in 8 cases.

There were no intraoperative and immediate postoperative complications. The average follow-up time after treatment was 24±24,2 months (1-84 months). Only 1 (9%) postoperative infection with dehiscence of the surgical scar was described in a case previously uninfected. The average time of healing was about 15-30 days. Complete healing occurred in 7 (67%) cases, relapses in 4 (33%) cases, after 18 months on average (1 at 5 months, and 3 after 1-2 years). Two patients underwent the excisional method, and two remain with asymptomatic cysts. In cases of recurrence, the mean age was 28 years-old, the mean cyst diameter was 2.8 cm, and all had a history of previous treatment.

Comparison between groups

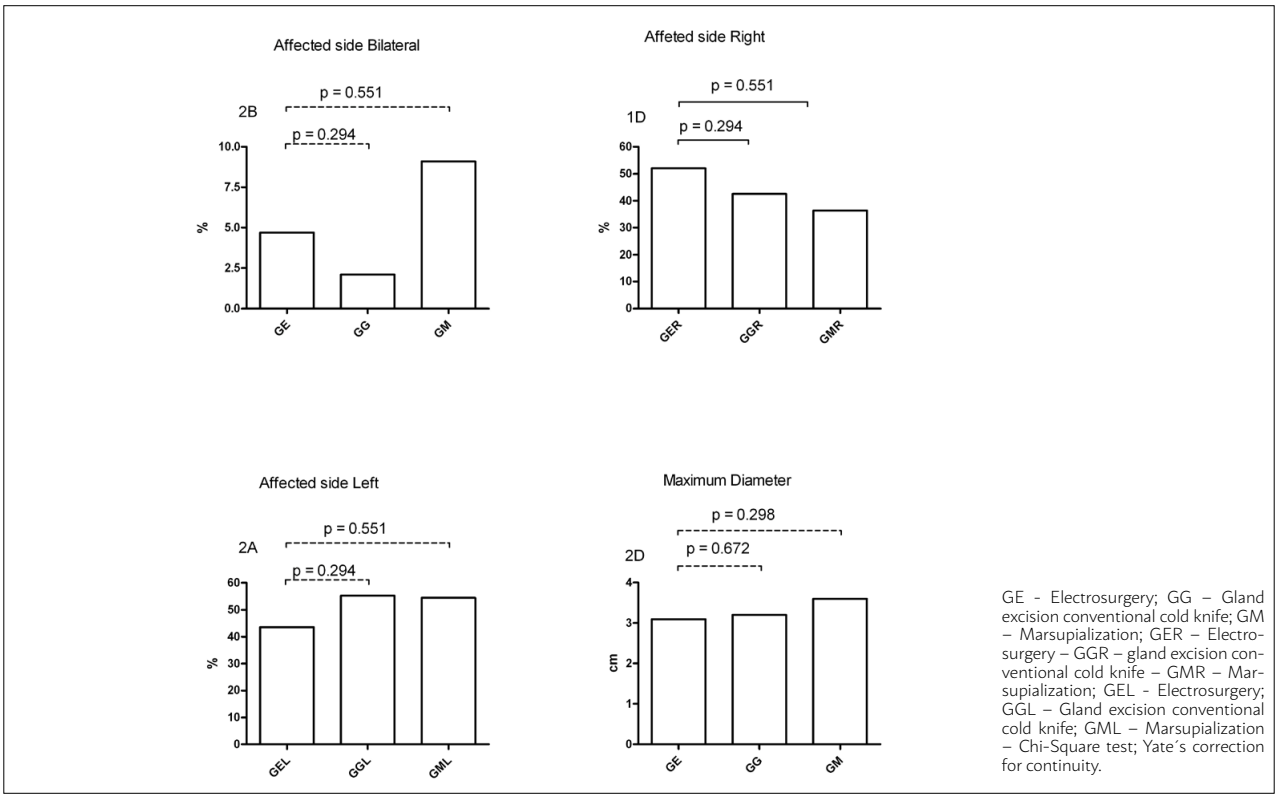
Figures 1, 2, and 3 summarize the comparative data between groups. There is a significant difference regarding age between groups 1 and 3 ($p = 0.046$), with younger patients in the Marsupialization group; groups 1 and 2 are homogeneous concerning this variable. The affected side was homogeneous in all groups ($p > 0.05$). The mean duration of the disease was different from the statistical point of view between groups 1 and 2, and it was greater in the first group ($p = 0.002$). The percentage of recurrences or pretreatment of Bartholin gland pathology was similar in all groups ($p > .05$), which translates homogeneity in the groups regarding this variable. We found a larger number of cysts in patients treated with Electrosurgery (96.4%) in comparison to those who were treated by the technique of Marsupialization (72.7%), $p = .012$. The size of the cysts was similar among the study groups, with no significant differences. The final results of Table 2 indicate that intraoperative complications were similar between the groups ($p > 0.05$). The immediate postoperative complications were lower in group 1 than in group 2 (significant hematoma in the immediate postoperative period occurred only in group 2, in 3 (6%) cases ($p = 0.012$), with no differences between groups 1 and 3, 0% in each group). All the patients had the same value for the average follow-up after surgery, with no statistical differences (15, 18, and 24 months for groups 1, 2, and 3, ($p > 0.05$). The percentage of postoperative infections was distributed equally between the study groups ($p > 0.05$). Complete healing in a single treatment session, no significant late complications, was obtained in 147 (87%) patients in group 1, 48 (94.1%) cases in group 2, and 7 (63.6%) patients in group 3 ($p = 0.159$, $p = 0.091$, respectively). The Electrosurgery technique presented the lowest percentage of recurrences compared to the Marsupialization technique ($p = .041$). The recurrence time, in terms of mean and standard deviation, was similar between the groups.

FIGURE 1. A – AGE (YEARS) - *P=0.046 – COMPARISON BETWEEN GE AND GM; B – DURATION OF BARTHOLIN PATHOLOGY (MONTHS) - *P=0.002 COMPARISON BETWEEN GE AND GG; C – PREVIOUSLY TREATED OR WITH RECURRENCE FOR BARTHOLIN PATHOLOGY (%); D – NUMBER OF CYSTS/NUMBER ABSCESSES (%) – *P=0.012 COMPARISON BETWEEN GE AND GM



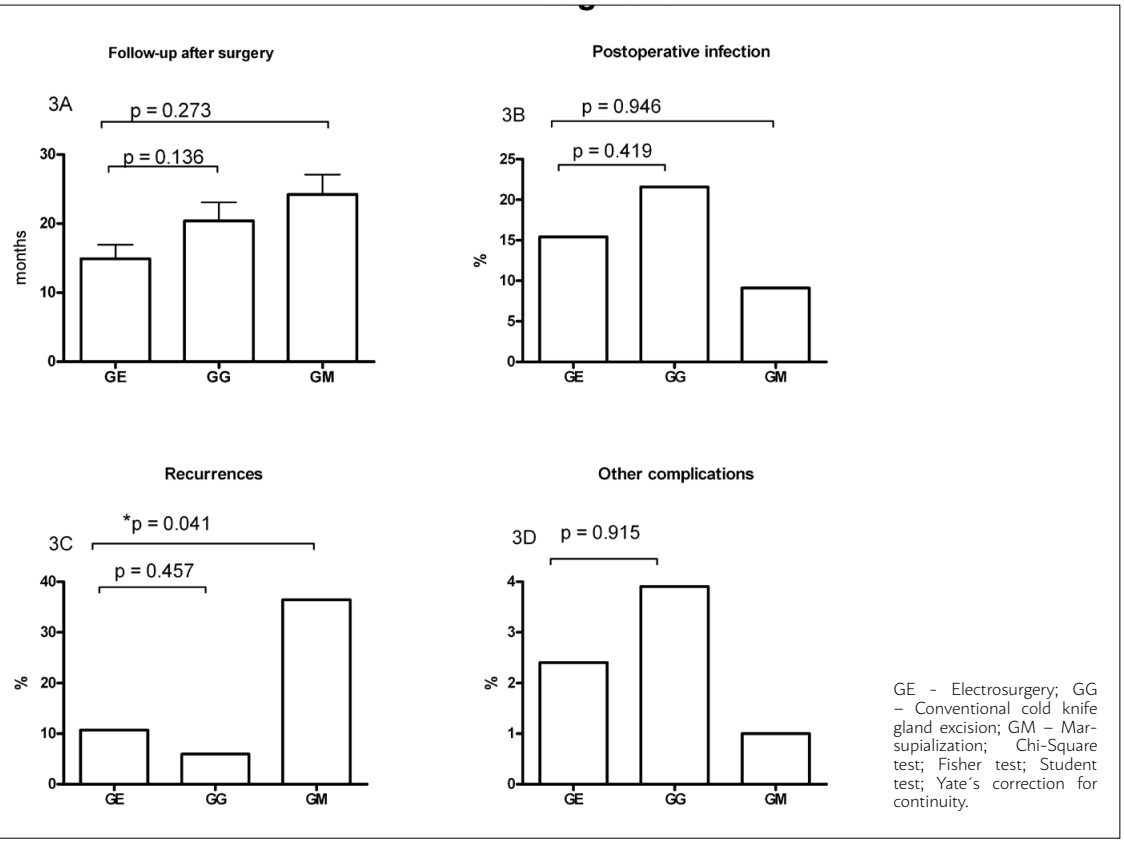
GE – Electrosurgery; GG – Conventional cold knife Gland excision; GM – Marsupialization. Chi-Square test; Yate´s correction for continuity.

FIGURE 2. A - AFFECTED SIDE BILATERAL OF SURGERY (%); B – AFFECTED SIDE RIGHT OF SURGERY (%); C – AFFECTED SIDE LEFT OF SURGERY (%); D – MAXIMUM DIAMETER OF SURGERY (CM);



GE - Electrosurgery; GG – Gland excision conventional cold knife; GM – Marsupialization; GER – Electrosurgery – GGR – gland excision conventional cold knife – GMR – Marsupialization; GEL - Electrosurgery; GGL – Gland excision conventional cold knife; GML – Marsupialization – Chi-Square test; Yate´s correction for continuity.

FIGURE 3. A – FOLLOW-UP AFTER SURGERY (MONTHS); B – POSTOPERATIVE INFECTION (%); C – RECURRENCES (%); D – OTHER COMPLICATIONS (%).



GE - Electrosurgery; GG – Conventional cold knife gland excision; GM – Marsupialization; Chi-Square test; Fisher test; Student test; Yate´s correction for continuity.

Finally, we studied other postoperative complications, finding no statistically significant differences between the different techniques.

DISCUSSION

The cysts of Bartholin's gland are common disorders, with important clinical implications because of the pain, dyspareunia, infection, and its recurrent character[16]. The long period between the onset of symptoms and access to definitive treatment stands out in these groups. Our data showed that the fulguration of the cystic capsule with electrosurgery might be effective for the treatment of cysts of Baratholin.

Our study showed that the Electrosurgical technique is innovative when considering its high efficiency associated with easy workability and cost, both compared to the conventional excisional, considered the gold standard in recurrent or persistent cases; it also presents less risk of intraoperative bleeding. This is particularly important considering the high demand for public services[17]. Marsupialization showed a higher percentage of recurrences compared to Electrosurgical technique and conventional excision. However, our study had some limitations concerning the surgical procedures, since they were performed by different medical staffs, and retrospective analysis[18].

Relapses in excisional and destructive techniques can be explained by the presence of other compartments, great extension of the capsule, deep location, fibrosis, difficult surgical access, probably as a consequence of repeated recurrences and infectious processes. The follow up of these patients should be long because of late recurrences, even over 3 years[1-6].

Comparative studies will be needed to assess the best initial approach technique in uninfected cysts and abscesses in order to decrease the time of disease progression and recurrence. The groups have the same baseline, which shows they were homogeneous at the beginning of the study. The Electrosurgery technique demonstrated that it was safe regarding the rate of intraoperative complications and immediate postoperative, infection, and other complications, it was also effective due to the low percentage of recurrence, in agreement with the contents of the scientific literature. The fulguration with Electrosurgery of the

capsule of the Bartholin cyst represents an effective method of treatment, with a better cost/benefit ratio when compared to conventional cold scapel gland excision and greater cure rate compared to Marsupialization. Recurrence can occur late in all methods, and a long follow-up period is advisable.

We conclude that, despite the limitations of this study, the electrosurgical technique can be recommended for the treatment of cysts of the Bartholin gland, as a great alternative to high-demand services.

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Competing interests

The authors declare they have no competing interests

Authors' contributions

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

CLB - was involved in data analysis and statistical analysis; in drafting the article or revising it critically for important intellectual content; and gave final approval to the version to be published.

RS - was involved in data analysis and statistical analysis; in drafting the article or revising it critically for important intellectual content; and gave final approval to the version to be published.

FPC - was involved in data analysis and statistical analysis; in drafting the article or revising it critically for important intellectual content; and gave final approval to the version to be published.

ECVA - was involved in data analysis and statistical analysis; in drafting the article or revising it critically for important intellectual content; and gave final approval to the version to be published.

JMSJ - made substantial contributions to the concept, study design, and definition of intellectual content; was deeply involved in drafting the article or revising it critically for important intellectual content; and gave final approval to the version to be published.

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

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RESUMO

OBJETIVO: *Avaliar a eficácia da fulguração da eletrocirurgia como tratamento para os cistos da glândula de Bartholin.*

MÉTODOS: *Estudo retrospectivo, grupo controle comparativo realizado no Hospital Brigadeiro e disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, de fevereiro de 2005 a março de 2009. Pacientes com cisto de glândula de Bartholin foram divididos em três grupos de tratamento: grupo 1 – eletrocirurgia (n = 169 casos); grupo 2 – excisão da glândula com técnica convencional utilizando bisturi frio (n = 51 casos); grupo 3 – marsupialização (n = 11 casos). Revisamos a história clínica e cirúrgica, o exame físico, a descrição da técnica cirúrgica, os resultados pós-operatórios (sucesso e complicações) e os dados de acompanhamento.*

RESULTADOS: *Não há diferença entre os grupos em relação ao sangramento intraoperatório, hematoma e cicatrização completa em uma única sessão de tratamento. No entanto, a eletrocirurgia mostrou o percentual mínimo de recidivas, 18 (10,7%), em relação à técnica de marsupialização (grupo 3, p = 0,031). Recorrências ocorreram em 18 (10,7%), três (5,9%) e quatro (36,4%) casos. Após o retratamento pela mesma técnica, houve taxa de cura completa: 90% (152/169) para o grupo 1 e 98% (50/51) para o grupo 2. O custo do grupo 1 foi menor do que os dos outros grupos.*

CONCLUSÃO: *A fulguração com eletrocirurgia da cápsula do cisto de Bartholin é um método efetivo de tratamento, mas o custo dessa técnica é menor do que a técnica de convenção e a marsupialização.*

PALAVRAS-CHAVE: *Glândulas vestibulares maiores. Cistos/diagnóstico/terapia. Eletrocirurgia.*

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ORIGINAL ARTICLE

NADPH Oxidase 5 upregulation is associated with lymphoma aggressiveness

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SUMMARY

OBJECTIVES: *Lymphomas are a heterogeneous set of malignant neoplasias of lymphoid B and NK/T mature and immature cells at various stages of differentiation. Genetic and molecular biology tools are used to appropriately classify the type and prognosis of the lymphomas, which have implications in therapeutic effectiveness. Among them, the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase (NOX5) enzymes have been explored. This study analyzed the expression of NADPH oxidase 5 in lymphoma tissue according to the degree of tumor aggressiveness.*

METHODS: *Slides from 64 patients with lymphoma who had paraffin-embedded tissue available were reviewed by two independent, experienced pathologists. They classified tumors according to the WHO classification (2017). NOX5 expression in tissues was assessed by immunohistochemical staining using a tissue microarray. The assay was interpreted using a scoring system of 0, 1, 2, and 3, for cytoplasmic staining of NOX5 corresponding to negative, weak, intermediate, and strong staining, respectively. We compared the expression of NOX5 in patients with aggressive versus non-aggressive lymphomas.*

RESULTS: *NOX5 expression was positive in 100% (27/27) of aggressive lymphomas and in 19% (7/37) of non-aggressive ones. The seven patients with positive expression of NOX5 presented intermediate staining (2); strong staining (3) was observed only in tissues of aggressive lymphomas, and negative and weak staining (0 and 1) were observed only in non-aggressive lymphomas.*

CONCLUSIONS: *Aggressive lymphomas overexpress NOX5 protein. The higher NOX5 expression in aggressive lymphomas can suggest an involvement of this enzyme on the acquisition of an aggressive phenotype in lymphoid neoplasia.*

KEYWORDS: *Lymphoma. NADPH Oxidase 5. Reactive oxygen species. Immunohistochemistry.*

INTRODUCTION

Lymphomas have a clinical behavior that ranges from the most indolent to the most aggressive human malignancies[1,2]. This heterogeneity is also observed in morphological, immunophenotypical, and genotypical aspects[1,3]. Clinical and biological markers of prognosis for both Hodgkin and non-Hodgkin's lymphomas have been sought in order to decide on the best appropriate treatment for each patient[4-8]. Specific markers

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could also improve the diagnosis and contribute to the development of therapeutic targets to be used in personalized therapy[9].

The NOX family is composed of seven members, NOX1-NOX5 and DUOX1/2, which are differentially expressed among tissues[10]. NOX are transmembrane proteins, which transport electrons across biological membranes in order to reduce molecular oxygen to superoxide anion or hydrogen peroxide.

Oxidative stress is characterized by a cellular redox imbalance and is involved in several steps related to the carcinogenic process due to increased reactive oxygen species (ROS) availability. The initiation step can be triggered by deoxyribonucleic acid (DNA) oxidation, leading to changes in the pattern of gene expression. In addition, ROS can alter cellular signaling pathways, leading to changes in cellular proliferation, apoptosis, angiogenesis, among others[11]. ROS, such as superoxide and hydrogen peroxide (H2O2), can be formed by xanthine-oxidase, cytochrome P-450, or mitochondrial electron transport chain, as a by-product, or directly by the NOX family of enzymes[12].

NOX overexpression promotes mutagenesis[13], chromosomal aberrations[14], proliferation[15], avoids mitotic control by inactivating tyrosine phosphatases[16], stimulates angiogenesis[17] and contributes to the acquisition of an invasive and metastatic phenotype[18]. Furthermore, increased expression of the NOX enzymes has been documented in a wide range of neoplasias, such as prostate tumors[19], and in tumor cell lines of various types[20].

NOX5 was the last member of the NOX family to be identified and compared with the other NOXs; little is known about its regulation and function in human physiology and diseases. NOX5 is highly expressed in testis, uterine smooth muscle, and in lymphocyte-rich areas of the spleen and lymph nodes, but several other cell types express this enzyme in a less expressive way[21]. The majority of cellular models show that NOX5 is present in intracellular membranes, but its presence in the cellular plasma membrane has already been reported[22]. Calcium is essential for the activity of NOX5 that contains two pairs of 4 EF-hands in its N-terminal region[23]. Antony et al.[24] showed by tissue microarray analysis that NOX5 is overexpressed in several human cancers when compared to their adjacent non-tumor tissues, such as breast, lung, prostate, brain, ovary, colon, malignant melanoma, and non-Hodgkin lymphoma.

Some studies have shown NOX5 as an important source of ROS in cancer cells and during the carcinogenic process. Li et al.[25] have shown that the activation of NOX5 after exposure to bile acid can cause DNA damage in Barrett's human adenocarcinoma cell line, FLO-123. Interestingly, the authors suggest that high levels of ROS derived from NOX5 could contribute to the progression of the disease from Barrett's esophagus to esophageal adenocarcinoma. In addition, the inactivation of NOX5 by RNA interference protects human primary fibroblasts from DNA damage induced by ionizing radiation, reinforcing the role of NOX5-derived ROS in genetic instability[26]. Another key point related to tumorigenesis is the disruption of physiological mechanisms related to proliferation, migration, and survival. Shigemura et al.[27] showed that NOX5 was upregulated in adult T-cell leukemia (TLA) compared to normal peripheral blood T cells. Since human T-cell leukemia virus type 1 (HTLV-1) infection is associated with human peripheral blood T-cell transformation and ATL development, the authors evaluated the expression of NOX5 in HTLV-1 transformed cell lines and observed a marked increase in their expression after infection, which was linked to increased cell growth, migration, survival, and tumorigenicity. ATL is a very aggressive form of leukemia/lymphoma. Very similar results were observed in prostate carcinoma cells in which shRNA-mediated silencing of NOX5 impaired cell proliferation and increased apoptosis of the cells studied[28].

The objective of this study was to analyze the expression of NOX5 in a greater sample of different types of lymphoma, and its association with the degree of tumor aggressiveness.

METHODS

Study design and setting

This was a retrospective study of specimens from patients with lymphomas diagnosed between January 1981 and December 2012 in Clementino Fraga Filho University Hospital, from the Federal University of Rio de Janeiro. All patients diagnosed with lymphoma of any type were eligible. Patients with available paraffin-embedded blocks were included.

Slides from the diagnosis period were reviewed by two independent and blinded pathologists who classified the lymphomas according to the criteria defined by the World Health Organization[1]. They were then divided into two groups: aggressive and non-aggressive.

The paraffin-embedded blocks were tested for NOX5 using the tissue microarray technique (TMA). A manual instrument (Beecher Instruments, Sun Prairie, WI, USA) was used to include at least two spots of the blocks, which were selected by the same pathologists during the slide review process. Paraffin sections were dehydrated and dewaxed according to standard procedures. Immunohistochemical processing was performed using the NOX5 antibody (rabbit polyclonal antibody raised, SANTA CRUZ BIOTECHNOLOGY, INC) in a 1:200 dilution, with overnight incubation. The secondary antibody used was EnVision®+Dual Link/Peroxidase (Dakocytomation®). Heat-induced antigen retrieval was then performed.

The assay was interpreted according to the criteria described by Antony et al.[24], which consists of a scoring system (0-3) for cytoplasmic staining corresponding to negative (0), weak positive (1), moderate positive (2), and strong positive (3) staining, respectively. Negative and weak staining were considered "negative", while intermediate and strong staining were considered positive.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 5.01, GraphPad Software Inc., San Diego, USA). All results were expressed as mean ± standard error of the mean (SEM). The histological quantification of intensity and proportion was analyzed using Fisher's exact test, and $p < 0.05$ was considered statistically significant.

RESULTS

Sixty-four patients were included, of which 27 (42,2%) had aggressive lymphomas [diffuse large B-cell lymphoma (18/27) 66,6% and Burkitt lymphoma (9/27) 33,4%] and 37 (57,8%) had non-aggressive lymphomas [follicular lymphoma (1/37) 2,7%, Hodgkin Lymphoma Mixed Cellularity (5/37) 13,5%, Hodgkin Lymphoma Nodular Sclerosis I (22/37) 59,5%, Hodgkin Lymphoma Nodular Sclerosis II (8/37) 21,6%, and Hodgkin Lymphoma Lymphocyte-Depletion (1/37) 2,7%].

The expression of NOX5 was positive in 27/27 (100%) tissues of aggressive lymphomas and 7/37 (19%) of non-aggressive lymphomas.

The positivity of Hodgkin's lymphoma was evaluated in the Reed-Sternberg cell and its variants. The inflammatory background was not considered for the evaluation. The cases of aggressive lymphomas were usually marked diffusely and, interestingly, all the cases were positive for NOX5 staining, but only 19% of non-aggressive scored as positive. (Figures 1 and 2)

The staining proportion was scored using a range from 0 to 3 for cytoplasmic staining. Negative and weak staining was considered negative, while intermediate and strong staining was considered positive. (Figure 3)

FIGURE 1

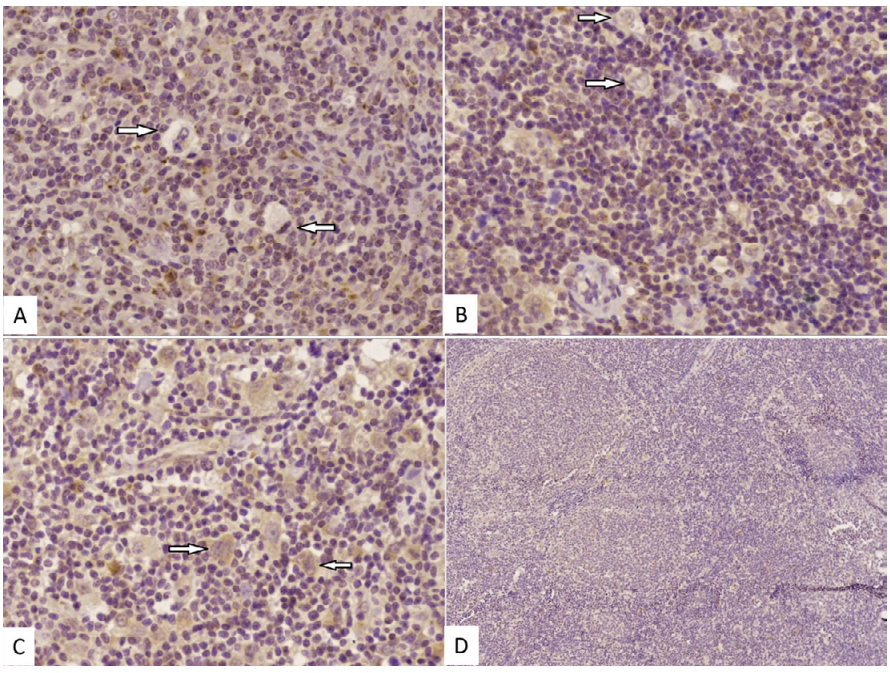
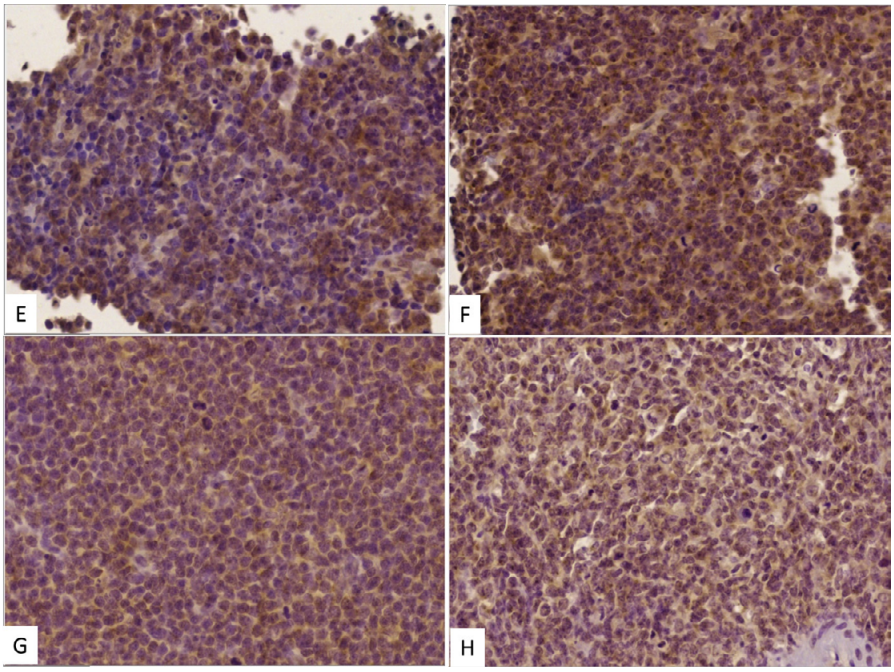


FIGURE 2

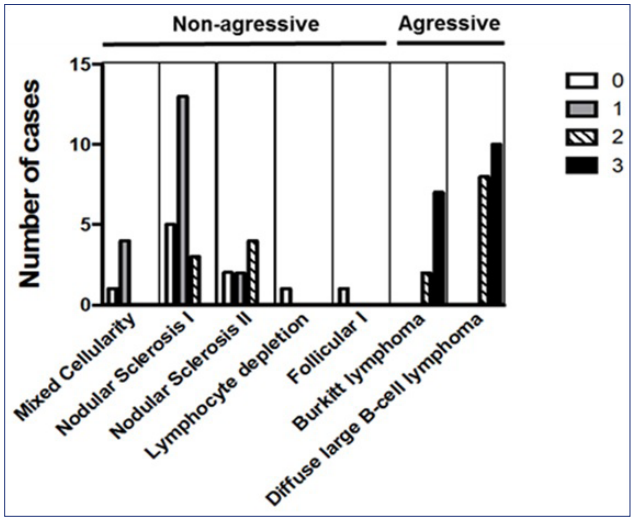


DISCUSSION

In this study, we have shown that strong staining (3) of NOX5 in Hodgkin's and non-Hodgkin's lymphomas can identify aggressive lymphomas and that the absence of staining or weak staining (1) can identify non-aggressive lymphomas.

Only a few articles have studied the expression of NOX in lymphomas. Antony et al.[24] showed for the first time, by tissue microarray analysis, that NOX5 is overexpressed in several human cancers when compared to their adjacent non-tumor tissues. Forty-three samples of non-Hodgkin Lymphomas were analyzed; 24 (56%) were negative (intermediate expression), while 19 (44%) were positive[24]. In this study, there was no distinction between the types of non-Hodgkin lymphomas. In our study, we separated cases that clinically presented as indolent lymphomas from another group with aggressive lymphomas.

FIGURE 3



Carnesecchi et al.[29] demonstrated that ROS derived from NOX5 are involved in blocking apoptosis of anaplastic large cell lymphoma cell lines positive for anaplastic lymphoma kinase (ALK) cells, the authors detected NOX5 mRNA only in ALK + ALCL cells, but not in any other Hodgkin's or non-Hodgkin's lymphoma. In our study, we found 17% and 97, 3% positivity in Hodgkin's and non-Hodgkin's lymphomas, respectively.

Burkitt's lymphoma, another aggressive lymphoma, has been little explored in the literature on NADPH oxidase derivatives. Klingerberg et al.[30] explored the therapeutic response of the NADPH oxidase 4 inhibitor imipramine-blue in the Burkitt cell line and observed decreased viability of cancer in vitro and in vivo in the cells. All 9 cases of Burkit's lymphoma present in our study showed NOX 5 positivity.

To the best of our knowledge, this is the first study that analyzes NOX5 expression in a set of different lymphoma types, according to their aggressiveness. Our data opens new perspectives that could be useful for the prognosis and future treatment of lymphomas.

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Conflicts of Interest

None

Ethics

The study was approved by the institutional research ethics review committee (CAAE 516504415.9.0000.5257).

Author's Contributions

João dos Santos Gonçalves: Selection of samples, morphological evaluation, immunohistochemistry, and text writing; Fabiano Lacerda Carvalho: Selection of samples, morphological evaluation, immunohistochemistry, and text writing; Igor Cabral do Rego Coutinho: Selection of samples, morphological evaluation, immunohistochemistry, and text writing; José Carlos Oliveira Morais: Morphological evaluation, immunohistochemistry, and text revision; Rodrigo S Fortunato: Selection of samples, morphological evaluation, immunohistochemistry, and text writing; Cristiane Bedran Milito: Selection of samples, morphological evaluation, immunohistochemistry, and text revision.

RESUMO

OBJETIVOS: *Os linfomas são um grupo heterogêneo de neoplasias malignas de células linfoides B e NK/T maduras e imaturas em vários estágios de diferenciação. Ferramentas de biologia molecular e genética são usadas para classificar adequadamente o tipo e o prognóstico dos linfomas, os quais têm implicações na eficácia terapêutica. Entre eles, as enzimas nicotinamida adenina dinucleótido fosfato oxidase (NADPH) oxidase (NOX5) foram exploradas. Este estudo analisou a expressão da NADPH oxidase 5 em linfomas de acordo com o grau de agressividade tumoral.*

MÉTODOS: *As lâminas de 64 pacientes com linfoma, que tinham tecido embebido em parafina disponível, foram revisadas por dois patologistas experientes independentemente. Eles utilizaram a classificação da OMS (2017). A expressão de NOX5 nos tecidos foi avaliada por coloração imuno-histoquímica utilizando microarray de tecido. O ensaio foi interpretado com um sistema de pontuação de 0, 1, 2 e 3, para coloração citoplasmática de NOX5 correspondente à coloração negativa, fraca, intermediária e forte, respectivamente. Comparamos a expressão de NOX5 em pacientes com linfomas agressivos versus não agressivos.*

RESULTADOS: *A expressão de NOX5 foi positiva em 100% (27/27) dos linfomas agressivos e em 19% (7/37) dos linfomas não agressivos. Os sete pacientes com expressão positiva de NOX5 apresentaram coloração intermediária (2); coloração forte (3) foi observada apenas em tecidos de linfomas agressivos, e negativos e fracos (0 e 1) observados apenas em linfomas não agressivos.*

CONCLUSÕES: *Linfomas agressivos superexpressam a proteína NOX5. A expressão aumentada de NOX5 em linfomas agressivos pode sugerir um envolvimento dessa enzima na aquisição de um fenótipo agressivo na neoplasia linfoide.*

PALAVRAS-CHAVE: *Linfoma. NADPH oxidase 5. Espécies reativas de oxigênio. Imuno-histoquímica.*

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ORIGINAL ARTICLE

Stiffness degree of ankle range of motion in diabetic patients with atypical amputation

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SUMMARY

In diabetics, foot deformities are risk factors that increase the risk of amputation as a result of developing ulcers. However, knowledge of the influence of plantar stiffness is still limited. The main objective was to describe connections between the degree of stiffness of the ankle, atypical amputation, and the Foot Posture Index (FPI).

METHODS: *62 diabetic patients, 58 with type 2 and 4 with type 1 (average age 63.35 years) were included. Records of foot deformities were included; A range of motion test of the ankle joint was used to determine the degree of stiffness. An exploratory analysis of the association of foot position and the degree of rigidity was performed.*

RESULTS: *The dorsal flexion range of the ankle was 9.6 ± 5.1 º, 13.8 ± 5.9 º and 17.2 ± 6.5 º and 20.5 ± 6.8 º to 45, 67, 89 and 111 N respectively in the amputated feet., And 14 patients (22.58%) had a high level of pronation of IPF with an average value of 3.7 ± 2.629, CI (3.032.-4.367) in amputated feet compared to non-amputees. We use the device "Iowa ankle range of motion" (IAROM) to determine the differences in ankle stiffness. Proper IPF was associated with the presence of amputation and an increase in stiffness*

CONCLUSIONS: *There was an increase in the degree of limitation of movement of the ankle, as a greater force was applied. Comparing FPI between the groups, there was a higher frequency of prone feet in the group of amputees*

KEYWORDS: *Diabetic foot. Risk factors. Amputation. Ankle joint/physiopathology.*

INTRODUCTION

The presence of minor amputations in patients with diabetes mellitus (DM) is a consequence of failed preventive measures to avoid this complication[1]. Plantar stiffness associated with amputation has consequences on feet posture, causing deformities that are more present in diabetic individuals[2]. The measurement of the FPI to determine the posture of the foot is a test that should be considered

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since feet in the prone position have more risk of ulceration[3].

Amputations can be classified as major (foot, leg or thigh) or minor (toe, metatarsals)[4].

Major amputations involve disability and mortality and generate expenses from your medical care[5,6]. It is important to consider that, in the USA, the costs generated by the amputation of diabetic feet range between 30,000 and 60,000 dollars.

Minor amputations also have repercussions, since even the loss of intermediaries toes (2nd, 3rd, 4th) causes functional and biomechanical problems[7]. For this reason, nowadays, to preserve the maximum bone length and obtain a better functional outcome, atypical amputations are performed, which by various strategies attempt to preserve the maximum tissue possible in the foot[8].

Plantar stiffness does not appear in diabetic patients with neuropathy, but in cases with ulcers. That is why early detection of rigidity could prevent the emergence of future complications[9].

Studies conducted to date to determine the classification of stiffness of movement at the ankle joint in patients with atypical distal amputation have focused mainly in the Goniometric measurement of the classification of joint movements that the patient is able to perform, leaving aside the degree of stiffness as a physical concept, as disregarding the FPI as a method for the clinical classification of the foot position, which can contribute as a determinant for predicted complication factors[10].

If we also consider that the comparison with the contralateral limb, race, when used, there are circumstances to justify our research, in which we present the results of a study conducted to determine the influence of ankle stiffness in patients with atypical distal amputation regarding the position of the foot[10,11].

This is an observational, descriptive, cross-sectional study on patients who underwent atypical distal amputation of one of their feet due to diabetes complications.

OBJECTIVE

Compare the classification of ankle joint movement of patients who underwent atypical distal amputation, with those that were not amputated to determine if there are any differences of rigidity and in FPI.

Assess changes in the pattern of foot posture in patients with atypical distal amputations.

METHODS

We present the results of an observational, descriptive, cross-sectional study on patients who underwent atypical distal amputation of one of their feet due to diabetes complications. This is based on 2 searches of the Angiology and Vascular Surgery Service at the University Hospital of Valdecilla Santander (Spain) and the Diabetic Foot Unit of the Endocrinology Service at the Hospital Athan Trias i Puyol, Catalonia (Spain), between January 2016 and October 2017. The measurements were recorded by a team, including a physician specialized in cardiovascular surgery, one specialized in plastic surgery, and a podiatrist, all with over 10 years of experience in the treatment of diabetic patients.

We requested approval from the Research Ethics Committee of the University of Extremadura, under Registration No. 84/2016. In addition, we respected the rights of patients pursuant to the ethical standards of the Declaration of Helsinki, and their personal data was protected by the organic law (15/1999).

For inclusion in the present study, patients had to be over 18 years and of both sexes, with DM type 1 or 2, the presence of neuropathy, and diabetic foot ulcer in advance, and with atypical unilateral forefoot amputation after at least one year of follow-up, which, at the time of measurement, showed ulceration in neither feet, without ischemia of the lower limbs, without the presence of limiting cognitive or functional impairment, such as of locomotion to participate in the study and had to agreed to participate in the study by means of informed consent.

Patients were excluded if they had Charcot foot or general signs related to systemic infection. Pregnant women or with suspicion of pregnancy were also excluded.

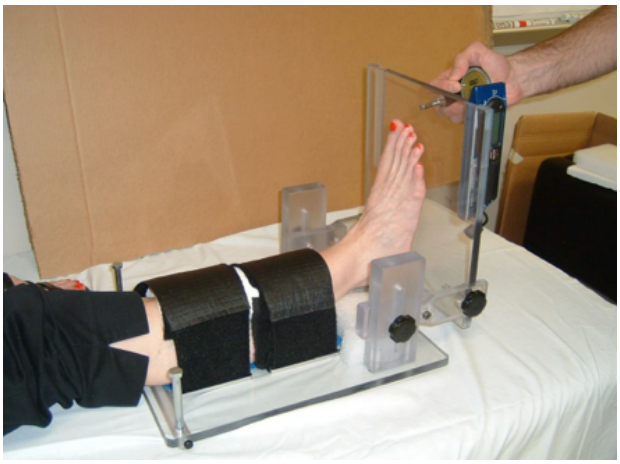
Sample characteristics

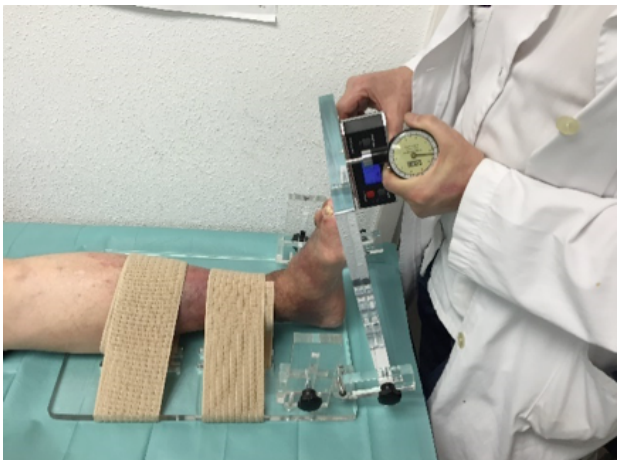
The sample included 62 subjects, of which 50 were men (80.6%) and 12 (19.4%) women. The average age of patients was 60.2 ± 8.5 years, with a height of 172.32 ± 7.9 cm, weight of 84.16 ± 9.95 kg, and BMI of 28.33 ± 3.7. The average feet figure was 42.32 ± 2.23 cm. The average glycated hemoglobin (HbA1C) value was 7.060±0.946 %

Evaluation of ankle stiffness

To measure the classification of ankle movement, we used the IAROM validated measuring system[12]. This device is mainly composed of two methacrylate

IMAGE 1. ON LEFT, THE IAROM DEVICE (12); ON THE RIGHT, THE ONE USED FOR THE PRESENT STUDY





panels bound by a mobile axis, which coincides with the ankle joint. The is placed in this device and secured with a band to make sure only pure movements of dorsal and plantar flexing are allowed by the ankle joint. (Image 1.)

To secure the foot, we used some velcro straps. We had the help of a digital magnetic inclinometer to measure the precise angle at the exact moment the prescribed force is applied.

To apply a known force and with good reproducibility, we had the help of a penetrometer (Wagner Force FDK Dial - 40). Thus, the force applied in all assessments was as accurate as possible[13].

All patients were placed in the supine position with the knee extended at the time of the assessment test for the classification of ankle movement.

We measured the articular classification of the movement of the ankle joint of the lower limb that underwent atypical distal amputation and the contralateral limb (without amputation).

The forces applied with the penetrometer were the following: 45, 67, 89, and 111N. Three measurements were performed for each applied force, recording them for statistical analysis.

TABLE 1. SOCIO DEMOGRAPHIC CHARACTERISTICS ACCORDING TO SEX

	Total (N=62) Men (n=50) Women (n=12) Mean ± SD (95% CI)
Age (years)	63.35.±8.495 (61.342-65.657)
Weight (kg)	84.16±12.229 (81.054-87.265)
Height (m)	172.32±7.981 (170.293-174.346)
BMI	28.33±3.704 (27.389-29.270)
HbA1C (%)	7.060±0.946 (6.797-7.334)

Abbreviations: SD: standard deviation; kg: kilogram; m: meters; BMI: Body Mass Index; Mean Glycosylated hemoglobin (HbA1C); IC95%: confidence interval at 95%; Statistical significance for a value of p<0.05, with a confidence interval of 95%.

Assessment of foot posture index

The measurement of the FPI was performed according to the criteria by Redmond et al.[14]. Patients were asked to stand, relaxed, and with bipodal support (with their arms relaxed on each side and looking forward).

RESULTS

The sample included 62 subjects, of which 50 were men (80.6%) and 12 (19.4%) women. In addition, we collected demographic data (age, sex, weight, size, and body mass index), and the FPI.

All variables were treated by the normality of distribution using the Kolmogorov-Smirnov test, and the data were considered normally distributed if $p > 0.05$.

The independent Student t-tests were calculated to determine if the differences were statistically significant when there was a normal distribution. (Table 1)

To determine the indexes of foot posture, we performed a descriptive analysis, expressed in the form of mean ± standard deviation. To determine the differences between the amputated foot and its contralateral, we used the parametric Student t-test for paired samples.

The value of global FPI for the amputated feet was 3.7 ± 2.6, while the non-amputated was 3.1 ± 3.4. The Student t-test for paired sample indicates the differences were not significant (p=0.221).

The joint classification of dorsal flexion of the ankle was 9.6 ± 5.1°, 13.8 ± 5.9° and 17.2 ± 6.5° and 20.5 ± 6.8° at 45, 67, 89, and 111N, respectively, in

TABLE 2. FPI CHARACTERISTICS AND ANKLE DEGREE OF FD IN UMPUTATED AND NON-AMPUTATED PATIENTS

Variable	Amputated (n=62) Mean ± SD (95% CI)	Non-Amputated (n=62) Mean ± SD (95% CI)	P-value/ Amputated vs Non-amputated (n=62)
FPI (Value 0-6)	3.7 ±2.629 (3.032. -4.367)	3.1±3,446 (2.224. -3.975)	0.221
Strength 45N Ankle FD degrees	9.689±5.183 (8.372. -11.005)	12.060±5.206 (1.737. -13.382)	0.001
Strength 67 N Ankle FD degrees	13,885±5,957 (12.372. -15.397)	16,941±5,846 (15.456. -18.425)	0,001
Strength 89 N Ankle FD degrees	17,206±6,512 (15.552. -18.859)	20,436±6,311 (18.333. -20.038)	0,001
Strength 111 N Ankle FD degrees	22.55±6,882 (18.802. -22.297)	23.688±6.431 (22.034. -25.301)	0.001

Abbreviations: SD, standard deviation; 95% CI, confidence interval at 95%; FPI (Foot posture Index). Statistical significance for a value of p<0.05, with a confidence interval of 95%.

TABLE 3. CORRELATION BETWEEN HBA1C AND THE DEGREES OF ANKLE DORSAL FLEXING

Variable Stiffness	Amputated (n=62) R HbA1c and ankle FD degrees	P-value (n=62)
Strength 45N	-0.240	0.439
Strength 67 N	-0.199	0.235
Strength 89 N	-0.219	0.100
Strength 111 N	-0.230	0.132

Abbreviations: HbA1C Mean Glycated Hemoglobin, Statistical significance for a value of p<0.05, with a confidence interval of 95%.

the amputated legs. The contralateral feet presented a classification of greater movement (± 3° approx.) in all the forces applied (p=0.001 in all cases)

Comparing the frequencies of FPO between the amputated and not amputated feet, we found a higher frequency of feet in the prone position in the group of amputees (p=0.001). (Table 2 and 3)

The statistical analysis was performed using SPSS v. 19.0 (SPSS, Chicago, IL)

DISCUSSION

Although demonstrated the first time in the hand[15], the limitation of mobility because of diabetes has been studied in multiple studies at the level of the foot and ankle[16-18].

The results show a position approximately one point more prone than in regular non-amputated individuals, but still within the parameters of a neutral foot (0 to 5)[19].

This finding is of great interest since feet in the prone position have an increased risk of ulceration, and, in our study, there is a higher frequency of feet in the prone position in the group of amputees[3].

The limitation of mobility in joints such as the tarsal was related to increased pressure in the metatarsal zone[20], which leads us to think that the stiffness may have significant consequences on joints such as the ankle, which, as we know, needs a classification of motion in joint from 12º of dorsal flexing during the support phase to 9° of plantar flexing at the start of the balancing phase to develop a physiological gait[21].

Rao et al.[18] compared ankle stiffness between patients with and without diabetes and demonstrated that diabetic patients had more stiffness in the ankle. Another study by the same author also concluded there was reduced mobility in the frontal plane of the calcaneal, which reduced flexibility in patients with diabetes mellitus compared with healthy patients[19]. In our sample, both feet behaved differently, since the classification of dorsal flexing movement of the ankle is up to 3 degrees higher in the non-amputated foot.

In our study, neither age, nor smoking, nor the BMI, nor Hb1Ac influenced the classification of ankle stiffness, in contrast to the non-enzymatic glycosylation phenomenon studied, which affects the body of diabetic patients and its relationship with the elasticity of tissues [22].

On the other hand, the results from Rao et al.[13] showed that people with diabetes mellitus had significantly lower dorsiflexion (between 5.1 and 11.5 degrees) and greater ankle stiffness (0,016 and 0,008 Nm/kg/degree) than non-diabetic individuals. In patients with diabetes mellitus, there was a positive relationship between glycemic control and the duration of diabetes mellitus and ankle stiffness, respectively; in our study, we did not find any correlation between these aspects.

CONCLUSIONS

Feet with atypical distal amputations feature classification of dorsal flexing movement in the ankle lower than the contralateral foot.

Amputated feet have a tendency of FPI in the prone position, as does the contralateral foot.

LIMITATIONS

In our study, all patients were white, which could be a factor to consider for further studies, since another study has found greater joint restriction in white diabetic patients than in African-Americans[23].

"All authors certify that we have participated substantially in the conception, design, analysis, and writing of this study, and we have approved the manuscript and agree with its submission to RAMB. This manuscript represents original, unpublished material and is not under consideration for publication elsewhere, nor has it been posted on the Internet for public access.

Contributorship statement: ENF in conceived and designed the study section and also in analyzed and interpreted the data may include ENF finally in draft-ead manuscript section must appears ENF.

RESUMO

Nos diabéticos, as enfermidades nos pés são fatores de risco, que aumentam o risco de sofrerem uma amputação, como resultado do desenvolvimento de úlceras. Contudo, o conhecimento sobre a influência da rigidez plantar ainda é limitado. O objetivo principal foi descrever conexões entre o grau de rigidez do tornozelo, a amputação atípica e o Foot Posture Index (FPI).

MÉTODOS: *62 diabéticos, 58 com tipo 2; e 4 com tipo 1 (idade média de 63.35 anos). Incluindo o registro de deformidades do pé; teste de classificação do movimento da articulação do tornozelo, para determinar o grau de rigidez. Realizou-se uma análise exploratória da associação da posição do pé com o grau de rigidez.*

RESULTADO: *A classificação de flexão dorsal do tornozelo foi de 9.6 ± 5.1 ^{0}, 13.8 ± 5.9 ^{0} e de 17.2 ± 6.5 ^{0} e 20.5 ± 6.8 ^{0} a 45, 67, 89 e 111 N respectivamente nos pés amputados, e 14 pacientes (22.58%) teve alto nível de pronação de FPI com um valor médio de 3.7 ±2.629, IC(3.032.-4.367) em pés amputados com relação aos não amputados. Utilizamos o dispositivo "Iowa ankle range of motion" (IAROM) para determinar as diferenças de rigidez do tornozelo. O FPI pronado foi associado à presença de amputação e um aumento da rigidez.*

CONCLUSÕES: *Aumento do grau de limitação do movimento do tornozelo; à medida que se aplicava uma força maior. Comparando FPI entre os grupos existentes maior frequência de pés pronados no grupo de amputados.*

PALAVRAS-CHAVE: *Pé diabético. Fatores de risco. Amputação. Articulação do tornozelo/fisiopatologia.*

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REVIEW ARTICLE

Impact of intermittent fasting on body weight in overweight and obese individuals

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SUMMARY

OBJECTIVE: *To verify the relationship of intermittent fasting in the bodyweight of overweight and obese individuals through a systematic literature review.*

METHODS: *This is a systematic review based on randomized controlled trials. The articles were consulted in the databases: Science Direct, PubMed e BVS. This review was evaluated through the PRISMA recommendation.*

RESULTS: *After the selection process, four articles were included in this review, comparing intermittent fasting (IF) with calorie restriction diet (CRD) as a control group. In 2 studies using similar protocols, there was no significant reduction in body weight of overweight or obese subjects. In the other two studies using different protocols, weight loss was significant in the IF group compared to the CRD group.*

CONCLUSIONS: *Results did not provide evidence of the effect of intermittent fasting on weight loss in overweight or obese individuals.*

KEYWORDS: *Fasting. Body Weight. Overweight. Obesity.*

INTRODUCTION

Intermittent fasting (IF) is a dietary practice in which periods of regular consumption of foods and beverages are interspersed with periods of severe energy restriction or by fasting, typically in 1 to 3 days per week. The objective of fasting is to reduce the total energy value, thus creating a negative energy balance, which results in weight loss.[1]

The excessive intake of energy is associated with the worldwide increase in the incidence of chronic diseases, including obesity, type II diabetes mellitus

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(DM type II), and metabolic syndrome. Caloric restriction by intermittent fasting increases longevity and reduces the incidence of chronic non-communicable diseases associated with aging, such as obesity, cardiovascular diseases, cancer, renal disease, and diabetes mellitus.[1,2]

Fasting is associated with substantial weight loss in short periods of time, around 8 to 12 weeks, accompanied by the control of dyslipidemia, arterial pressure, and changes in body composition.[3-5] In addition, increases in insulin sensitivity have also been demonstrated as impacts of intermittent fasting practice.[4,5]

As stated above, the dietary practice of using intermittent fasting has an impact on the bodyweight of overweight or obese individuals, as well as in reducing the risk of health problems. Therefore, the objective of this study was to verify the relationship of intermittent fasting on the bodyweight of overweight and obese individuals through a systematic review of the literature.

METHODS

We performed a systematic review of randomized clinical trials based on the recommendations of PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses).[6]

We used the PICO strategy to draw up the main question of the present study, which culminated with the delimitation of the following: Does intermittent fasting have any effect on bodyweight in overweight or obese individuals? Each PICO dimension was equivalent to the following elements: (P) overweight or obese individuals, (I) Intermittent Fasting, (C) calorie-restricted diet, and (O) changes in bodyweight of overweight or obese individuals.

The online search was performed in the PubMed, Science Direct, and Virtual Health Library (VHL) databases, from July to August of 2019, by two authors independently (C.H.R.L and I.K.F.). The connective "and" was used in combination with the Medical Subject Headings (MeSH terms): "Fasting". "Body Weight", "Overweight", and "Obesity". With their respective analogs in Portuguese and Spanish.

The search for articles in the different databases was performed with the following combinations of descriptors: SEARCH 1: Body composition AND Fasting OR fasting intermittent; SEARCH 2: Obesity OR Adiposity AND Overweight AND Fastin intermittent. The same search combinations were used in Spanish and Portuguese.

The clinical trials searched had a limits of publication date between 2015 and 2019, and had to have been published in English, Portuguese, or Spanish, involving overweight or obese individuals, without restriction of gender, ethnicity, and age over 18 years, available in its entirety, and including intermittent fasting as a dietary practice.

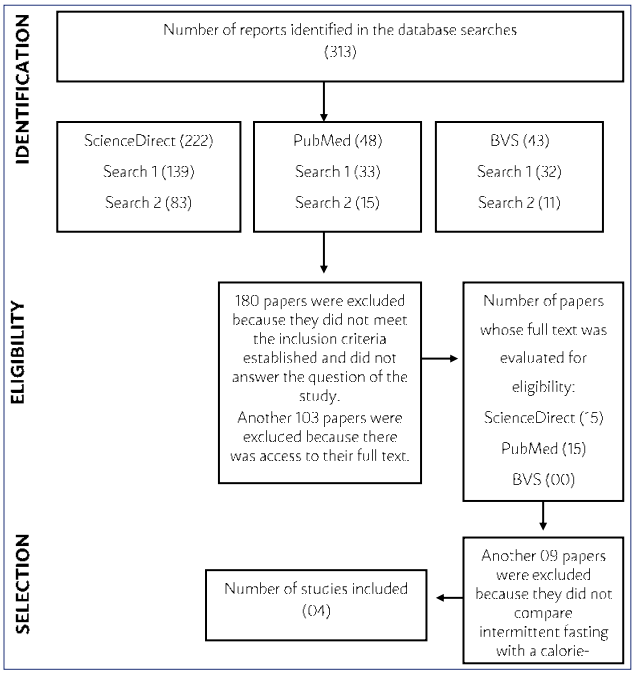
Were excluded studies that included pregnant women, heart disease, publications whose full text was inaccessible, chapters of books, manuals, congress publications, theses, dissertations, articles, reviews, articles outside of the databases, and *in vitro studies* involving animals. The details of the selection of articles are described in Figure 1.

After defining the inclusion and exclusion criteria, the articles were read and analyzed by two authors independently (C.H.R.L and I.K.F.), initially by reading the title, then the title and abstract, and finally, the full text. In the event of a discrepancy between the evaluators, a third researcher was consulted.

The data extracted from the studies included: author, year of publication, location, sample size, variables analyzed, interventions, and outcomes.

To ensure the methodological quality of this review, we used the Jadad scale,[7] employed by two authors independently for qualitative classification.

FIGURE 1. FLOWCHART OF THE IDENTIFICATION, ELIGIBILITY, AND SELECTION OF THE PAPERS INCLUDED IN THE SYSTEMATIC REVIEW.



Scores were assigned to the studies (0-5) based on the following criteria: method of randomization, use of blinding, and description of the proportion of losses during follow-up.

RESULTS

We found 313 articles in the databases searched: ScienceDirect (222), PubMed (48), and BVS (43). After the screening process, 04 articles were considered eligible. The articles included in the last stage of selection were a consensus among researchers. Figure 1 shows the characteristics of the articles included.

The studies were conducted in overweight or obese individuals of both sexes and different locations. All studies used intermittent fasting (IF) as the intervention in comparison with a calorie-restrictive diet (CRD) in the control group. As to the duration of the intervention using intermittent fasting, it ranged from 08 weeks to 04 months.

The papers met most of the criteria established by the tool for evaluation of the methodological quality of randomized clinical trials proposed by Jadad et al.[7] with a score of 3 in all 4 papers, which indicates that the studies had a reliable methodological quality reliable that could be extrapolated to other research scenarios (Table 1).

Table 2 shows the results of four studies that used randomized clinical trials to evaluate the effect of intermittent fasting on the bodyweight of overweight or obese individuals, using different times of fasting and intervention duration.

Trepanowski et al.[8] conducted a study in Chicago with obese individuals for 24 weeks to compare strategies between the IF and CRD groups. In this protocol, they used fasting for 24 hours on alternate days, in which period of fasting, in the IF group, energy consumption was only 25%, while in the CRD group, it was 75%.

The results of this intervention did not demonstrate significant differences in bodyweight loss, drawing attention to the figures of such reduction (-7.7kg ±1.0) in both groups after the intervention with fasting.

Corroborating the previous study, a survey carried out in the Colorado region, USA, by Catenacci et al.[9] also revealed no significant effect of fasting on the bodyweight of overweight or obese individuals in the IF group (-8.2 ± 0.9 kg), when compared to the CRD in this same population (-7.1kg ± 1.0).

The protocol used consisted in providing a diet with a calorie deficit of 400 kcal/day, based on the estimated energy needs of the participants included in the CRD group, whereas for the participants of the IF group the protocol was fasting with total calorie restriction for 24 hours on alternate days.

Two studies assessed the effect of intermittent fasting in overweight or obese individuals comparing IF groups with CRD groups, using different protocols. In both studies, the weight reduction was significant in the IF group in comparison to the CRD group.[10,11]

In the first study, the protocol consisted of a calorie restriction of 500 kcal/day based on the energy needs in the CRD and IF groups; participants in the IF group were instructed to fast for 18 hours/day for the 24 weeks of intervention. In addition, the participants of the IF group had two meals per day only, while the ones in the CRD group had their six meals regularly. The findings of this research have demonstrated a reduction in bodyweight of -4,0kg ± 1.0 in the IF group in comparison with -2,0kg ± 1.0 in the CRD group.[10]

In the second study, the protocol was carried out in participants of both sexes, according to the Buchinger method, which consists of fasting from solid foods for 7 consecutive days in the IF group and a balanced diet in the CRD group. In this protocol, the intervention lasted for 4 months. The outcomes of this research revealed a significant impact on weight reduction in the IF group (-3.5 ± 4.5) in comparison to the CRD group (-2.0 ± 4.8).[11]

DISCUSSION

The main difference between the calorie-restrictive diet protocols and the frequency of fasting involves schemes with restriction of calories without the need to eliminate one or more meals during the day. In contrast, intermittent fasting requires deprivation of food on alternate days during the week.[12]

The results of this revision corroborate other studies that evaluated the loss of bodyweight using intermittent fasting as a strategy. The authors showed that the loss varies from 2.5 to 9.9%, including the loss of associated body fat. These results are consequences of metabolic changes caused by fasting, which lead to an increase in lipolysis, proteolysis, and the depletion of glycogenolysis.[13]

According to Azevedo et al.,[14] the metabolic alterations are observed soon after the beginning of the fasting period. Plasma glucose levels fall and remain low during this period; lipolysis, ketogenesis, and gluconeogenesis increase while glycogenolysis decreases.

TABLE 1. PAPERS RELATED TO THE IMPACT OF VITAMIN D IN THE GLUCOSE PROFILE OF PRE-DIABETIC INDIVIDUALS: CHARACTERISTICS OF THE STUDIES INCLUDED IN THE SYSTEMATIC REVIEW.

Authors/Year of publication	Location of the Study	Partic.	Sex	Age (years)	Intervention		Variable	Outcomes
		CRD/IF			Fasting	Duration		
Trepanowski et al., 2017.	Chicago	34/35	M/F	18-65	24 hours	24 weeks	Bodyweight (kg)	No significant reduction in bodyweight was found.
Catenacci et al., 2016.	Colorado	14/15	M/F	18-55	24 hours	08 weeks	Bodyweight (kg)	No significant reduction in bodyweight was found in this study.
Kahleova et al., 2014	Czech Republic	27/27	M/F	30-70	18 hours	24 weeks	Bodyweight (kg)	Intermittent fasting has a significant effect on the loss in bodyweight.
Chenying et al., 2017	Berlin	23/23	M/F	25-75	168 hours	4 months	Bodyweight (kg)	The study revealed significant bodyweight loss using intermittent fasting.

M- Male. F - Female. Kg - kilogram.

TABLE 2. DISTRIBUTION OF STUDIES INCLUDED IN THE REVIEW ACCORDING TO THE METHODOLOGICAL QUALITY OF THE RANDOMIZED CLINICAL TRIALS, PER JADAD ET AL.

Items	E1	E2	E3	E4
Was the study described as randomized?	Yes	Yes	Yes	Yes
Was the study described as double-blind?	No	No	No	No
Was there a description of exclusions and losses?	Yes	Yes	Yes	Yes
Was the randomization method described and appropriate?	Yes	Yes	Yes	Yes
Was the double-blind method described and appropriate?	No	No	No	No
Points	3	3	3	3

E - Study. E1 - Trepanowski et al.[8]; E2 – Catenacci et al.[9]; E3 – Kahleova et al.[10]; E4 - Chenying et al.[11].

The increase in lipolysis and fat oxidation provides the substrate for gluconeogenesis and compensates for the decline in carbohydrate oxidation and glycogenolysis. This process also causes a moderate increase in proteolysis and protein oxidation.

Intermittent Fasting also negatively regulates the expression in muscle tissue of mTOR, the gene responsible for modulating nutritional signaling, which decreases protein synthesis and increases the expression of carnitine palmitoyltransferase I (CPT-1) in muscle tissue, as well as lipid oxidation.[14]

These metabolic processes lead to weight loss, as demonstrated by the results of two studies included in this review, but it is worth noting that these studies followed different protocols; the other two studies included had similar protocols showed no significant results.

Varady et al.[4] supports intermittent fasting as an efficient dietary practice that can culminate in health benefits. This conclusion is based on their study on 16 obese individuals submitted to eight weeks of intermittent fasting, consuming 25% of their basal energy needs. The fasting protocol promoted significant weight loss, reduced adipose tissue mass, blood pressure, and heart rate, improved the lipid profile, reduced the total cholesterol and LDL-c, and increased the levels of HDL cholesterol.

This metabolic regulation, according to Santos and Macedo[15], can increase the liver production of apolipoprotein A (apo A) and decrease apolipoprotein B (apo B). The production of apo A increases HDL-c since it is its precursor. The increased expression of PPARa is also responsible for the increase in serum HDL. The reduced production of apo B also promotes a decrease in serum levels of VLDL, LDL, and small and dense LDL (sdLDL).

The limitations of this review are related to the profile of the articles included, considering they used different protocols, different ages, and different genres. Another limitation is regarding the sample size of the studies, which were restricted.

The strengths are related to the information that although intermittent fasting is disseminated among the general population as an effective dietary practice for weight loss, randomized clinical trial studies comparing IF are not conclusive in demonstrating better effects for weight loss in relation to calorie-restrictive diets.

CONCLUSION

The results of this systematic review did not provide conclusive evidence of greater benefits from intermittent fasting on the bodyweight of overweight or obese individuals when compared with a diet of calorie restriction in different periods of intervention. Therefore, further interventional studies on intermittent fasting are needed with larger sample sizes and standardization of protocols.

Author's contributions

The authors Carlos Henrique Ribeiro Lima and Iara Katrynne Fonsêca Oliveira contributed to the writing of the text of the article; the other co-authors contributed to the evaluation and revision of the article.

RESUMO

OBJETIVO: *Verificar a relação do jejum intermitente no peso corporal de indivíduos com sobrepeso e obesidade por meio de uma revisão sistemática da literatura.*

MÉTODOS: *Trata-se de uma revisão sistemática, baseada em ensaios clínicos randomizados. Os artigos foram consultados nas bases de dados: Science Direct, PubMed e BVS. A avaliação dessa revisão ocorreu por meio da recomendação Prisma.*

RESULTADOS: *Após o processo de seleção, quatro artigos foram incluídos nesta revisão, comparando o jejum intermitente (JI) com dieta de restrição calórica (DRC) como grupo controle. Em dois estudos com utilização de protocolos semelhantes não houve redução significativa no peso corporal dos indivíduos com sobrepeso ou obesidade. Já nos outros dois estudos com utilização de protocolos distintos, a perda de peso foi significativa no grupo JI em comparação ao grupo DRC.*

CONCLUSÕES: *Os achados desta revisão não fornecem evidências plausíveis do efeito do jejum intermitente na perda de peso em indivíduos com sobrepeso ou obesidade.*

PALAVRAS-CHAVE: *Jejum. Peso corporal. Sobrepeso. Obesidade.*

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REVIEW ARTICLE

Serum ferritin levels is associated with acute myocardial infarction: a meta-analysis

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SUMMARY

BACKGROUND: *An association between increased serum ferritin levels and acute myocardial infarction (AMI) remains controversial. The purpose of this meta-analysis is to confirm the association between increased serum ferritin levels and AMI.*

METHODS: *We searched PubMed and China National Knowledge Infrastructure (CNKI) for relevant articles that assessed the association between serum ferritin and acute myocardial infarction using terms that included serum ferritin and acute myocardial infarction up to February 13, 2019. Results: A total of 11 studies were identified for analysis. All pooled analysis was based on a random-effects models. The variance was exhibited using a forest plot, and the heterogeneity among studies was examined using the I2 index, the publication bias was evaluated using a funnel plot. The pooled standard mean difference of ferritin levels between AMI and controls was 0.78 (95%CI,0.68-0.88).*

CONCLUSION: *The results of this meta-analysis demonstrate that serum ferritin in acute myocardial infarction patients is higher than that of healthy controls.*

KEYWORDS: *Myocardial infarction. Ferritins. Meta-analysis.*

INTRODUCTION

Acute myocardial infarction(AMI), a severely wretched status of heart attack, with the classic symptoms of sudden, severe, and persistent pain in the back of the chest (accompanied by pain radiating to the shoulder and sometimes the arm). AMI is currently a cause of mortality and morbidity with high economic costs worldwide[1]. The damage or death of cardiomyocytes is irreversible. Therefore, we must take medical measures to interfere with its progression. Its wide range of risk factors includes smoking, hypertension, obesity, and dyslipidemia, which have been established as useful predictors for acute myocardial infarction[2]. In addition to these classical risk factors, obesity, fatigue, insufficient sleep, are also risk factors for AMI[3]. Some studies demonstrate that serum ferritin could be an independent factor in

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predicting the risk of AMI. Serum ferritin is a kind of intracellular protein, which regulates the homeostasis of serum iron. Serum iron is essential for oxygen metabolism, especially in the chain that generates adenosine triphosphate through oxidative respiration in the mitochondria[4]. There was been no positive association between serum iron and the risk of cardiovascular diseases in recent studies[5,6]. The objective of this paper was to perform a meta-analysis on the association between serum ferritin and acute myocardial infarction.

METHODS

Search strategy and data sources

We searched PubMed and the China National Knowledge Infrastructure (CNKI) for relevant articles that assessed the association between serum ferritin and acute myocardial infarction up to February 13, 2019, using the following medical subject heading (MeSH) terms: "ferritin", and "acute myocardial infarction". In addition, we reviewed the reference lists of the original articles retrieved. There were no language restrictions in the search strategy. Two independent reviewers examined the abstracts of relevant articles and reviewed the full texts in detail. This meta-analysis was performed according to the PRISMA statement, which evaluated the methodological quality[7].

Study selection

The following were the inclusion criteria for eligible studies: (1) studies in humans; (2) observational studies, including case-control, cohort studies, and cross-sectional studies; (3) studies conducted to assess the association between ferritin and acute myocardial infarction. The exclusion criteria were: (1) study in animal models or animal experiments; (2) reviews or editorials; (3) case reports or case series; (4) study subjects without acute myocardial infarction.

Data extraction and Statistical analyses

In Table 1, we present the general characteristics, including the family name of the first author, year of publication, country, study design, source of the paper, the number of participants, and serum ferritin levels.

All statistical analyses were performed using the standardized mean difference (SMD) methodology in MetaXL. The SMD is the pooled difference between AMI groups and control groups on mean values across a group of studies using a different scale of measurement for the outcome. 95% confidence intervals (CI) were used to measure the precision of the summary estimates. The random-effects model was used to calculate pooled SMD in the presence of heterogeneity. The variance was exhibited using a forest plot. The overall heterogeneity among studies was examined using the I^2 index. The publication bias was evaluated using the funnel plot. If the value of I^2 was greater than 50%, and the value of p was less than 0.05, the meta-analysis was considered heterogeneous.

RESULTS

A total of 236 papers were retrieved from PubMed and the CNKI. A total of 199 results were eliminated

TABLE1. BASIC CHARACTERISTICS OF THE ARTICLES INCLUDED

Author	Year	country	Study design	Size	Experiment/Control		
					N	Mean (ug/l)	SD (ug/l)
Ramesh et al.[8]	2018			102	51/51	264.2/225.51	40.6/45.35
Yiping[9]	2014	China	Case-control	222	112/110	237.5/171.5 (g/l)	48.4/48.4 (g/l)
Shipra et al.[10]	2014	India	Case-control	100	50/50	268.43/110.96 (ng/ml)	30.17/56.59 (ng/ml)
Lqbal et al.[11]	2013	Pakistan	Case-control	408	203/205	158.7/87.1 (ng/ml)	136.7/74.7 (ng/ml)
Wadhwa et al.[12]	2013	India	Case-control	80	40/40	279.33/245.15 (mg/l)	46.69/56.94 (mg)
Holay et al.[13]	2012	India	Case-control	150	75/75	324.4/155.65	256.8/79.76
Wenxing et al.[14]	2005	China	Case-control	42	22/20	333.59/166.1	324.6/167.1
Silvia et al.[15]	2003	India	Case-control	145	100/45	257.35/155.42	76.34/76.1
Claeys et al.[16]	2002	Switzerland	Case-control	266	177/89	176/131	155/106
Klipstein-Grobusch et al.[17]	1999	Netherlands	Case-control	172	60/112	183/144	168/142
Moroz et al.[18]	1997	Israel	Case-control	26	20/6	113.5/110 (ng/ml)	58/58 (ng/ml)

after reading the abstracts. From the remaining papers, six reviews and two animal models were excluded, and 18 references failed to meet the criteria due to lack of data on standard deviation. Finally, 11 articles were included in the study (Figure 1). Four studies were conducted in India, one in China, one in Pakistan, one in Switzerland, one in the Netherlands, and one in Israel.

A total of 1469 participants were analyzed. In Figure 1, the pooled standard mean difference of ferritin levels between AMI and controls was 0.78 (95%CI, 0.68-0.88), p=0.00, I^2=92%. In Figure 2 and Figure 3, we present the funnel plot for serum ferritin and acute myocardial infarction. Each spot indicates one study with standard error representing the weight of the study and its odds risk. The dashed lines indicate 95% confidence interval lines, which reflect the symmetry of the pooled estimates.

FIGURE 1. FOREST PLOT FOR THE POOLED DIFFERENCE OF FERRITIN LEVELS

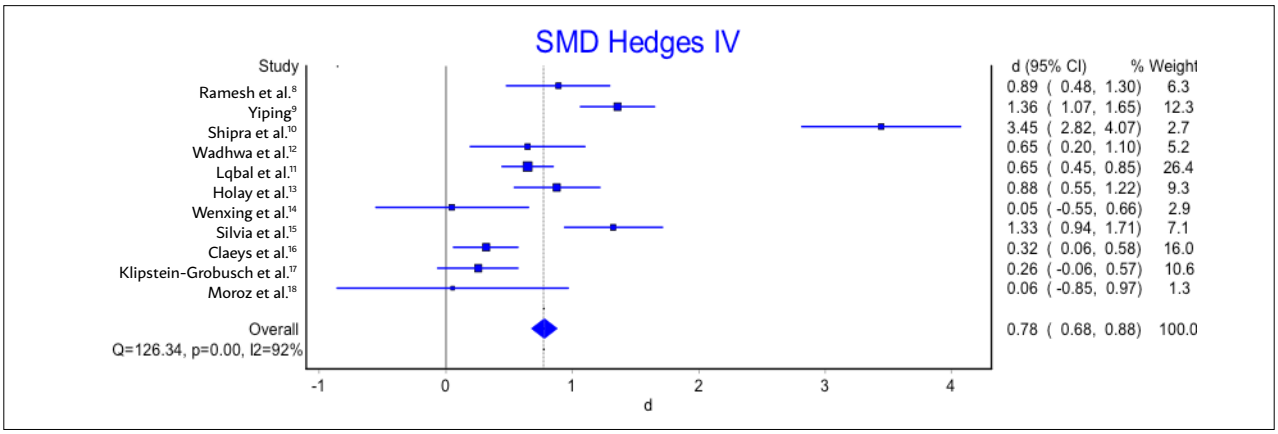


FIGURE 2. PUBLICATION BIAS EVALUATION FOR STUDIES INCLUDED

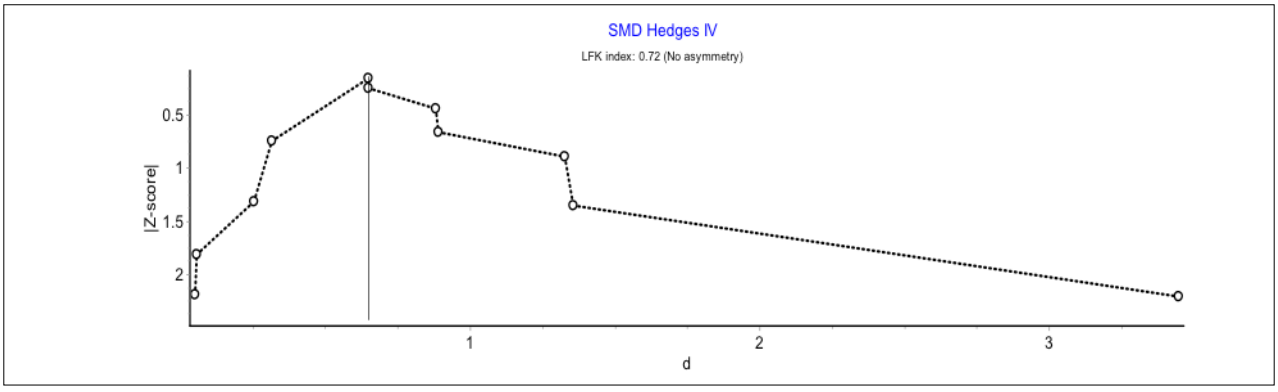
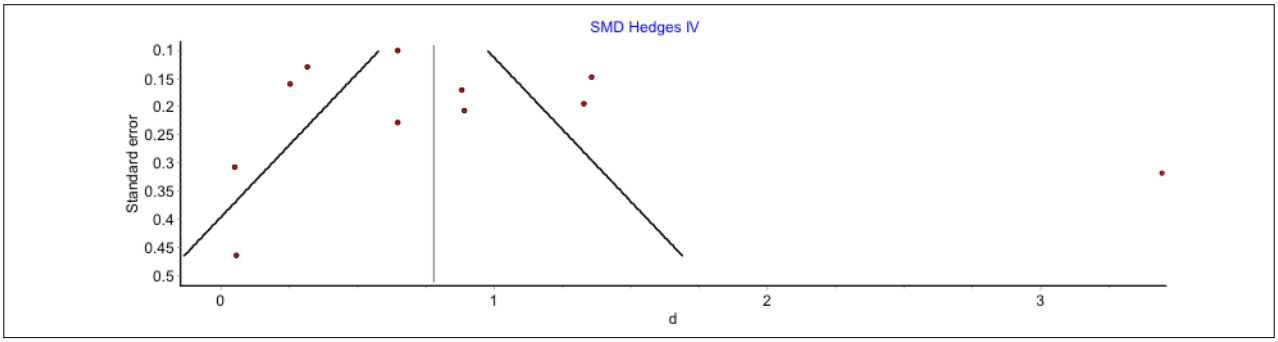


FIGURE 3. FUNNEL PLOT FOR FERRITIN AND ACUTE MYOCARDIAL INFARCTION



DISCUSSION

The possibility that high serum ferritin has a role in AMI was first postulated in 1985.[19] Later, few studies supported the association of high ferritin and acute myocardial infarction, including: (1) Elevated ferritin concentration as a marker of high levels of stored iron, which is a strong risk factor related to acute myocardial infarction. Men with high serum ferritin, not less than 200ug/compared with lower serum ferritin.[20] (2) A study reported an association between reduced stored serum iron through blood donation and the risk of myocardial infarction; however, a study did not support it.[21,22] (3)Increased serum levels of ferritin as an independent predictor of the occurrence of acute myocardial infarction, especially ST-elevation acute myocardial infarction.[23] Our paper is the first meta-analysis that provides few case-control studies on serum ferritin related to acute myocardial infarction and provides insight into its associated risks. In this paper, there is a significantly positive association between serum ferritin and acute myocardial infarction. There is a distinction of serum ferritin in the body between the premenstrual and menstrual stage for the same female participant. In general, the levels of serum ferritin are higher in men than women. The method used to measure the phase of acute myocardial infarction and the corresponding therapeutic measures were different; the followed levels of serum ferritin were not standardized. From all studies included, except for the study by Claeys et al.[16], the level of LDL-cholesterol in controls was greater than in the cases in the studies by Yiping[9] and lqbal et al.[11], respectively, the others were the opposite. In the study by Salonen et al.[21], the increased serum ferritin accelerated the oxidative metabolism of LDL-cholesterol and induced the process of atherosclerosis, indirectly.[24] Therefore, the levels of LDL-cholesterol found in all participants of the studies included were a potential factor, which played a role in increased heterogeneity. In the stage of health examination, high levels of serum ferritin, combined with other physical symptoms, could be an indicator of the risk of acute myocardial infarction.

Limitation

Subjects in our study are moderate; therefore, the limitation is inevitable. There are no follow-up values of serum ferritin from the participants in case groups to compare with the values in the phase of AMI. The sources of heterogeneity remain residual, even though the sensitivity analysis was conducted; thus, the heterogeneity was inevitable in the paper. Nine studies of the eleven included are from Asia; therefore, more studies are needed from other countries in Europe, America, and Africa. Therefore, further studies about the association of serum ferritin and acute myocardial infarction are required.

CONCLUSION

In sum, the results of this meta-analysis demonstrate that serum ferritin in acute myocardial infarction patients is higher than that of healthy controls.

Author contributions

SW and LPH designed and organized the research. LPH supervised the study. LY and CWL acquired, analyzed, and interpreted data. LPH performed statistical analysis. SW and LY wrote the manuscript. CWL revised the review. All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval to the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

RESUMO

Antecedentes: a correlação entre o aumento do nível de proteínas de ferro no soro e o infarto agudo do miocárdio (AMI) continua controversa. O objetivo desta análise é confirmar a relação entre o aumento dos níveis de proteínas de ferro no soro y o AMI. Metodologia: busca de artigos sobre Pubmed e a infraestrutura nacional de conhecimentos da China (cnki) para avaliar a relação entre a proteína de ferro no soro e o infarto agudo do miocárdio, incluída a proteína de ferro no soro e o infarto agudo de miocárdio, até 13 de fevereiro de 2019. Resultado: foram identificados 11 estudos para sua analise e todas as análises resumidas tiveram base em modelos de efeitos aleatórios. Foram utilizados mapas florestais para mostrar as margens, foi utilizado o índice 12 para examinar a heterogeneidade dos estudos e foram utilizados mapas de funil para avaliar os desvios publicados.
A diferença entre a norma de fusão dos níveis de proteína de ferro do Grupo ami e o Grupo de controle é de 0,78 (intervalo de confiança de 95% 0,68-0,88). Conclusão: nos resultados das análises da meta indicam que os pacientes com infarto agudo do miocárdio têm proteínas de ferro superiores às do Grupo de controle de saúde.

PALAVRAS CHAVE: *Infarto do miocárdio. Ferritinas. Metanálise.*

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COMMENTARY

Investigating gender differences for effectiveness and side effects of varenicline during smoking cessation treatment

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Pharmacological treatments for smoking cessation are effective and can increase quitting rates by 30%[1]. However, women do not benefit from it as much as men do[2,3]; for them, to quit smoking can be as difficult as for low-income populations or people with severe mental illness[4,5]. Both sex (biological) and gender (cultural) differences may be responsible for these discrepancies. For example, women metabolize nicotine more quickly, which causes more severe withdrawal symptoms and less response to nicotine replacement therapy[3]. Fear of stigmatization and custody issues, especially if smoking during pregnancy or exposing children to second or thirdhand smoking, can be important barriers when seeking help[6].

In addition, women have been excluded from clinical trials for many years due to the belief that biological differences or the need to care for their children would bias the results or prevent them from participating in studies[6]. Therefore, many treatment guidelines and public health policies have been developed based on studies that cannot be generalized to the female population[7]. Results from a recent meta-analysis have shown that varenicline is more effective in women and may help balance the differences in smoking cessation rate[8]. However, it is more expensive than bupropion and nicotine replacement therapy and is not currently available in the Brazilian public health care system[7,9].

Nonetheless, economic implications due to these gender differences cannot be ignored. Smoking-attributable hospitalizations for former smokers are 70% lower in comparison to those who did not quit smoking, and this difference is larger for women aged 35 to 54 years[10]. In addition, health problems can be extended to the fetus or newborn, and the harmful effects of nicotine use during pregnancy and breastfeeding have been well described in the literature[6].

Therefore, the work of Castellani et al.[11] makes some valuable contributions. First, it is in line with an international movement that encourages research on women and addiction for the reasons explained above[6]. Second, replicating findings of varenicline's effectiveness among women in a Brazilian clinical sample may support the inclusion of the medication in treatment programs. For low- and middle-income countries, this is especially important since the rational use of financial resources is crucial. However, public health policies that aim to encompass women's needs more effectively may assure equity and diminish the economic burden associated with tobacco use.

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COMMENTARY

Two criteria of oral glucose tolerance test to diagnose gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is the most common metabolic disease of pregnancy and has been associated with short and long-term adverse health outcomes for the mother and offspring. Its incidence depends on the population and the diagnostic criteria (2.4-37.7%) and its prevalence is significantly increasing, mostly due to the obesity epidemic[1]. Since appropriate glycemic control decreases the risk of GDM-related complications, early diagnosis and treatment are very important.

Since 1954 after the first use of the term meta-gestational diabetes by Hoet, guidelines for the diagnosis of GDM have changed many times, beginning with O'Sullivan & Mahan and followed by Carpenter & Coustan, the World Health Organization, American Diabetes Association, and lastly to that of International Association of Diabetes in Pregnancy Study Group (IADPSG). There is currently no consensus on the definition, screening, diagnosis, and management strategies regarding GDM. Today, there are more than 17 different guidelines for screening and diagnosing GDM by national and international diabetes organizations, health societies, endocrine groups, and obstetric associations[2]. A disagreement is present even between obstetric and diabetes organizations of the same country (e.g., the American Diabetes Association (ADA) and the American College of Gynecology and Obstetrics (ACOG))[3,4]. The diversity of recommendations results in different approaches, even within the same hospital. Since this lack of consensus creates major problems in addressing prevalence, complications, the efficacy of treatment, and follow-up of GDM, the need for consensus has been repeatedly expressed by the experts. There is a need for standardization so to have global uniformity in diagnosing GDM.

There are several retrospective and prospective studies to improve the efficiency of GDM diagnosis and find the real prevalence of GDM. However, most studies were performed in local populations only, thus cannot be considered as international, and include different screening strategies, which makes it impossible to reveal the true prevalence. In addition, it is very hard to follow and compare these various studies.

The study by Nunes et al.[5] in this issue approaches GDM screening through two different criteria. The authors compared the International Association of Diabetes and Pregnancy Study Groups (IADPSG)/American Diabetes Association (ADA) criteria with the Brazilian Federation of Gynecology and Obstetrics Association (FEBRASGO)/Brazilian Diabetes Society (SBD) criteria. The second criteria had 3 points elevated threshold values in the values of fasting and post glucose load in the second hour, which results in a lesser prevalence of GDM (12.5% vs. 5.8% respectively).

However, no statistically significant increase in adverse obstetric outcomes was found in the patients diagnosed with GDM through the first criteria, and not by the second. Thus, lowering the threshold increases prevalence, resulting in unnecessary interventions (diet and treatment).

Despite the almost six decades of research, multiple international conferences, and major collaborative trials, GDM remains a complex and very challenging obstetric and public health issue that certainly deserves to be further discussed and studied. Furthermore, the lack of consensus confuses health care providers of obstetric health who look to the experts for guidance. Therefore, a single acceptable evidence-based global guideline, which is simple, easy to follow, and validated by confirmative research, is essential.

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COMMENTARY

The relationship between tumor budding and survival in colorectal carcinomas

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Dear Editor

I have reviewed the article entitled '*The Relationship Between Tumor Budding and Survival in Colorectal Carcinomas*', by Peltek Ozer et al.[1] for the Revista da Associação Médica Brasileira The authors investigated tumor budding (TB) by its new definition and reported that TB was an independent prognostic factor in colorectal carcinoma, which was also associated with perineural invasion, lymph node metastasis, lymphovascular invasion, and survival. The association with TB and perineural invasion has been reported in only a few studies; thus, Peltek Ozer et al.'s[1] study made a significant contribution to the literature.

Tumor budding has been considered a prognostic factor in many cancers recently. It was first described in the 1990s, followed by great interest from researchers. In 2016, The International Tumor Budding Consensus Conference[2] defined tumor budding as a single tumor cell or a cell cluster of up to 4 cells. Tumor budding may reflect the aggressive character of tumors. In a recent study, the authors reported that the cell proliferation index was higher in samples with high-intensity tumor budding than in samples with low-intensity or no tumor budding in subjects with oral squamous cell carcinoma[3].

Numerous studies have shown that tumor budding is an independent prognostic factor associated with lymph node metastasis, local recurrence, and survival. Zhu et al.[4] reported that TB was significantly associated with poor survival and lymph node metastasis in subjects with head and neck squamous cell carcinomas. In another study, TB was associated with prognosis in patients with oral squamous cell cancer[5]. Recently, another study reported TB as a risk factor for lymph node metastasis[6]. Accordingly, the guidelines of the European Society for Medical Oncology now include tumor budding as a criterion for identifying high-risk patient groups.

In conclusion, TB should be considered an independent prognostic factor in colorectal carcinomas, and it should be included in the pathology reports along with lymphovascular and perineural invasion.

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COMMENTARY

Impact of intermittent fasting on the body weight of overweight and obese individuals

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The efficacy of intermittent fasting (IF) on weight loss and metabolic parameters compared to continued caloric restriction is controversial. Although it has gained many followers among patients, literature is still conflicting due to some unclear aspects. First, there are different definitions of what is considered IF. Most studies started observing the effects of religious fastings, such as Ramadan. Later, other protocols emerged mostly investigating the 5:2 diet, characterized by two days a week considered as "fasting" days, and alternate-day energy restriction, alternating between days considered as "fast" or "feed".[1] Other protocols with time-restricted eating, typically adopting 16h fasting and 8h eating, has also being applied with varied results.[2] Due to the different approaches regarding IF, it is difficult to compare studies and discuss possible benefits.

Secondly, still considering methodological aspects, studies on IF usually include a small number of subjects, have short-term interventions, and often lack an appropriate control group, such as continued caloric restriction. This is evident in the systematic review by Lima et al.[3], which, after a criterion approach, resulted in the inclusion of four studies. In overweight or obese subjects, most interventions that somehow promote caloric restriction or increased expenditure tend to result in short-term weight loss. Adherence to IF for longer periods should be evaluated, especially when transposing clinical studies to a free-living setting. Nevertheless, the adoption of IF has shown some benefits on weight loss and control of some metabolic parameters, such as glycemic control and parameters related to cardiovascular diseases.[4,5]

Finally, an important aspect that should be mentioned when considering IF is related to practical issues. Obesity is a multifactorial disease that is not caused solely by a misbalance between calories consumed and spent; it evolves hormonal and behavioral mechanisms. Individuals do not eat only to suppress physiological hunger. There are important cultural, social, and economic aspects influencing food choices and eating behavior. There is no clear evidence that adopting IF could be a trigger for eating disorders but the presence of disordered eating behaviors should be considered by health professionals when deciding whether or not IF could be an option for weight loss strategy.[6]

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Erratum

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Regarding the article "Application value of magnetic resonance hydrography of the inner ear in cochlear implantation" with DOI number: http://dx.doi.org/10.1590/1806-9282.66.1.74, published in Journal of the Brazilian Medical Association, 2020;66(01), page 74, authors name order, affiliation and Corresponding Author info in the article changed from:

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Regarding the article "Esophageal manometry in systemic sclerosis: findings and association with clinical manifestations" with DOI number: https://doi.org/10.1590/1806-9282.66.1.48 , published in Journal of the Brazilian Medical Association, 2020;66(01), page 48, figure 1 changed from:

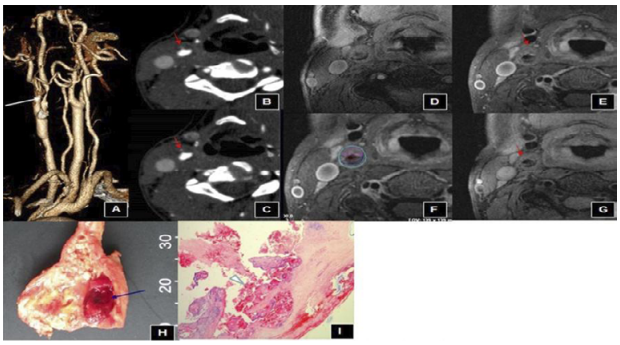

FIGURE 1. NORMAL CONVENTIONAL ESOPHAGEAL ELECTROMANOMETRY. PERISTALTIC CONTRACTIONS IN THE ESOPHAGEAL BODY (GREEN ARROWS), NORMOTONIC LOWER ESOPHAGEAL SPHINCTER WITH ADEQUATE RELAXATION DURING SWALLOWING (RED ARROWS)

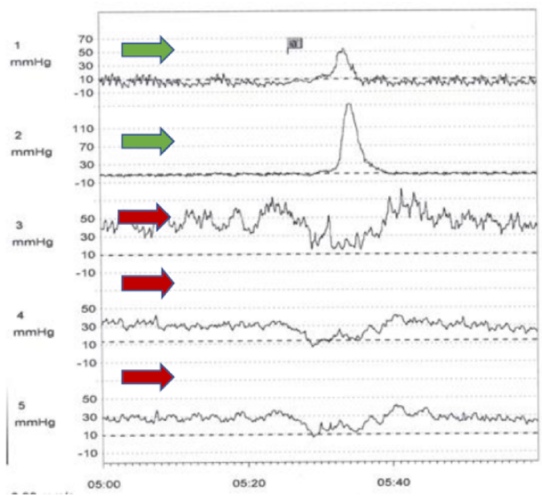

FIGURE 1- NORMAL CONVENTIONAL ESOPHAGEAL ELECTROMANOMETRY. PERISTALTIC CONTRACTIONS IN THE ESOPHAGEAL BODY (GREEN ARROWS), NORMOTONIC LOWER ESOPHAGEAL SPHINCTER WITH ADEQUATE RELAXATION DURING SWALLOWING (RED ARROWS

Regarding the article "Bronchodilator test in extreme old age: Adverse effects of short-acting beta-2 adrenergic agonists with clinical repercussion and bronchodilator response" with DOI number: http://dx.doi.org/10.1590/1806-9282.65.11.1343, published in Journal of the Brazilian Medical Association, 2019;65(11), page 1343, figure 1 and summary (in portuguese and english) changed from:

SUMMARY
OBJECTIVE: To evaluate chronological age as a limiting factor to perform the bronchodilator test, determine significant adverse effects of short-acting beta 2 agonists with clinical repercussions, and assess bronchodilator response in extreme-old-age patients who undergo the spirometry test.
METHODS: This is a cross-sectional and retrospective study. The sample was extracted from the database (spirometer and respiratory questionnaire) of a pulmonary function service. Patients over 90 years old were included in the research, and we evaluated their bronchodilator response and its significant adverse effects that may have clinical repercussions related to the bronchodilator.
RESULTS: A sample of 25 patients aged 92.12 ± 2.22 years (95% CI, 91.20 - 93.04), with a minimum age of 90 years and a maximum of 97 years and a predominance of females with 72% (18/25). The bronchodilator test was performed in 84% (21/25) of the patients. The bronchodilator response was evaluated in 19 of the 21 patients (90.47%) who underwent the bronchodilator test. Two tests did not meet the criteria of acceptability and reproducibility. No clinical adverse effects were observed with the bronchodilator medication (salbutamol) during or after the exam.
CONCLUSIONS: Chronological age is not a limiting factor for the bronchodilator test, short-acting beta-2 agonists did not present adverse effects with significant clinical repercussion and were useful in the diagnosis and therapeutic guidance of extreme-old-age patients.
KEYWORDS: Bronchodilator agents; aged; aging; respiratory function tests; Spirometry; longevity

RESUMO
OBJETIVOS: Avaliar se idade cronológica é um fator limitante para realizar prova broncodilatadora, determinar efeitos adversos significativos com repercussão clínica dos beta-2 agonistas de curta ação e avaliar a resposta broncodilatadora na espirometria, na velhice extrema.
MÉTODOS: Estudo transversal, retrospectivo. Amostra extraída do banco de dados (espirômetro e questionário respiratório) de um serviço de

função pulmonar. Incluídos na pesquisa pacientes com ≥90 anos, sendo avaliados a resposta broncodilatadora e efeitos adversos significativos com repercussão clínica ao broncodilatador.
RESULTADOS: Amostra de 25 pacientes com idade de 92,12 ± 2,22 anos (IC 95%; 91,20 – 93,04), idade mínima de 90 anos e máxima de 97 anos, predominando o sexo feminino, com 72% (18/25). A prova broncodilatadora foi realizada em 84% (21/25) dos pacientes. A avaliação da resposta ao broncodilatador foi feita em 19 dos 21 pacientes (90,47%) que realizaram a prova broncodilatadora, uma vez que dois desses exames não preencheram os critérios de aceitabilidade e reprodutibilidade. A resposta broncodilatadora foi significativa em 10,52% (2/19) dos pacientes, ambos portadores de pneumopatia obstrutiva. Não foram observados efeitos adversos com repercussão clínica da medicação broncodilatadora (salbutamol) durante ou após sua realização.
CONCLUSÕES: A idade cronológica não é um fator limitante para a realização da prova broncodilatadora, os beta-2 agonistas de curta ação não apresentaram efeitos adversos com repercussão clínica significativa e foram bastante úteis para auxiliar no diagnóstico e orientação terapêutica na velhice extrema.
PALAVRAS-CHAVE: Broncodilatadores; Idoso; Envelhecimento; Testes de função respiratória; Espirometria;

FIGURE 1 (BLANK)

TO

SUMMARY
OBJECTIVE: To evaluate chronological age is a limiting factor to perform bronchodilator test, to determine significant adverse effects that may have clinical repercussions of short-acting beta 2 agonists and to assess the bronchodilator response in fourth age patients who submit the spirometry test.
METHODS: Cross-sectional and retrospective study. The sample extracted from the database (spirometer and respiratory questionnaire) of a pulmonary function service. Patients over 87 years were included in the research and were evaluated the bronchodilator response and significant adverse effects which may occasion clinical repercussion related to bronchodilator.
RESULTS: A sample of 75 patients aged 89.34 ± 0,29 years (95% CI, 88,74 – 89,94), minimum age of 87 years and maximum of 97 years, predominance of female with 58,7% (44/75). The bronchodilator test was performed in 86.6% (65/75) of the patients. The bronchodilator response was evaluated in 63 of the 65 patients (96.92%) who underwent the bronchodilator test. The bronchodilator response was significant in 20.63% (13/63) of the patients. No clinical adverse effects were observed with bronchodilator medication (salbutamol) during or after exam.
CONCLUSIONS: Chronological age is not a limiting factor for the bronchodilator test, short-acting beta-2 agonists did not present adverse effects with significant clinical repercussion and were useful to help in diagnosis and therapeutic guidance in the fourth age group.
KEYWORDS: Bronchodilator agents; aged; aging; respiratory function tests; Spirometry; longevity

RESUMO
OBJETIVOS: Avaliar se idade cronológica é um fator limitante para realizar prova broncodilatadora, determinar efeitos adversos com repercussão clínica significativa dos Beta 2 agonistas de curta ação e avaliar a resposta broncodilatadora na espirometria, na quarta idade.
MÉTODOS: Estudo transversal, retrospectivo. Amostra extraída do banco de dados (espirometro e questionário respiratório) de um serviço de função pulmonar. Incluídos na pesquisa pacientes com ≥ 87 anos sendo avaliado a resposta broncodilatadora e efeitos adversos com repercussão clínica significativa ao broncodilatador.
RESULTADOS: Amostra de 75 pacientes com idade de 89,34 ± 0,29 anos (IC 95% 88,74 – 89,94), idade mínima de 87 anos e máxima de 97 anos, predominando o sexo feminino com 58,7 % (44 / 75). A prova broncodilatadora foi realizada em 86,6 % (65 / 75) dos pacientes. A avaliação da resposta ao broncodilatador foi feita em 63 dos 65 pacientes (96,92%) que realizaram a prova broncodilatadora. A resposta broncodilatadora foi significativa em 20,63 % (13 / 63) dos pacientes. Não foi observado efeito adversos com repercussão clínica significativa da medicação broncodilatadora (salbutamol) durante ou após sua realização.
CONCLUSÕES: A idade cronológica não é um fator limitante para realização da prova broncodilatadora, os agonistas beta-2 de curta ação não apresentaram efeitos adversos com repercussão clinica significativa e foram úteis para auxiliar no diagnóstico e orientação terapêutica, na quarta idade.

PALAVRAS-CHAVE: Broncodilatadores; Idoso; Envelhecimento; Testes de função respiratória; Espirometria;

Figure 1:

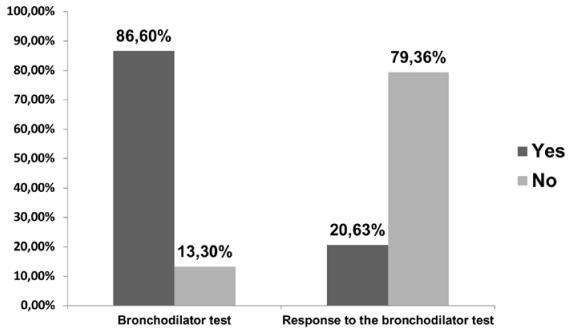

FIGURE 1.: BRONCHODILATOR TEST AND ASSESSMENT OF SIGNIFICANT RESPONSE IN THE SAMPLE

Footnote: [1]Values as %. The bronchodilator test was performed on 65 patients of the general sample (n: 75); and the assessment of the bronchodilator response was conducted in 63 of the 65 patient (due to exclusions).