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Correspondence to:

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The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): the best science is providing health for all

Paulo Andrade Lotufo[I]

Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo (SP), Brazil

[I]MD, DrPH. Full Professor, Department of Internal Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo (SP), Brazil.
orcid.org/0000-0002-4856-8450

Two situations: March 2018. Duke University, United States. The head of the United States National Health Institutes opens a summit conference of world epidemiology, by presenting the 60 most essential cohort studies currently in existence. Among the first slides, there is a world map with the area corresponding to Brazil, showing the presence in this country with a cohort study. It is the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a research project that originated in and was financed in this country that has met all the quality requirements to be in the first team of world science. August 2004. Auditorium of Hospital Universitário, University of São Paulo (USP), Brazil. Researchers from USP, FIOCRUZ (Rio de Janeiro) and the Federal Universities of Minas Gerais, Rio Grande do Sul, Espírito Santo and Bahia launch a proposal to study the determinants of heart disease and diabetes in a Brazilian population, after two days of meetings. The plan of what would become ELSA-Brasil was born.

Between these two times, many things have happened. In 2005, the Brazilian Ministry of Health incorporated the proposal that had been drawn up in August 2004 and, together with the Ministry of Science and Technology, issued a call for financing a multicenter study on cardiovascular diseases and noncommunicable diseases. This public call was won by the six universities that began to organize ELSA-Brasil.

In 2006, 2007 and the first half of 2008, questionnaires, manuals and research protocols were created. Facilities for the clinical investigation were either built or renovated in the six universities. An unprecedented cryobiology center was established to serve the entire study. In parallel, ultrasound and electrocardiogram reading centers started to work.

Thus, in August 2008, everything was ready for the first major epidemiological project in Brazil. The main thing that was still missing was the ELSA participants. When the invitations to participate were issued, there was an immediate and immense response in the six universities. The number of participants was so large that the organizers were forced to stop admitting participants when the total reached 15,105 because of the limit on resources. The initial contact at the workplace, followed by the extended battery of examinations, was very well received.

After ten years, the main players in this project, i.e. the ELSA-Brasil subjects, were still all actively participating through responding to telephone contacts year after year. A second wave of visits was organized in 2012-14 and a third wave of visits in 2017-18. So far, more than 200 original articles using data from ELSA-Brasil have been published; 100 theses and dissertations have been defended; more than 1,000 master's and doctoral students and clinical research fellows have been trained and then incorporated into the labor market; and Brazilian epidemiology has been integrated with the main scientific centers worldwide.

The impact of ELSA-Brasil cannot be measured solely in terms of its publications. Brazilian studies have begun to adopt the research methods of this study and the cryobiology model. Clinical laboratories have adopted standards that were obtained through ELSA-Brasil, rather than using those coming from foreign populations.

During this time, intensive collaborations have been established with universities overseas, such as Harvard University (Boston, USA), Brown University (Providence, USA), Johns Hopkins University (Baltimore, USA), University of Miami (Miami, USA), University of North Caroline

(Chapel Hill, USA), University of Wisconsin (Madison, USA), Erasmus University (Rotterdam, Netherlands) and University of Amsterdam (Amsterdam, Netherlands).

However, the most important outcome has been the production of guidelines for application in the Brazilian National Health System relating to diet, hypertension, diabetes, renal disorders, mental disorders, cognitive dysfunction, headache and thyroid diseases. It completes a virtuous cycle: funding from the Ministry of Health for science has produced returns that benefit all citizens through a new approach towards non-communicable diseases.

The trajectory of ELSA-Brasil shows that it is possible for Brazilian scientists to work together with common goals over the long term, while revealing the high proportion of selfless people who are willing to volunteer with actions that will benefit everyone for a long time.

Going back to what happened at the summit meeting of world epidemiology in the United States in March 2018: Brazil does not have a nuclear arsenal, but it is armed with science to improve the living conditions of humanity, thereby contributing towards understanding the relationship between health and disease.

References: Access to the complete collection of ELSA-Brasil papers can be requested from palotufo@usp.br

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Address for correspondence:
Centro de Pesquisa Clínica e Epidemiologia, Hospital Universitário (HU),
Universidade de São Paulo (USP)
Av. Prof. Lineu Prestes, 2.565
Butantã — São Paulo (SP) — Brasil
Tel. (+55 11) 3091-9300
E-mail: palotufo@usp.br

Central hemodynamic parameters to predict cardiovascular outcomes and mortality among the elderly: protocol for a systematic review

Grasiele Sausen[I], Tarsila Vieceli[II], Clarissa Garcia Rodrigues[III], Daniel Kipper[IV], Airton Tetelbom Stein[V], Guilherme Brasil Grezzana[VI]

Clínica Del Cuore, Antonio Prado (RS); Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre (RS); Fundação Universitária de Cardiologia (IC-FUC), Porto Alegre (RS); and Global Research and Innovation Network (GRINN), Porto Alegre (RS), Brazil

[I]PhD. Coordinator of the Experimental Research Section, Instituto de Cardiologia do Rio Grande do Sul, Fundação Universitária de Cardiologia (IC-FUC), Porto Alegre (RS), Brazil.
orcid.org/0000-0002-0995-6688

[II]MSc. Medical Student, School of Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre (RS), Brazil.
orcid.org/0000-0001-6274-1320

[III]PhD. Chief Executive Officer, Board of Directors, Global Research and Innovation Network (GRINN), Porto Alegre (RS), Brazil.
orcid.org/ 0000-0002-9636-4938

[IV]MD. Physician, Clínica Del Cuore, Antonio Prado (RS), Brazil.
orcid.org/0000-0002-4714-8526

[V]PhD. Provost of Research and Graduate Programs. Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre (RS), Brazil.
orcid.org/ 0000-0002-8756-8699

[VI]PhD. Physician and Director, Clínica Del Cuore, Antonio Prado (RS), Brazil.
orcid.org/ 0000-0003-1310-9075

KEY WORDS:
Blood pressure.
Hypertension.
Aged.
Mortality.

ABSTRACT

BACKGROUND: Central blood pressure is a factor that may predict cardiovascular events. However, its use in clinical practice is not well consolidated. Therefore, the aim of our study will be to summarize the use of central hemodynamic parameters to predict cardiovascular-related outcomes and all-cause mortality.

DESIGN AND SETTING: Protocol for systematic review of longitudinal observational studies conducted in healthcare institutions, as presented in the studies included.

METHODS: We will perform a systematic search in the electronic databases MEDLINE (via PubMed), EMBASE and LILACS (via Virtual Health Library (VHL)), using health descriptors terms for elderly people and for hemodynamic indices of central blood pressure. We will include articles that evaluated hemodynamic indices and at least one of the following outcomes: all-cause mortality, total cardiovascular death, total non-cardiovascular death, myocardial infarction, stroke, coronary artery restenosis after percutaneous coronary intervention, revascularization and aortic syndromes. Two independent reviewers will conduct analysis on the abstracts selected and on the full-text articles. Two reviewers will independently perform data extraction and evaluate the methodological quality of the articles selected, and a third reviewer will evaluate any divergences. The methodological quality of the studies will be assessed in accordance with the ROBINS-I tool (Risk Of Bias In Non-randomized Studies of Interventions).

RESULTS AND CONCLUSIONS: Through this systematic review, we intend to summarize evidence that supports the use of central hemodynamic parameters for central blood pressure to diagnose and perform prognostics on arterial hypertension in elderly patients within clinical practice and predict future cardiovascular events in this population.

REGISTRATION: Prospero - CRD42018085264.

INTRODUCTION

Brachial blood pressure (BP) is a parameter for predicting cardiovascular injury, morbidity and mortality[1] that is widely used in clinical practice to assess cardiovascular risk. However, brachial BP does not correspond to the central BP that is assessed in the carotid artery and ascending aorta,[2] which is an independent prediction factor for cardiovascular clinical events.[3] Considering the aging process and the presence of some cardiovascular risk factors, the differences between central and brachial BP, and between central and wrist-assessed BP, tend to become smaller.[4] However, central BP better reflects the pressure load associated with the left ventricle and coronary circulation and is therefore a potentially accurate risk marker and pressure target for efficiency assessments on therapeutic interventions.[5] Moreover, the pharmacological superiority of vasodilator drugs for cardiovascular outcomes suggests that these will have a distinct effect on central BP, although the effects on brachial BP will be similar.[6] Thus, this suggests that peripheral BP measurements are not a proper substitute for assessing the antihypertensive effects on arterial hemodynamics.[7]

Despite the applicability of central hemodynamic assessments, with diagnostic, therapeutic and prognostic scope, many aspects of these assessments continue to be pending matters. This is reflected in less widespread incorporation of this method into clinical practice. One of these issues is the lack of recommendations for use of central BP assessments to diagnose arterial hypertension within regular practice in the current guidelines. There are also deficiencies regarding standardization and validation for non-invasive central BP assessment instruments,

and regarding the definitions for cutoff values for normal BP in different populations and among individuals of different ages.[8] Furthermore, there is a need to clarify the reference interval for indirectly assessed central BP values, the age-related physiological increase in BP and the pathological increase in BP that relates to higher risk of cardiovascular disease.[8]

Lastly, the evidence available increasingly corroborates widespread usage of central BP assessment as a substitute tool for prediction of future cardiovascular events.[6] Therefore, this topic deserves further investigation through the approach of conducting a systematic review of the literature.

OBJECTIVES

Thus, the aim of the present protocol for a systematic review will be to summarize the evidence regarding the use of central hemodynamic parameters to predict cardiovascular-related outcomes such as total cardiovascular death, total non-cardiovascular death, myocardial infarction, stroke, coronary artery restenosis after percutaneous coronary intervention, revascularization and/or aortic syndromes, and all-cause mortality.

METHODS

Protocol and registration

This systematic review will be reported in accordance with the guidelines of the Meta-Analysis Of Observational Studies in Epidemiology (MOOSE) group.[9] It has been registered in the International Prospective Register of Systematic Reviews (Prospero) database,[10] under the code CRD42018085264.

Eligibility criteria

We will include full peer-reviewed publications from longitudinal observational comparative studies, in which patients aged 60 years or older were included (we will consider the mean age presented in the study). The studies included need to report at least one of the following indexes of central hemodynamics: central systolic blood pressure (SBP), central pulse pressure (PP), central augmentation index (AIx), aortic pressure, wave reflections (WR), pulse wave velocity (PWV) and/or carotid systolic blood pressure (CSBP). Additionally, these studies need to report at least one of the following outcomes: total (all-cause) mortality, total cardiovascular death, total non-cardiovascular death, myocardial infarction, stroke, coronary artery restenosis after percutaneous coronary intervention, revascularization and/or aortic syndromes. We will exclude studies if they reported results from duplicate populations.

Information sources

We will search the following electronic databases: MEDLINE (via PubMed), EMBASE (via Elsevier) and Virtual Health Library (VHL). This last platform contain citations from LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde), IBECS (Índice Bibliográfico Español en Ciencias de la Salud), MEDLINE, Cochrane Library and SciELO. In addition, we will manually search the references of the articles included and we will perform a citation analysis on the studies included, using Google Scholar. Gray literature will not be searched. We will also ask for experts' suggestions, through email communications.

Search

The initial search will comprise the Mesh terms "Aged", "Aged, 80 and over", "Pulse wave analysis" and related entry terms; other terms relating to central hemodynamics such as "Central systolic blood pressure", "Central pulse pressure", "Central augmentation index", "Central pressures", "Aortic pressure" and "Wave reflections"; and a sensitive search strategy for observational studies.

Study selection

The titles and abstracts of the articles retrieved will be independently evaluated by two reviewers (GS and TV). Abstracts that do not provide enough information regarding the eligibility criteria will be kept for full-text evaluation. The reviewers will independently evaluate full-text articles and determine study eligibility. Any disagreements will be resolved by reaching a consensus and, if disagreement persists, these two reviewers will ask a third reviewer for an opinion (GG).

Quality of studies

Risk of bias will be evaluated by ranking each study in accordance with the ROBINS-I tool (Risk Of Bias In Non-randomized Studies of Intervention).[11] The following types of bias will be considered: bias due to confounding, bias in selecting participants for the study, bias in classifying interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measuring outcomes, bias in selecting the reported result and overall bias. Each item will be classified as presenting low, moderate, serious or critical risk of bias; or as presenting "no information" when the article provides no information on which to base a judgement about the risk of bias for this domain.

Appraisal of uncertainty of the evidence

GRADEpro GDT[12] will be used for appraisal of uncertainty of the evidence from the studies included. Tables summarizing the findings will be built in order to grade the evidence available. In relation to each outcome, these factors will include risk of bias, inconsistency (heterogeneity), indirectness, imprecision, and bias of publication. Each type of evidence will be graded as very low, low, moderate or high.

Data extraction

Two reviewers (GS and TV) will independently conduct data extraction and any disagreements will be resolved by bringing in a third reviewer (GG). The general characteristics of the studies will be noted, such as: study title, author, journal and year of publication, study design, inclusion and exclusion criteria, outcome definitions, outcome measurements and follow-up. In addition, we will extract specific information about indexes of central hemodynamics and their predictive values (when available).

Data analysis

Descriptive analysis will be performed on the studies, including study characteristics and main results. We plan on performing meta-analyses, if appropriate. The risk estimates for each study will be reported as hazard ratios, relative risks (RRs) or odds ratios. Hazard ratios will be treated in the same way as RRs. Adjusted risk estimates from multivariate models will be used to control for possible selection bias in the original studies. Heterogeneity across studies will be quantified by means of the I^2 statistic, and the random-effects model will be used to obtain the pooled RRs. The RRs and confidence intervals (CIs) of comparable studies will be illustrated using forest plots. We will generate funnel plots to assess the presence of publication bias, and Duval and Tweedie's trim-and-fill method will be used to assess the implications of publication bias. Results will be considered statistically significant at $P < 0.05$. All analyses will be performed using R language.

DISCUSSION

Evaluation of central hemodynamic measurements may be an effective way to obtain an accurate diagnosis of arterial hypertension and, consequently, may lead to appropriate therapeutic and prognostic decisions. There is an independent relationship between central pressure findings and future cardiovascular events, independently of assessments on peripheral BP, including in elderly populations with coronary arterial and chronic renal disease.[6] Thus, the main potential of the present study is that, through a large and systematic review of the literature, it may bring to light the current evidence supporting the use of central BP parameters as a constant practice for diagnostics, therapeutics and prognostic evaluation in the context of arterial hypertension.

The main limitation of the present study will be that it uses the findings of diagnostic, therapeutic and prognostic evidence from longitudinal studies, to the detriment of more robust studies for supporting broad indication of evaluation of central BP parameters in the context of arterial hypertension. Another limitation to be considered is the fact that the present study proposes to evaluate the central hemodynamic parameters for BP in elderly populations. This aspect of the study design therefore does not allow us to evaluate the pathological condition of systolic arterial hypertension in isolation, in younger individuals.

Lastly, choosing a tool to assess risk of bias was a challenging task, given that different tools can lead to different results.[13] We acknowledge that the ROBINS-I is an instrument primarily built to assess risk of bias of non-randomized studies of interventions, which is not the case of our study. However, it assesses a causal relationship and, thus, it can be used to assess risk of bias of other type of study designs as well. We accept that ROBINS-E (Risk Of Bias In Non-Randomized Studies of Exposures)[14] would potentially be the ideal tool; however, this tool is still under development and therefore we did not consider using it in our study since it has not been validated yet. We considered using the NOS (Newcastle-Ottawa Scale) because we have experience of using this instrument in previous studies. However, it has been well reported that NOS has several limitations including low reliability between individual reviewers and lack of evidence that NOS can identify studies with biased results, which underscores the need for revisions and/or more detailed guidance for systematic reviewers.[15-17] In this light, even though ROBINS-I was primarily developed for intervention studies, we considered it to be the best available option for assessing risk of bias in our study.

CONCLUSION

Through this systematic review, we intend to summarize evidence that supports the use of central hemodynamic parameters for central blood pressure to diagnose and perform prognostics on arterial hypertension in elderly patients within clinical practice and predict future cardiovascular events in this population.

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Address for correspondence:
Guilherme Brasil Grezzana
Rua Oswaldo Hampe, 258
Centro — Antônio Prado (RS) — Brasil
CEP 95250-000
Tel. (+55 54) 98149-9977
E-mail: gbgrezzana@yahoo.com.br

Evaluation of podiatric disorders in a sample of children with intellectual disabilities: an analytical cross-sectional study

Laura Cala-Pérez[I], Marta Elena Losa-Iglesias[II], David Rodríguez-Sanz[III, VI], César Calvo-Lobo[IV], Daniel López-López[V], Ricardo Becerro-de-Bengoa-Vallejo[VI]

Cala Clinic of Podiatry and Surgery, Asturias, Spain

[I]DP, MSc, PhD. External Collaborator, Department of Nursing, Faculty of Health Sciences, Universidad Rey Juan Carlos, Madrid, Spain.
orcid.org/0000-0002-3075-5047

[II]RN, DP, BSc, MSc, PhD. Professor, Department of Nursing, Faculty of Health Sciences, Universidad Rey Juan Carlos, Madrid, Spain.
orcid.org/0000-0001-7588-2069

[III]DP, PT, MSc, PhD. Assistant Professor, Faculty of Sport Sciences, Universidad Europea de Madrid, Madrid 28670, Spain.
orcid.org/0000-0002-3629-6590

[IV]PT, MSc, PhD. Assistant Professor, Department of Nursing and Physical Therapy, Faculty of Health Sciences, Universidad de León, Ponferrada, León, Spain.
orcid.org/0000-0002-6569-1311

[V]DP, BSc, MSc, PhD. Professor and Researcher, Health and Podiatry Research Unit, Department of Health Sciences, Faculty of Nursing and Podiatry, Universidade da Coruña, Spain.
orcid.org/0000-0002-9818-6290

[VI]PhD. Full Professor, School of Nursing, Physiotherapy and Podiatry, Universidad Complutense de Madrid, Spain.
orcid.org/0000-0003-1568-7602

KEY WORDS:
Disabled children.
Foot.
Foot injuries.
Intellectual disability.
Musculoskeletal disease.

ABSTRACT

BACKGROUND: Intellectual disabilities (IDs) usually derive from neurodevelopmental disabilities. They limit intellectual functioning and cause adaptive behaviors and orthopedic problems. These disabilities have harmful effects on health, everyday practical skills and social functioning, and they diminish quality of life. The goal of our research was to perform podiatric evaluations on schoolchildren with and without ID and ascertain their records of foot disorders.

DESIGN AND SETTING: Analytical cross-sectional study conducted at a podiatric clinic in the city of Piedras Blancas, province of Asturias, Spain.

METHODS: An analytical cross-sectional study on 82 schoolchildren affected by ID, compared with 117 healthy schoolchildren, was conducted at a podiatric clinic. Demographic data, clinical characteristics and measurements relating to podiatric examinations were recorded among the participants who completed all phases of the tool that was used in the study process.

RESULTS: Almost 90% of the schoolchildren with and without ID presented foot disorders relating to smaller toes, nail disorders, flat feet or lower-limb alterations.

CONCLUSIONS: The participants showed elevated prevalence of foot disorders. Podiatric evaluations are a significant means for preventing the appearance of medical conditions and/or foot problems, and they also improve general health.

INTRODUCTION

Intellectual disabilities (IDs) are a grouping of developmental diseases characterized by impairment of cognitive functions. They give rise to learning difficulties, adaptive behavior and diminished abilities.[1] The prevalence of ID is around 1-2% in countries of various income levels.[2,3] Presence of IDs has been correlated with a myriad of conditions, including genetic syndromes,[4] genitourinary system diseases, physical health problems,[5] psychiatric alterations,[6] seizure disorders[7] and foot problems,[8] among others.[9]

Currently, over 72% of people with IDs present foot disorders. However, management of these individuals' foot health is often ignored, underestimated or neglected. These individuals tend not to have good access to foot care through regular podiatric examinations.[10] Moreover, foot conditions may have a negative impact on overall health. These individuals may present higher incidence of orthopedic foot surgery, relating to pathological conditions of greater severity.[11,12]

Furthermore, foot disorders are associated with fatigue, difficulties in walking,[13] postural problems[14] and foot pain. These conditions affect people both with and without ID regarding their activities of daily life.[15] The value of taking IDs into account is recognized by clinicians and healthcare policymakers,[6] given the high incidence of foot disorders.[8] Nonetheless, the prevalence of foot alterations and disorders in the ID population is unknown because of the scarcity of epidemiological studies. Hence, early diagnosis and control over foot disorders, general disorders and musculoskeletal conditions, and avoidance of use of inappropriate shoes, have important benefits for overall health, social functioning and mobility among people with IDs.[8]

OBJECTIVE

The goal of our study was to perform podiatric evaluations on schoolchildren with and without ID and to compare their records of foot disorders. We hypothesized that schoolchildren both

with and without ID would show presence of increased prevalence of foot conditions, in this period of life of the school-age population.

METHODS

Participants

This was an analytical cross-sectional study conducted at a podiatric clinic in the city of Piedras Blancas, province of Asturias, Spain, between January 2013 and January 2015. A non-randomized and consecutive sampling method was used to select schoolchildren who were affected by ID, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5),[16] in comparison with healthy schoolchildren. The sample size calculation is described below.

Three follow-up visits were made during the period of this study: after one year (first follow-up visit in 2013), after two years (second follow-up visit in 2014) and after three years (third follow-up visit in 2015). The parents and/or legal guardians of these children provided informed consent for them to take part in the study.

The inclusion criteria for cases in this study were that they needed to present deficits in intellectual functioning and adaptive functioning that were registered in their historical medical records, in accordance with the criteria of the Diagnostic and Statistical Manual of Mental Disorders,[17] but without any clinical signs of dementia, or any previous surgery or other significant orthopedic treatments. The subjects that participated as controls had no register of ID or medical problems in their medical records.

The exclusion criteria included the following situations: previous history of cardiovascular disease, being immunocompromised, having suffered foot trauma or undergone previous foot surgery, having major orthopedic malformations, presence of a neurological condition, being non-autonomous or semi-autonomous in daily activities, refusal of parents to sign the informed consent form and incapacity to comprehend the instructions relating to the investigation and/or carry them out. Controls were matched to cases, in conformity with their ages.

Procedure

At enrolment, anthropometric measurements were made, data were collected and a detailed podiatric examination was performed, always by the same trained podiatric evaluator. The first step, before any measurements were made, was to record information regarding the subjects' general health status, demographic characteristics, gender, age and history of injuries, at their visit to the podiatric clinic. This was done for all participants, with or without ID. After participants had been found to be eligible for inclusion in the study, their anthropometric data were collected. The body mass index (BMI) was calculated from the subjects' weight in kilograms, divided by the square of their height in meters, i.e. BMI = weight/height^2 (kg/m^2).[18]

The participants were then divided into two groups of schoolchildren: with and without ID, matched according to age. They took off their shoes and socks for podiatric examinations to be conducted. These examinations followed the protocol described by Concolino et al.,[19] using a Waldrop scale,[20] in which a single researcher assessed the legs using a LED podoscope device (Herbitas.com, Polígono Industrial, Foios, Valencia, Spain). For this appraisal, each child stood barefoot in a bipodal relaxed posture, with feet slightly apart and weight evenly distributed. Static and dynamic examinations were performed to enable detection of biomechanical abnormalities over various periods of time in the gait cycle. This evaluator could not be blind to the subject's ID.

Joint hyperlaxity was then evaluated. The range of foot movement was assessed in terms of abduction, adduction, dorsiflexion, plantar flexion, muscle tone (analyzed through testing manual counter-resistance), integrity of the ankle (evaluated through a drawer test) and patellar subluxation. Moreover, the foot examination involved recognition of the general appearance of the feet, abnormalities of the toes, condition of the toenails, rotation of the feet, presence of arches, foot type and skin pathology.[19]

Sample size calculation

The sample frame was analyzed using the clinical epidemiology research software of the University of Coruña (http://www.fisterra.com/mbe/investiga/9muestras/9muestras2.asp). The statistical treatment was based on schoolchildren living in the city of Piedras Blancas, in the province of Asturias, northwestern Spain, where the total number of schoolchildren on January 1, 2013, was 123,833 (http://www.ine.es/jaxiT3/Datos.htm?t=9681).

The sample size calculation assumed the following: a two-tailed test, an alpha level of 0.05, a desired power analysis level of 90% with a beta level of 5%, a precision of 3% for a proportion of 50% (P = 0.5) and a loss of schoolchildren of 15%. From this, it was determined that at least 156 subjects would need to be analyzed.

Ethics considerations

This study protocol was reviewed and approved by the local research ethics committee (Universidad Rey Juan Carlos, Spain), under registration number 050520165316, with the application date of June 24, 2016. All the parents and/or legal guardians of the participating children signed a written informed consent statement before these participants with or without ID were brought into the study.

Statistical analysis

Demographic and clinical characteristics were analyzed, including the participants' heights, weights, ages and BMI. The quantitative

variables were summarized as means and standard deviations (SD), and maximum and minimum values, and comparisons were made between the two groups (with or without ID). Categorical variables were shown as total values and percentages.

All the variables were examined for normality of distribution using the Kolmogorov-Smirnov test. Independent Student t tests were used to find out whether differences were statistically significant when normal distribution was shown. Measurements that were not normally distributed were tested using the nonparametric Mann-Whitney U test. Fisher's exact test was used to compare categorical qualitative variables.

In all of the analyses, the statistical significance level was established as $P < 0.05$. All the analyses were performed using a statistical software package (SPSS 19.0, Chicago, IL, USA).

RESULTS

Sample characteristics

A total of 197 children could be included in this study: 80 with ID who were capable of understanding the instructions necessary to carry out the present study; and 117 in the control group. All 197 individuals completed all the stages of the study process: 107 were male (54.31%) and 90 were female (45.68%). Their ages ranged from 4 to 15 years and their mean age was 8.76 ± 2.33 years.

Table 1 shows the descriptive statistics on successive evaluations of age and anthropometric data. Only the variables of weight, height and BMI had normal distribution. The age of the group of participants with disabilities was significantly greater than that of the control group at the beginning of the study, as indicated by the corresponding Student t test for two independent samples. For this reason, the initial weight of the group with disability was significantly greater than that of the control group. In addition, the initial height of the group with disability was greater and the BMI of the group of children with disabilities was also greater than that of the control group.

Overall, 89.84% of the participants (n = 177) stated that they had suffered from foot problems. Subsequent physical examination revealed that all of them presented non-neutral calcaneal stance, 69 (38.98%) had hallux deformities, 85 (48.02%) had metatarsus adductus and 52 (28.37%) had lower limb pain.

The frequencies of foot and leg pathological conditions, comprising hallux deformities, wide spacing between the 1st and 2nd toes, abnormalities of the 3rd, 4th and 5th toes, flat foot and lower-limb pain, were greater in the group with intellectual disabilities than in the control group, as shown in **Table 2**.

The data relating to the range of motion of the foot are shown in **Table 3**. The dorsiflexion values for the ankle with knee flexed or extended, inversion, eversion and dorsiflexion of the first metatarsophalangeal joint were similar between the two groups. The plantarflexion values for the ankle were higher in the control group, but the group with intellectual disability had a greater range of plantar flexion of the 1st metatarsophalangeal joint.

No significant differences were observed between the two groups regarding the degree of relaxed calcaneal stance or discrepancy between the lower limbs. However, the Chippaux-Smirak index values were found to be greater in the group with intellectual disabilities than in the control group, and ankle plantarflexion was less in the group with intellectual disabilities than in the control group.

DISCUSSION

We conducted a cross-sectional study consisting of podiatric evaluations on schoolchildren with and without ID and ascertained their records of foot disorders. Today, in Spain, schoolchildren generally do not have good access to foot care through regular podiatric examinations, even in situations in which foot alterations and disorders are present. These are often ignored because of the existence of other major complicating diseases.

Most previous studies on this issue have addressed detection of foot problems in children with ID.[19,21,22] However, to our knowledge, there are no studies demonstrating that careful podiatric examination among schoolchildren with and without ID shows higher incidence of foot disorders during this period of life of the school-age population.

Table 1. Sociodemographic and clinical characteristics of the sample population

Variable	Total group Mean ± SD (range) N = 197	ID group Mean ± SD (range) N = 80	Control group Mean ± SD (range) N = 117	P-value
Age (years)	8.76 ± 2.33 (4.0-15.0)	9.1 ± 3.08 (4.0-15.0)	8.52 ± 1.61 (6.0-11.0)	0.092**
Weight (kg)	35.04 ± 13.62 (16.60-85.30)	39.39 ± 17.58 (16.6-85.3)	32.1 ± 9.0 (17.7-56.4)	< 0.001*
Height (cm)	134.73 ± 13.61 (108.70-180.10)	136.90 ± 17.30 (108.2-180.1)	133.25 ± 10.17 (112.0-155.0)	0.064*
BMI (kg/m^2)	18.64 ± 3.99 (11.16-32.50)	19.93 ± 4.82 (11.16-32.50)	17.76 ± 3.03 (12.16-26.12)	< 0.001*

ID = intellectual disabilities; BMI = body mass index; SD = standard deviation.

In all the analyses, $P < 0.05$ (with a 95% confidence interval) was considered statistically significant. P-values are from an independent Student t test* or a nonparametric Mann-Whitney U test**.

Table 2. Frequency of foot and leg pathological conditions in the sample population

	Total group N = 197 n (%)	ID group N = 80 n (%)	Control group N = 117 n (%)	P-value
Relaxed calcaneal stance (left foot not neutral)	177 (89.84%)	70 (87.6%)	107 (91.45%)	0.472
Relaxed calcaneal stance position (right foot not neutral)	169 (85.78%)	67 (83.75%)	102 (87.5%)	0.537
Hallux deformities	69 (34.67%)	38 (46.3%)	31 (26.5%)	0.006
Wide space between 1st and 2nd toes	52 (26.39%)	14 (17.5%)	38 (35.8%)	0.021
Deep crease between 1st and 2nd	32 (16.08%)	15 (18.5%)	17 (16.0%)	0.438
Abnormalities of 2nd toe (left foot)	20 (10.15%)	9 (11.25%)	11 (9.4%)	0.810
Abnormalities of 2nd toe (right foot)	21 (11.65%)	12 (15.0%)	9 (7.8%)	0.156
Abnormalities of 3rd toe (left foot)	30 (15.07%)	20 (24.4%)	10 (8.5%)	0.002
Abnormalities of 3rd toe (right foot)	33 (16.58%)	21 (25.6%)	12 (10.3%)	0.005
Abnormalities of 4th toe (left foot)	45 (22.84%)	24 (30.0%)	21 (17.9%)	0.057
Abnormalities of 4th toe (right foot)	49 (24.62%)	28 (34.1%)	21 (17.9%)	0.007
Abnormalities of 5th toe (left foot)	48 (24.12%)	25 (30.5%)	23 (19.7%)	0.066
Abnormalities of 5th toe (right foot)	49 (24.87%)	26 (32.5%)	23 (19.7%)	0.045
2nd toe longer than 1st toe	42 (21.31%)	14 (17.5%)	28 (23.9%)	0.294
Metatarsus adductus (left foot)	83 (42.13%)	27 (33.75%)	55 (47.0%)	0.077
Metatarsus adductus (right foot)	84 (42.63%)	33 (41.25%)	51 (43.6%)	0.770
Flatfoot (left foot)	22 (11.05%)	19 (23.5%)	3 (2.6%)	0.001
Flatfoot (right foot)	17 (8.54%)	14 (17.3%)	3 (2.6%)	0.001
Negative foot progression angle	25 (12.56%)	9 (11.3%)	16 (13.7%)	0.668
Lower limb pain	52 (26.13%)	12 (26.7%)	40 (30.2%)	0.003

Fisher's exact test was performed. In all the analyses, $P < 0.05$ was considered statistically significant with a 95% confidence interval.

Table 3. Range of motion of foot and leg: joint pathological conditions in the sample population

	Total group Mean ± SD (range) N = 197	ID group Mean ± SD (range) N = 80	Control group Mean ± SD (range) N = 117	P-value
Left ankle dorsiflexion (knee extended)	14.32 ± 4.48 (0.0-28.0)	14.5 ± 5.3 (0.0-27.0)	14.2 ± 3.8 (3.0-28.0)	0.246
Right ankle dorsiflexion (knee extended)	14.33 ± 4.54 (0.0-30.0)	14.5 ± 5.4 (0.0-30.0)	14.2 ± 3.9 (2.0-26.0)	0.237
Left ankle dorsiflexion (knee flexed)	19.26 ± 4.53 (0.0-33.0)	19.4 ± 5.5 (0.0-32.0)	19.1 ± 3.7 (7.0-33.0)	0.485
Right ankle dorsiflexion (knee flexed)	19.35 ± 4.51 (0.0-35.0)	19.7 ± 5.5 (0.0-35.0)	19.1 ± 3.7 (5.0-31.0)	0.114
Left ankle plantarflexion	54.34 ± 8.18 (0.0-81.0)	52.9 ± 10.3 (0.0-81.0)	55.4 ± 6.1 (45.0-74.0)	0.038
Right ankle plantarflexion	16.27 ± 2.98 (4.0-27.0)	53.4 ± 9.9 (5.0-27.0)	56.2 ± 6.1 (4.0-24.0)	0.027
Eversion (left foot)	17.03 ± 3.28 (0.0-30.0)	17.2 ± 4.2 (0.0-30.0)	16.9 ± 2.5 (5.0-24.0)	0.886
Eversion (right foot)	16.84 ± 3.11 (0.0-28.0)	17.0 ± 3.7 (0.0-28.0)	16.7 ± 2.6 (4.0-25.0)	0.664
Inversion (left foot)	37.46 ± 3.69 (5.0-51.0)	37.3 ± 5.0 (5.0-47.0)	37.6 ± 2.5 (30.0-51.0)	0.818
Inversion (right foot)	37.51 ± 3.15 (20.0-50.0)	37.5 ± 4.0 (20.0-49.0)	37.6 ± 2.5 (33.0-50.0)	0.606
Plantarflexion of 1st metatarsophalangeal joint (left foot)	45.87 ± 3.45 (30.0-67.0)	46.3 ± 4.0 (30.0-67.0)	45.6 ± 3.0 (35.0-63.0)	0.016
Plantarflexion of 1st metatarsophalangeal joint (right foot)	45.98 ± 3.47 (30.0-68.0)	46.3 ± 3.9 (30.0-65.0)	45.8 ± 3.2 (35.0-68.0)	0.050
Dorsiflexion of 1st metatarsophalangeal joint (left foot)	77.43 ± 10.07 (20.0-93.0)	77.3 ± 11.2 (20.0-93.0)	77.5 ± 9.2 (40.0-92.0)	0.599
Dorsiflexion of 1st metatarsophalangeal joint (right foot)	77.59 ± 10.14 (15.0-93.0)	77.2 ± 11.7 (15.0-93.0)	77.8 ± 9.0 (44.0-93.0)	0.506
Relaxed calcaneal stance position (grades) (left foot)	4.74 ± 3.26 (-5.0-13.0)	4.7 ± 3.7 (-5.0-13.0)	4.8 ± 2.9 (-2.0-12.0)	0.932
Relaxed calcaneal stance position (grades) (right foot)	3.85 ± 3.11 (-5.0-12.0)	4.2 ± 3.6 (-3.0-12.0)	3.6 ± 2.8 (-5.0-12.0)	0.616
Chippaux-Smirak index (left foot)	34.53 ± 17.66 (0.0-84.0)	40.7 ± 19.1 (0.0-84.0)	30.2 ± 15.3 (0.0-73.0)	< 0.001
Chippaux-Smirak index (right foot)	37.28 ± 18.55 (0.0-90.0)	43.3 ± 19.4 (7.0-90.0)	33.1 ± 16.8 (0.0-81.0)	< 0.001
Lower limb length discrepancy (mm)	8.4 ± 6.50 (3.0-30.0)	14.0 ± 9.5 (5.0-30.0)	6.0 ± 2.5 (3.0-10.0)	0.062

ID = intellectual disabilities; SD = standard deviation. In all the analyses, $P < 0.05$ (with a 95% confidence interval) was considered statistically significant. P-values are from Mann-Whitney U test.

Our research demonstrated that careful podiatric examination during the school-age period showed elevated incidence of foot conditions. Most of the anomalies that we found may have been secondary to hypotonia and laxity of the muscles and ligaments.[19,23] This was similar to the findings from other studies that have investigated foot problems, and it suggests that the most critical problems are based on other, less common orthopedic abnormalities.[24,25]

There are important variations relating to the morphology and function of the lower extremities during the school-age period. These contribute towards changes relating to postural sway, variations in plantar loading patterns during gait and presence of flatter feet or greater pronation in the foot, with higher prevalence of bunions, pain, muscle weakness and smaller-toe alterations, increased plantar pressure and difficulty in putting shoes on.[26]

This study had some limitations that need to be acknowledged. Firstly, this investigation was conducted at a podiatric clinic with a relatively small number of subjects. Secondly, a larger and more diverse sample (including children with ID in different countries) would be beneficial, to improve the strength of the study and make it possible to identify more of the mechanisms involved. Thirdly, there was only a single non-blinded evaluator analyzing the participants' feet. Future studies should have at least two blinded evaluators: one evaluator to examine the alterations and deformities of the feet and another to manage the disorders of the lower limbs, in comparison with the blinded data that has been recorded. Despite the existence of obvious demographic differences between the ID and control groups, future studies should consider using normalized demographic data in order to compare the group with ID with a matched paired control group.

The issues highlighted above show that there is a need for further continuous research on this trend, in order to analyze different foot conditions and the therapeutic interventions that physicians could use to improve foot health during the school-age period.

CONCLUSION

Our study showed that clinical signs such as hallux deformities, abnormalities of the third, fourth and fifth toes, flat feet, limited range of motion for ankle plantarflexion and for first metatarsophalangeal joint and higher Chippaux-Smirak index (i.e. showing flat feet) were clinical characteristics with higher prevalence among children with intellectual disabilities, compared with a control group.

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Address correspondence:
Daniel López López
Health and Podiatry Research Unit, Department of Health Sciences, Faculty of Nursing and Podiatry, Universidade da Coruña
Campus Universitário de Esteiro s/nº
15403 Ferrol — Spain
E-mail: daniellopez@udc.es

Association of alanine aminotransferase concentration with cardiometabolic risk factors in children and adolescents: the CASPIAN-V cross-sectional study

Roya Kelishadi[I], Mostafa Qorbani[II], Ramin Heshmat[III], Nazgol Motamed-Gorji[IV], Mohammad Esmaeil Motlagh[V], Hasan Ziaodini[VI], Majzoubeh Taheri[VII], Gita Shafiee[VIII], Tahereh Aminaee[IX], Zeinab Ahadi[X], Motahar Heidari-Beni[XI]

Isfahan University of Medical Sciences, Isfahan, Iran

[I]MD. Professor, Department of Pediatrics, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0001-7455-1495

[II]PhD. Epidemiologist and Assistant Professor, Non-communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran; and Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
orcid.org/0000-0001-9465-7588

[III]MD. Associate Professor, Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
orcid.org/0000-0002-8134-7940

[IV]MD. Researcher, Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
orcid.org/0000-0002-9426-5194

[V]MD. Professor, Department of Pediatrics, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
orcid.org/0000-0002-6101-548X

[VI]MD. Researcher, Office of Health and Fitness, Ministry of Education, Tehran, Iran.
orcid.org/0000-0002-7421-0196

[VII]MD. Pediatrician and Researcher, Bureau of Population, Family and School Health, Ministry of Health and Medical Education, Tehran, Iran.
orcid.org/0000-0002-0873-4338

[VIII]MD. Researcher, Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
orcid.org/0000-0003-3441-3578

[IX]BSc. Researcher, Bureau of Population, Family and School Health, Ministry of Health and Medical Education, Tehran, Iran.
orcid.org/0000-0002-8808-7328

[X]MSc. Researcher, Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.
orcid.org/0000-0002-2176-7206

[XI]PhD. Nutritionist and Assistant Professor, Department of Pediatrics, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.
orcid.org/0000-0001-5543-5536

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Risk factors.
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ABSTRACT

BACKGROUND: It has been suggested that the levels of some liver enzymes, and especially alanine aminotransferase (ALT), might be correlatable with cardiometabolic risk factors. We investigated the relationship between ALT concentration and cardiometabolic risk factors among children and adolescents.

DESIGN AND SETTING: This nationwide study in Iran was conducted within the framework of the fifth survey of a national surveillance program known as the Childhood and Adolescence Surveillance and Preventlon of Adult Non-communicable disease study (CASPIAN-V).

METHODS: The participants comprised 4200 students aged 7-18 years, who were recruited through multi-stage random cluster sampling in 30 provinces in Iran. Physical examinations and laboratory tests were conducted in accordance with standard protocols.

RESULTS: Overall, 3843 students (participation rate: 91.5%) completed the survey. Mean ALT levels were significantly higher in individuals with dyslipidemia, in terms of elevated total cholesterol (TC) or LDL-cholesterol or triglycerides (TG), excess weight and dyslipidemia. Some cardiometabolic risk factors were associated with higher levels of ALT, with the following odds ratio (OR) and 95% confidence interval (CI): metabolic syndrome (OR: 1.013; 95% CI: 1.001-1.025); elevated TC (OR: 1.060; 95% CI: 1.039-1.081), elevated LDL (OR: 1.031; 95% CI: 1.016-1.046), elevated TG (OR: 1.056; 95% CI: 1.040-1.072) and dyslipidemia (OR: 1.051; 95% CI: 1.034-1.068).

CONCLUSION: This large population-based study revealed that some cardiometabolic risk factors were significantly associated with ALT levels. These findings suggest that an association with fatty liver is an underlying mechanism for development of cardiometabolic risk factors.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder. Recently, an association between NAFLD and metabolic syndrome (MetS) has been demonstrated. However, it is unclear whether NAFLD causes metabolic dysfunction, or whether metabolic disorders are responsible for fatty liver disease, or whether both of these might occur.[1] Cardiovascular diseases (CVDs) lead to death among individuals with NAFLD. However, it is less clear whether NAFLD increases morbidity or mortality relating to CVD.[2]

Several methods are used to diagnose NAFLD. Although liver biopsy is the standard for diagnosing of hepatic steatosis, ultrasonography and biochemical tests are easy to use in clinical plans. Serum biomarkers, particularly alanine aminotransferase (ALT) levels, are sensitive for detecting NAFLD in both obese and non-obese patients.[3] Because ALT is closely related to liver fat accumulation, ALT is used as a surrogate marker for NAFLD in epidemiological studies.[4]

It has been suggested that serum ALT is the liver enzyme most closely correlated with liver fat content. Elevated ALT levels in cases of obesity have physiological significance in terms of the potential effect of fatty liver, which commonly occurs in cases of MetS. Thus, it has been shown that elevated ALT levels are associated with the incidences of MetS, diabetes mellitus[5,6] and cardiovascular disease, independently of traditional risk factors.[7]

In addition, correlations between ALT levels and both waist circumference and higher circulating insulin levels have been found. Hepatic insulin resistance leads to subsequent decline in hepatic insulin sensitivity.[8,9] Recent studies have indicated that higher levels of abdominal fat,

particularly visceral fat, are closely correlated with occurrences of NAFLD. However, data on the relationship between ALT concentrations, visceral fat accumulation and cardiometabolic risk factors remain rare. These associations are considerable and need to be studied further.[10,11]

The Framingham Heart Study showed that ALT levels were associated with multiple cardiometabolic risk factors.[6] In the Korean National Health and Nutrition Examination Surveys, an association between increasing ALT levels and the presence of MetS was found.[12] In addition, a cross-sectional study on a rural Chinese population showed that increased ALT levels were correlated with a worse cardiometabolic risk profile.[13] A study on 5,586 adolescents reported that a relationship existed between ALT levels and waist circumference and insulin levels.[14] An independent correlation between elevated liver enzyme levels and adverse CVD events was demonstrated in a meta-analysis on 10 pooled studies.[15] These findings confirmed the potential for ALT levels to act as a biomarker for the risk of metabolic disease. Recently, NAFLD has come to be considered to be a new and important cardiovascular risk factor. Insulin resistance, obesity and increased triglyceride levels are important determinants of NAFLD and lead to increased risk of CVD.[16]

The relationship between ALT levels and some cardiometabolic risk factors among children and adolescents is unclear. To investigate this hypothesis, we studied the relationship between ALT levels and risk factors for MetS and CVD in the Childhood and Adolescence Surveillance and PreventIon of Adult Noncommunicable disease (CASPIAN-V) study.

METHODS

The data for this study were collected as part of the "National Survey of School Student High-Risk Behaviors" (2014-2015). This constituted the fifth survey of the school-based surveillance system, known as the Childhood and Adolescence Surveillance and PreventIon of Adult Noncommunicable disease (CASPIAN-V) study. This school-based nationwide health survey was conducted in 30 provinces in Iran. Details of the study protocol have been discussed previously,[17] and are reported here briefly as well.

Study population and sampling

The study population consisted of students aged 7-18 years in primary and secondary schools in urban and rural areas across the country. They were selected using a multistage stratified cluster sampling method. Sampling within each province was conducted according to the student's place of residence (urban or rural) and level of education (primary and secondary) using the proportional-to-size method and with a 1:1 sex ratio.

The desired number of samples was achieved using cluster sampling in each province, with equal cluster sizes. This was a multistage stratified cluster sampling method in which the clusters were determined at school level. The size of each cluster was 10 students, meaning that a total of 10 sampling units (including 10 students and their parents) would be considered in each cluster. The sample size of the main survey included 480 students in each province (48 clusters of 10 students), i.e. a total of 14,400 students at national level. In each province, 14 out of the 48 clusters were randomly selected for biochemical tests. Therefore, the sample size of the current study was estimated to be 4200.

Procedures and measurements

Questionnaires

Two questionnaires were used: one for students and the other for their parents. The students' questionnaire was derived from the World Health Organization Global School Student Health Survey (WHO-GSHS) (Persian-translated version). The validity and reliability of this questionnaires had been assessed previously.[18,19] An expert panel approved the validity and, in the phase of content validity assessment, questions with a score of more than 0.75 were approved as having optimal content validity.

After eligible students had been identified, the mission and purpose of the interview was explained to them. They were told that the questions were about the health status and health-related behaviors of children and adolescents. Interviews were conducted in a peaceful environment. The questions were read out to the students using simple words, and they could not see the questions. The whole process was supervised and controlled by a team of healthcare professionals. This questionnaire included questions about physical activity status and screen time status.

After the eligible students had been identified, their parents were invited to complete the parents' questionnaire. The presence of at least one of the parents was necessary and sufficient. The parents were informed that the questions were about health status and health-related behaviors in the students' families. The parents' questionnaire also sought information on family characteristics, namely household size, number of students and socioeconomic variables.

Physical measurements

A team of trained healthcare experts recorded information. They performed the examinations in accordance with standard protocols and used calibrated instruments. Weight was measured to the nearest 0.1 kg on a scale placed on a level floor, while the subjects were wearing light clothes; and height were measured to the nearest 0.1 cm, without shoes.[19] Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). We used the WHO growth charts to categorize BMI.[20]

Waist circumference was measured to the nearest 0.1 cm using a non-elastic tape, at a point midway between the lower border

of the rib cage and the iliac crest at the end of normal expiration. Hip circumference was measured to the nearest 0.1 cm, at the widest part of the hip at the level of the greater trochanter.[21] Wrist circumference was measured to the nearest 0.1 cm on the dominant arm using a non-elastic tape. The subjects were asked to rest their arm on a flat surface, such as a table. The upper edge of the tape measure was placed just distally to the prominences of the radial and ulnar bones. Neck circumference was measured to an accuracy of 0.1 cm, taking the most prominent portion of the thyroid cartilage as a landmark.

Blood pressure was measured in the sitting position on the right arm using a mercury sphygmomanometer with an appropriate cuff size. It was measured twice, with a five-minute interval between the measurements. Systolic and diastolic pressures were recorded and the average was registered.[22]

Laboratory sampling

Selected students were referred to the laboratory (i.e. the laboratory that was the nearest to the school) for blood sampling, and one of each student's parents accompanied him/her. A sample of 6 ml of venous blood was collected after 12-hour overnight fasting. All collection tubes were centrifuged at 2500-3000 x g for 10 minutes. Immediately after centrifugation, the serum samples were aliquoted into 200-microliter tubes and were stored at -70°C. All the samples were transferred by means of a cold chain to Isfahan Mahdieh Laboratory. A cold chain is a system used for keeping biological samples in good conditions and this system consists of a series of links that are designed to keep biological samples within the temperatures ranges recommended by the World Health Organization, from the point of manufacture to the point of administration. One common temperature range for a cold chain is 2 to 8°C. Alanine aminotransferase (ALT), fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) were measured enzymatically using a Hitachi auto-analyzer (Tokyo, Japan).[23,24]

Definitions

WHO growth curves were used to define age and sex-specific BMI categories. Underweight was defined as BMI < 5th percentile, overweight as BMI as 85th-95th, excess weight as BMI > 85th and generalized obesity as > 95th. On the other hand, abdominal obesity was defined as waist-to-height ratio (WHtR) greater than or equal to 0.5.[25]

ALT > 35 IU/l was considered high. Fasting blood glucose (FBG) ≥ 100 mg/dl, serum triglycerides (TG) ≥ 100 mg/dl, total cholesterol (TC) ≥ 200 mg/dl, LDL-C ≥ 110 mg/dl and HDL-C < 40 mg/dl (except for boys aged 15-18 years, for whom it was < 45 mg/dl) were considered abnormal.

Elevated blood pressure was defined as either high systolic blood pressure (SBP) or high diastolic blood pressure (DBP) ≥ 90th percentile for age, sex and height. Metabolic syndrome was defined in accordance with the modified NCEP ATP III criteria.[26]

MetS was defined as clustering of at least three of the five cardiometabolic risk factors, including abdominal (central) obesity, elevated BP, elevated fasting plasma glucose (FPG), high serum TG and low serum HDL. WHtR > 0.5 was considered to represent abdominal obesity. Dyslipidemia was determined as the presence of at least one abnormal lipid profile component (i.e. TG, TC, HDL or LDL).

Physical activity (PA)

It was considered that the students were doing enough PA if they did exercises lasting at least 30 minutes per day (the response options ranged from 0 to 7 days) that led to sweating and large increases in respiratory or heart rate.[27] Evaluation of PA was done via two questions: 1) "On how many days were you physically active for an overall 30 minutes per day during the past week?"; and 2) "On a regular basis, how much time do you spend in physical education (PE) classes at school per week?". PA of less than 2 hours per week was considered low, while 2-4 hours a week was considered moderate and more than 4 hours a week was considered high.

Screen time (ST)

To assess ST behavior, the students were asked how many hours per day they were spending watching television and/or videos, using a personal computer or playing electronic games. From this, the total cumulative time spent as ST was calculated.

Socioeconomic status (SES)

SES scores were estimated using the principle component analysis (PCA) method, based on parents' education and job, type of school (private or governmental), type of home (private/rented) and family assets (private car and computer). The SES score for each student was a weighted average of the SES variables. The weighted averages of these variables were summarized under one main component named SES score. Students were classified as having low, moderate or high socioeconomic status, based on this component.

Ethical concerns

The study protocols were reviewed and approved by the ethics committees of Isfahan University of Medical Sciences. The Research Ethics Council of Isfahan University of Medical Sciences approved the study (project number: 194049). After complete explanation of the study objectives and protocols, written informed consent and verbal consent were obtained from the parents and students, respectively.

Statistical analysis

The data were expressed as means and standard deviations (SD) for continuous variables, and as numbers (percentages) for categorical variables. ALT data were transformed to normalize their skewed distribution. The Student t test was used to compare variables with two groups. The ANOVA test was used to compare means for variables with more than two groups. Associations between qualitative variables were assessed using the Pearson chi-square test. Back transformation was applied to the final findings.

Logistic regression analysis was performed to evaluate associations between ALT and abnormal cardiometabolic outcomes (presence of high BP, high fasting blood sugar (FBS), high TG, high TC, high LDL, low HDL, overweight, obesity, excess weight, abdominal obesity, MetS and dyslipidemia). Two models were defined for each association: model I represented the crude association; and model II was adjusted for age, sex, ST, PA, SES and living area. The results from the logistic regression were represented as odds ratios (OR) and 95% confidence intervals (CI).

Linear regression analysis was conducted to evaluate associations between ALT and the quantitative values of cardiometabolic risk factors, including BMI, waist circumference, neck circumference, wrist circumference, hip circumference, SBP, DBP, HDL, LDL, TG, TC and FPG. The data were displayed via the β-coefficient and standard error (SE). Again, two models were defined for each association: model I represented the crude association; and model II described the model I associations after adjustment for age, sex, ST, PA, SES and living area.

A P-value of < 0.05 was considered statistically significant in all measurements. All statistical measurements were estimated using survey data analysis methods. The data were analyzed by using the STATA package, version 11.0 (Stata Statistical Software: Release 11; Stata Corp LP, College Station, TX, USA).

RESULTS

Overall, 3,843 students (out of 4,200 students who had been selected) completed the survey (response rate: 91.5%). The general characteristics of the participants are presented in **Tables 1** and **2**. Overall, the mean age of the students was 12.58 years (± 3.15). 52.6% of the sample consisted of boys, and 72.7% lived in urban regions.

Nearly 60% of the students had dyslipidemia, although the majority of the students affected by this only had one abnormal component (TG, HDL, LDL or TC). Low HDL was seen in approximately 30% of the students. Overall, 20% of the subjects presented excess weight (BMI > 85th percentile), and 10% had high blood pressure (either systolic or diastolic, or both). High ALT was seen in only 13 students (0.3%).

Overall, 188 students out of 3731 (5.039%) were classified as having MetS. In comparing cardiometabolic risk factors between the groups with and without MetS, most of the cardiometabolic risk factors were significantly more prevalent in the MetS group, including abdominal obesity (70.7%), generalized obesity (36.2%), high BP (44.7%), low HDL (81.9%), high TG (85.1%), high FBG (25%) and high ALT (1.6%) (**Table 1**). **Table 2** displays the continuous values for cardiometabolic outcomes according to MetS status. Overall, the MetS and non-MetS groups showed significant differences in almost all risk factors (except for TC and LDL), thus suggesting MetS is associated with higher values for cardiometabolic risk factors (**Table 2**).

The mean ALT levels according to the cardiometabolic risk factors and characteristics of the study population are demonstrated in **Table 3**. These levels were significantly higher in individuals with high TC, high LDL, high TG, excess weight and dyslipidemia.

Table 1. Participants' demographic characteristics in numbers and percentages, according to presence of metabolic syndrome: the CASPIAN-V study

Variables*		Overall (total = 3,731) n (%)	MetS (total = 188) n (%)
Gender	Boys	1,964 (52.6)	108 (57.4)
	Girls	1,768 (47.4)	80 (42.6)
Region	Urban	2,714 (72.7)	154 (81.9)
	Rural	1,018 (27.3)	34 (18.1)
Dyslipidemia		2,085 (55.9)	187 (99.5)
Dyslipidemia components	0	1,647 (44.1)	1 (0.5)
	1	1,353 (36.3)	53 (28.2)
	2	590 (15.8)	108 (57.4)
	3	122 (3.3)	20 (10.6)
	4	20 (0.5)	6 (3.2)
Abdominal obesity		756 (20.3)	133 (70.7)
Generalized obesity		408 (10.9)	68 (36.2)
Excess weight		747 (20.0)	96 (51.1)
High systolic BP		93 (2.5)	20 (10.6)
High diastolic BP		345 (9.2)	81 (43.1)
High BP		374 (10.0)	84 (44.7)
High TC, mg/dl		185 (5.0)	9 (4.8)
Low HDL-C, mg/dl		1,106 (29.6)	154 (81.9)
High LDL-C, mg/dl		659 (17.7)	30 (16.0)
High TG, mg/dl		1,029 (27.6)	160 (85.1)
High FBG, mg/dl		158 (4.2)	47 (25.0)
High ALT		13 (0.3)	3 (1.6)

*Data are presented as number (percentage).
MetS = metabolic syndrome (defined according to ATP-III criteria); SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; FBG = fasting blood glucose; ALT = alanine aminotransferase; dyslipidemia: at least one of the components (TG, HDL, LDL or TC) was abnormal; excess weight: BMI > 85th percentile; obesity, BMI > 95th percentile; low HDL: < 40 mg/dl (except among boys aged 15-19 years, for whom the cutoff was < 45 mg/dl); high LDL: > 110 mg/dl; high TG: 100 mg/dl; high TC: > 200 mg/dl; high FBS > 100 mg/dl; high blood pressure: > 90th percentile (adjusted according to age, sex and height); high ALT: > 35 IU/l.

The mean ALT level increased monotonically with increasing numbers of MetS components and with increasing dyslipidemia (**Table 3**).

Table 4 shows the associations between ALT levels and cardiometabolic risk factors in two different logistic regression models. Presence of MetS (OR: 1.013; 95% CI: 1.001-1.025), high TC (OR: 1.060; 95% CI: 1.039-1.081), high LDL (OR: 1.031; 95% CI: 1.016-1.046), high TG (OR: 1.056; 95% CI: 1.040-1.072) and dyslipidemia (OR: 1.051; 95% CI: 1.034-1.068) were significantly associated with high ALT levels (**Table 4**). **Table 5** presents the associations

Table 2. Participants' demographic characteristics in means and standard deviations, according to presence of metabolic syndrome: the CASPIAN-V study

Variables*	Overall (total = 3,731) mean (SD)	MetS (total = 188) mean (SD)	Non-MetS (total = 3,543) mean (SD)	P-value
Age, years	12.283 (3.158)	12.409 (2.893)	12.443 (3.046)	0.882
BMI, kg/m^2	18.510 (4.713)	21.998 (9.754)	18.296 (4.197)	< 0.001
Waist circumference, cm	66.723 (12.177)	76.704 (13.995)	66.160 (11.658)	< 0.001
Hip circumference, cm	79.144 (14.642)	86.556 (15.455)	79.142 (14.342)	< 0.001
Neck circumference, cm	29.845 (3.999)	31.650 (4.057)	29.860 (3.909)	< 0.001
Wrist circumference, cm	14.722 (1.891)	15.800 (1.985)	14.713 (1.783)	< 0.001
ALT IU/l	8.332 (7.071)	9.760 (7.309)	8.288 (7.141)	0.008
SBP, mmHg	99.170 (13.095)	106.143 (14.401)	98.323 (12.711)	< 0.001
DBP, mmHg	63.836 (10.435)	71.531 (11.773)	63.122 (9.931)	< 0.001
TC, mg/dl	153.85 (27.424)	152.780 (27.231)	153.94 (27.504)	0.573
HDL-C, mg/dl	46.190 (9.976)	37.210 (7.259)	46.64 (9.922)	< 0.001
LDL-C, mg/dl	90.052 (22.609)	87.145 (22.800)	90.249 (22.656)	0.067
TG, mg/dl	88.04 (45.180)	142.14 (61.844)	85.23 (42.656)	< 0.001
FBG, mg/dl	91.65 (12.111)	98.34 (14.861)	91.29 (11.899)	< 0.001

*Data are presented as mean ± standard deviation.
MetS = metabolic syndrome (defined according to ATP-III criteria); ALT = alanine aminotransferase; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; FBG = fasting blood glucose.

Table 3. Mean alanine aminotransferase (ALT) levels according to cardiometabolic risk factors: the CASPIAN-V study

Outcomes		ALT		
		Mean (IU/l)	SD	P-value
Gender	Boy	8.49	5.11	0.14
	Girl	8.15	8.73	
Age	7-10	8.43	6.77	0.83
	11-14	8.26	4.79	
	15-18	8.33	9.88	
Region	Urban	8.41	7.9	0.13
	Rural	8.11	4.16	
Physical activity	High	8.42	5.37	0.52
	Low	8.27	8.09	
Socioeconomic status	High	8.15	5.29	0.77
	Medium	8.37	7.42	
	Low	8.21	5.74	
High SBP	Yes	8.244	6.337	0.879
	No	8.358	7.150	
High DBP	Yes	7.971	4.972	0.290
	No	8.395	7.318	
High TC	Yes	13.052	23.302	0.004
	No	8.088	4.840	
Low HDL-C	Yes	8.586	10.755	0.280
	No	8.226	4.747	
High LDL-C	Yes	9.623	8.522	< 0.001
	No	8.058	6.691	
High TG	Yes	9.590	10.369	< 0.001
	No	7.850	5.210	
High FBG	Yes	8.422	4.738	0.869
	No	8.328	7.156	
Abdominal obesity	Yes	8.866	7.721	0.250
	No	8.220	6.933	
Generalized obesity	Yes	8.920	6.422	0.081
	No	8.277	7.175	
Excess weight	Yes	8.808	7.965	0.045
	No	8.231	6.861	
Dyslipidemia	Yes	8.848	8.806	< 0.001
	No	7.684	3.828	
Number of MetS components	0	7.856	4.301	< 0.001
	1	8.300	5.167	
	2	9.104	12.550	
	≥ 3	9.402	6.623	
Number of dyslipidemia components	0	7.684	3.828	< 0.001
	1	8.125	4.882	
	2	9.239	7.164	
	≥ 3	14.11	7.755	

MetS = metabolic syndrome (defined according to ATP-III criteria); SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; FBG = fasting blood glucose; ALT = alanine aminotransferase; dyslipidemia: at least one of the components (TG, HDL, LDL or TC) was abnormal; excess weight: BMI > 85th percentile; obesity, BMI > 95th percentile; low HDL: < 40 mg/dl (except among boys aged 15-19 years, for whom the cutoff was < 45 mg/dl); high LDL: > 110 mg/dl; high TG: 100 mg/dl; high TC: > 200 mg/dl; high FBS > 100 mg/dl; high blood pressure: > 90th percentile (adjusted according to age, sex and height). The Student t test was used to compare variables with two groups. The analysis of variance (ANOVA) test was used to compare means for variables with more than two groups.

between ALT levels and cardiometabolic risk factors in linear regression models. In this analysis, high BMI (β: 0.013; SE: 0.010), high DBP (β: -0.056; SE: 0.023), high TC (β: 0.471; SE: 0.064), high LDL (β: 0.282; SE: 0.053) and high TG (β: 1.125; SE: 0.102) were significantly associated with high ALT levels (**Table 5**).

DISCUSSION

The main finding from the present study is that there are associations between higher ALT levels and some cardiometabolic risk factors including presence of MetS, high TC, high LDL, high TG and dyslipidemia.

A limited number of cohort studies have reported that NAFLD is an independent risk factor for incidence of CVDs.[1,28] Since the prevalence of NAFLD is increasing among children and adolescents, it is important to investigate whether presence of NAFLD independently enhances the future risk of CVDs in this population.[29]

The relationship between liver enzyme levels and cardiometabolic risk factors has been extensively assessed in relation to adults. However, information regarding the distribution of liver enzymes and their association with cardiometabolic risk factors is scarce in relation to children and adolescents.[30]

ALT is a specific liver enzyme that relates to the cytosolic component of hepatocytes.[5] On the other hand, other liver enzymes, including aspartate transaminase (AST), are also released from other organs such as the heart, skeletal muscles, kidneys, brain, pancreas and red blood cells.[6] Liver enzyme levels are monitored routinely as part of clinical evaluations, and this form of assessment is accessible for many people.[31]

The use of ALT to screen for NAFLD is recommended in several national guidelines regarding overweight and obese children.[32,33] According to thresholds derived from the National Health and Nutrition Examination Survey (NHANES), the sensitivities of ALT for detection of NAFLD are 72% for boys and 82% for girls; while the specificities are 79% for boys and 85% for girls.[34]

Table 4. Association of alanine aminotransferase (ALT) with cardiometabolic risk factors in logistic regression models: the CASPIAN V study

Outcomes	Model I[a]			Model II[b]		
	OR	95% CI	P-value	OR	95% CI	P-value
MetS	1.013	1.001-1.025	0.029	1.013	1.001-1.025	0.033
High BP	0.990	0.969-1.011	0.337	0.982	0.958-1.007	0.165
High SBP	0.997	0.965-1.031	0.878	0.985	0.939-1.034	0.543
High DBP	0.987	0.964-1.010	0.261	0.982	0.965-1.008	0.170
High TC	1.062	1.042-1.082	< 0.001	1.060	1.039-1.081	< 0.001
Low HDL-C	1.007	0.997-1.016	0.173	1.009	0.998-1.019	0.102
High LDL-C	1.032	1.017-1.047	< 0.001	1.031	1.016-1.046	< 0.001
High TG	1.051	1.036-1.066	< 0.001	1.056	1.040-1.072	< 0.001
High FBG	1.002	0.982-1.022	0.869	1.00	0.977-1.024	0.983
Abdominal obesity	1.011	1.001-1.021	0.049	1.009	0.999-1.020	0.079
Generalized obesity	1.009	0.998-1.020	0.111	1.007	0.996-1.019	0.208
Excess weight	1.009	0.999-1.020	0.072	1.007	0.997-1.017	0.158
Dyslipidemia	1.045	1.029-1.060	< 0.001	1.051	1.034-1.068	< 0.001

[a]Crude model; [b]Adjusted for age, sex, PA, ST, SES and living area.
MetS = metabolic syndrome (defined according to ATP-III criteria); SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; FBG = fasting blood glucose; ALT = alanine aminotransferase; dyslipidemia: at least one of the components (TG, HDL, LDL or TC) was abnormal; excess weight: BMI > 85th percentile; obesity, BMI > 95th percentile; low HDL: < 40 mg/dl (except among boys aged 15-19 years, for whom the cutoff was < 45 mg/dl); high LDL: > 110 mg/dl; high TG: 100 mg/dl; high TC: > 200 mg/dl; high FBS > 100 mg/dl; high blood pressure: > 90th percentile (adjusted according to age, sex and height).

Table 5. Association of alanine aminotransferase (ALT) levels with cardiometabolic risk factors in linear regression models: the CASPIAN V study

Outcomes	Model I[a]			Model II[b]		
	β	SE	P-value	β	SE	P-value
BMI, kg/m^2	0.015	0.011	0.149	0.013	0.010	< 0.001
Waist circumference, cm	0.059	0.028	0.033	0.044	0.025	0.070
Wrist circumference, cm	0.006	0.004	0.123	0.005	0.004	0.139
Hip circumference, cm	0.065	0.034	0.054	0.049	0.028	0.082
Neck circumference, cm	0.011	0.009	0.250	0.006	0.008	0.464
SBP, mmHg	-0.029	0.030	0.323	-0.043	0.028	0.124
DBP, mmHg	-0.052	0.023	0.026	-0.056	0.023	0.015
TC, mg/dl	0.481	0.062	< 0.001	0.471	0.064	< 0.001
HDL-C, mg/dl	-0.026	0.023	0.246	-0.036	0.023	0.122
LDL-C, mg/dl	0.284	0.051	< 0.001	0.282	0.053	< 0.001
TG, mg/dl	1.119	0.102	< 0.001	1.125	0.102	< 0.001
FBG, mg/dl	0.013	0.028	0.633	0.008	0.029	0.770

[a]Crude model; [b]Adjusted for age, sex, PA, ST, SES and living area.
MetS = metabolic syndrome (defined according to ATP-III criteria); ALT = alanine aminotransferase; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglycerides; FBG = fasting blood glucose.

Some studies have demonstrated that undesirable changes in liver enzyme levels and liver fat content can be correlated with presence of diabetes mellitus and cardiovascular risk factors.[29,31] However, the findings have been divergent and are thus incompatible in this regard. It is debatable whether a linear relationship exists between ALT levels and cardiovascular risk factors, given that some findings have demonstrated a U-shaped association between ALT levels and total mortality.[1] However, some other studies have reported that ALT levels were associated with NAFLD, obesity, some features of MetS, fasting insulin levels and HOMA-IR as a marker for insulin resistance.[4,35] One prospective study on Swedish men[36] and another on Pima Indians[37] found that high ALT levels, but not any other enzyme, including gamma-glutamyl transferase (GGT), was a risk factor for development of diabetes. Increased liver enzyme activity may lead to inflammation that destroys insulin signaling. It has been shown that subjects with high ALT levels have higher levels of high-sensitivity C-reactive protein (hsCRP). HsCRP is an independent predictor for chronic disorders such as diabetes and cardiovascular disease.[38]

The Framingham Offspring Study demonstrated that enhanced ALT levels were related to an increase in risk factors for CVD after 20 years of follow-up, after adjusting for age and sex. However, this association disappeared with further adjustment.[5]

A positive correlation between the concentrations of some enzymes, including GGT, ALT, AST and ALP, and the risks of acquiring MetS and type 2 diabetes was shown in the Mexico City Diabetes Study.[39] A study on Chinese patients with newly diagnosed type 2 diabetes reported that high ALT levels were strongly associated with some components of MetS and insulin resistance.[35]

A study on 1084 adolescents aged from 12.5 to 17.5 showed that presence of adverse cardiometabolic risk factors increased ALT and GGT levels and decreased the AST/ALT ratio in both genders. Overweight and obese adolescents had higher ALT and GGT levels and lower AST/ALT ratios than did leaner adolescents. There was an association between presence of liver biomarkers and central and overall adiposity in both genders. However, the correlations between presence of liver enzymes and the levels of blood pressure, insulin resistance and blood lipids were different between boys and girls. It was shown that ALT levels were correlated with higher overall cardiometabolic risk only in males. The AST/ALT ratio thresholds for determining that adolescents presented high cardiometabolic risk were 1.00 for younger males, 0.74 for older males, 0.86 for younger females and 0.87 for older females.[30]

Mohammadi et al. reported that there was a strong association between high levels of ALT, AST and ALT/AST ratio and most cardiometabolic risk factors. This relationship was independent of anthropometric indexes. These authors showed that high levels of liver enzymes can be considered to be a cardiometabolic risk factor during childhood.[40]

Studies that have investigated the association between NAFLD and CVD risk factors are scarce. However, one review article reported that presence of NAFLD is independently correlated with increased risk factors for CVD, according to a prospective study.[41] A meta-analysis of population-based studies showed that there was a correlation between high GGT levels and increased risk factors for CVD. However, no correlation relating to ALT levels was found.[15]

We found that there was a significant association between ALT levels and BMI in linear regression models after adjusting for confounders. The coefficient of regression for waist circumference was borderline. Obesity is one of the risk factors for increased serum liver enzyme levels, particularly ALT, in both adults and children. An association between anthropometric measurements and biochemical complications, including increased ALT levels, was demonstrated among obese Japanese children.[38]

Measurement of ALT levels is commonly used in pediatrics. ALT measurement constitutes a readily available and low-cost blood test. It is used as a valuable screening test for detection of liver disease. However, the threshold value for high ALT levels and hence for diagnosing liver disease in pediatrics remains uncertain. In addition, the appropriate explanation for the results from ALT assays that are performed on children is unclear. Because of this indeterminacy, laboratories need to define what the normal range for ALT levels is, according to findings from their local populations.[34]

Clinicians need to be aware that high-normal ALT levels may be present in children with underlying chronic liver disease. There is evidence to suggest that lowering the cutoff for high ALT may improve sensitivity without a significant impact on specificity.[34,42,43]

The strength of the present study is its large sample size. Some limitations that need to be acknowledged are its cross-sectional nature, lack of imaging procedures and observational design. Thus, we were unable to investigate causality. In addition, some potential confounders might have affected our findings.

CONCLUSION

The present study showed that there were associations between higher ALT concentrations and the presence of MetS, high TC, high LDL, high TG and dyslipidemia. The findings suggest that a high level of ALT is beneficial for making pediatric clinical assessments on patients who may present high cardiometabolic risk. Clinicians who manage NAFLD patients should not focus on liver disease alone. They should also consider the increased chance of presentation of cardiometabolic risk factors.

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Address for correspondences:
Motahar Heidari-Beni
Department of Pediatrics, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran
Fax. +983137925280
Tel. +983137925284
E-mail: motahar.heidari@nutr.mui.ac.ir

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Evaluation of foot functionality in cases of rheumatoid arthritis through the FFI-BR and FHSQ-BR questionnaires: a cross-sectional observational study

Elinah Narumi Inoue[I], Agnes Patricia de Andrade[II], Thelma Skare[III]

Rheumatology Department, Hospital Universitário Evangélico de Curitiba, Curitiba (PR), Brazil

[I]Undergraduate Medical Student, Faculdade Evangélica do Paraná (FEPAR), Curitiba (PR), Brazil.
orcid.org/0000-0002-7135-3634

[II]Undergraduate Medical Student, Faculdade Evangélica do Paraná (FEPAR), Curitiba (PR), Brazil.
orcid.org/0000-0002-3801-5051

[III]MD, PhD. Professor, Department of Rheumatology, Hospital Universitário Evangélico de Curitiba, Curitiba (PR), Brazil.
orcid.org/0000-0002-7699-3542

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ABSTRACT

BACKGROUND: Rheumatoid arthritis (RA) can affect the feet, thus compromising the patient's gait and autonomy. In this study, we investigated foot disability in RA patients using the Brazilian versions of the Foot Health Status Questionnaire (FHSQ-BR) and Foot Function Index (FFI-BR).

DESIGN AND SETTING: Cross-sectional, observational study conducted in a tertiary care hospital.

METHODS: Two hundred individuals were studied: 100 with RA and 100 controls. Demographic variables and FFI-BR and FHSQ-BR scores were analyzed. In relation to RA patients, data on medications used and on the following clinical variables were collected: Disease Activity Score-28-ESR; erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level and rheumatoid factor (RF) level. The groups were compared and the scores and clinical variables were correlated.

RESULTS: RA patients' scores in the pain, difficulty and disability domains of the FFI-BR questionnaire were worse ($P < 0.0001$). The FHSQ-BR showed that there were differences between RA patients and controls in relation to the pain and foot function domains: shoes ($P < 0.0001$), foot health ($P < 0.0001$), general health ($P = 0.0002$), physical activity ($P < 0.0001$), social capacity ($P = 0.0006$) and vigor ($P = 0.01$). There were correlations between FFI-BR and DAS-28-ESR scores (rho = 0.45), ESR (rho = 0.27) and CRP (rho = 0.24). According to the FHSQ-BR questionnaire, there was a correlation between DAS-28-ESR and worse foot health (rho = 0.29).

CONCLUSION: RA patients' scores in the foot health assessment questionnaires were worse than those of controls. A correlation between inflammatory activity and worse foot function was found.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disease that can cause limitations and difficulties in activities of daily living (ADLs), due to pain, gait impairment and difficulties with self-care.[1] Damage to the ankles and feet occurs in 85% to 100% of these patients and contributes greatly to their loss of quality of life.[2] Typically, foot involvement indicates more aggressive disease and requires more than just pharmacological care.[3]

Studying functional disability of the feet is important because, in addition to causing the abovementioned limitations, this form of disability has been correlated with higher risk of falls due to stiffness, pain, muscle weakness and balance disorders, which increases mortality, health resource utilization and loss of patients' work capacity.[4,5] Although RA patients frequently complain about their feet, such complaints are often overlooked by healthcare professionals.

Specific instruments for foot health assessment, including the Brazilian versions of the Foot Health Status Questionnaire (FHSQ-BR) and the Foot Function Index (FFI-BR), measure the day-to-day impact of involvement of the feet in RA. These instruments provide data that help in understanding the repercussions of this involvement and the respective therapeutic indications. This can improve the quality of life of the individuals affected.[3]

OBJECTIVE

The objectives of the present study were to assess the disability that originated through foot involvement among RA patients, as indicated using the FFI-BR and FHSQ-BR questionnaires, and to evaluate the influence of inflammatory activity on the dysfunction of foot joints.

METHODS

This was a cross-sectional observational study on individuals diagnosed with RA and controls. It used a convenience sample that included all consecutive RA patients who went for consultations between July 2015 and February 2017 in the same university hospital. They were included in the study in the order of their appointments, according to their willingness to participate in the study.

For subjects to be included in the patient group, they needed to have reached a score of at least six points in the 2010 classification criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR).[6] For subjects to be included in the control group, they needed to be free from chronic inflammatory disease, orthopedic or neuromuscular disease, or any other condition affecting foot health; and to have come for consultation at the dermatology clinic for cosmetic reasons. Controls were matched with RA patients for age, gender and body mass index (BMI).

Approval for this study was granted by the local research ethics committee (date: May 11, 2016; number: 1.560.418). After participants had signed a free and informed consent statement, they were invited to answer two questionnaires, to evaluate the limitations and disabilities caused by foot health problems. The FFI (validated in Portuguese for use in Brazil as FFI-BR) assesses foot functionality; and the FHSQ (validated in Portuguese for use in Brazil as FHSQ-BR) assesses how foot pain impacts on patients' quality of life. Both of these questionnaires not only evaluate joints but also have already been used to study the foot in general, including skin and nail diseases.[3]

The FFI-BR questionnaire is divided into three domains: disability, difficulty and pain. The responses are given on a numerical scale, from 0 to 10. The total score is obtained for each patient as the arithmetic mean of the three domains and is multiplied by 100 to obtain a percentage. Higher scores mean that foot health is worse and, consequently, so is the individual's quality of life.[7]

The FHSQ-BR questionnaire also presents three domains. The first assesses foot health in four sections: foot pain, foot function, footwear and general foot health. The second evaluates the patient's general health, also in four sections: general health, physical activity, sociability and vigor. The third considers the epidemiological characteristics of the interviewees. The FHSQ-BR questionnaire scores were obtained using the Foot Health Status Questionnaire software, version 1.03. The responses were entered in a computer program and a score ranging from 0 to 100 was generated for each domain, such that 0 was the worst condition of the foot, and 100 was the best. The scores reflected the patients' difficulty in daily activities and the consequences of the disease for the foot.[3]

The RA patients' medical records were reviewed for epidemiological, clinical and treatment data, namely: age, sex, ethnicity, smoking, weight, height, length of time with the disease, disease activity measured using DAS-28-ESR (Disease Activity Score using 28 joints and erythrocyte sedimentation rate, ESR), ESR, C-reactive protein (CRP), rheumatoid factor (RF), antinuclear antibody (ANA) and medication use. The DAS-28-ESR values were interpreted as follows: < 2.6 = clinical remission; ≤ 3.2 = mild disease activity; < 5.2 = moderate disease activity; and > 5.2 = severe disease activity.[8]

Data were compiled in frequency and contingency tables. Frequencies were expressed as percentages, while central trend measurements were expressed as means and standard deviations (SD) or as medians and interquartile ranges (IQRs), according to the sample distribution. Comparative analyses on nominal data were done using the chi-square test and Fisher's test. Numerical data were compared by means of unpaired t and Mann-Whitney tests. The Spearman test was used to make correlations among numerical variables. The significance level was taken to be 5%. The calculations were done with the help of the GraphPad Prism software, version 5.0.

RESULTS

a) Description of study sample

In the study period, 200 participants were included, 100 in each group. The pairing of the RA patients and controls and the epidemiological data on these subjects are shown in **Table 1**.

The median duration of the disease among the sample of RA patients was 9.5 years (range: 1 to 54 years). Among these patients, 56.3% were positive for RF and 32% were positive for ANA.

The median DAS-28-ESR score was 3.13 (IQR = 2.45-4.75), with a range from 0.49 to 8.49. The median ESR was 26 mm/h, with

Table 1. Comparison of epidemiological data between rheumatoid arthritis (RA) patients and controls

	RA n = 100	Controls n = 100	P
Female gender (%)	82	82	1.0(*)
Mean age in years (range)	55.2 ± 11.6 (20-76)	53.5 ± 8.98 (36-76)	0.25 (**)
Ethnic background (%)	Caucasians: 69 African descendants: 29 Asians: 2	Caucasians: 69 African descendants: 16 Asians: 3	0.08 (*)
Tobacco exposure (%)	24	19	0.46 (*)
Mean BMI in kg/m^2 (range)	27.7 ± 4.41 (15.8-43.9)	27.02 ± 4.96 (17.7-46.8)	0.28 (**)

(*) Chi-square test; (**) unpaired t test; BMI = body mass index; n = number.

a range from one to 105 mm; and the median CRP concentration was 6 mg/dl, with a range from 0.1 to 54 mg/dl.

The treatment profile showed that 18% of the RA patients were treated with antimalarial drugs, 72% with methotrexate, 42% with leflunomide, 31% with anti-TNFα and 1% with other biological drugs.

b) Comparison of FFI-BR and FHSQ-BR results between RA patients and controls

The comparison between the scores of RA patients and controls, from the FFI-BR and FHSQ-BR instruments, is shown in **Table 2**. This table shows that the RA patients presented worse foot function in all the domains studied, in both questionnaires.

c) Correlation between foot function and RA variables

The correlations relating to both questionnaires are shown in **Table 3**. These showed that all the domains of the FFI-BR correlated with the disease activity measured using DAS-28-ESR. This correlation was also shown for all FHSQ-BR domains except for the patient's social capacity and use of shoes.

Table 2. Scores from the Brazilian versions of the Foot Function Index (FFI-BR) and Foot Health Status Questionnaire (FHSQ-BR) compared between rheumatoid arthritis (RA) patients and controls

	RA (*) n = 100	Control (*) n = 100	P (Mann-Whitney test)
FFI-BR			
Median incapacity	19.0 (0-37.5)	0 (0-0)	< 0.0001
Median difficulty	53.3 (24.4-73.3)	8.33 (0-22.2)	< 0.0001
Median pain	51.4 (28.5-71.4)	15.0 (1.78-28.5)	< 0.0001
FHSQ-BR			
Foot pain	54.3 (18.75-78.13)	79 (54.0-97.0)	< 0.0001
Foot function	68.7 (37.5-87.5)	94 (75-100)	< 0.0001
Shoes	25 (0-58.3)	50 (25.0-75.00)	< 0.0001
General foot health	25 (18.75-60)	60 (25.0-85.0)	< 0.0001
General health	50 (30.0-70.0)	60 (50.0-90.0)	0.0002
Physical activity	50 (27.78-66.67)	83 (67-94)	< 0.0001
Social capacity	75 (37.5-100)	88 (75-100)	0.0006
Vigor	50 (25-75)	62 (38-75)	0.01

(*) All values are stated as medians and interquartile ranges.

It was also found that the total score of the FFI-BR questionnaire did not have any association with body mass index (BMI) (P = 0.17), presence of RF (P = 0.49) or presence of ANA (P = 0.30).

There was no association between the domains of the FHSQ-BR and the presence of RF and ANA (P = 0.49 and 0.30 respectively). BMI showed a modest correlation with foot pain (Spearman rho = -0.24; 95% CI = -0.45 to -0.04; P = 0.01), but this was unrelated to the other domains studied, for which P was non-significant in all cases.

DISCUSSION

In this study, foot dysfunction evaluated using the FFI-BR questionnaire was higher in the RA group than in the control group. The FFI-BR questionnaire evaluates simple activities such as walking around the house and on uneven ground, standing on tiptoes and getting up from a chair. It was observed that the RA patients reported difficulties that were almost seven times greater than those of the controls, with median scores of 53.3 versus 8.3, respectively. Reported foot pain, as assessed using the FFI-BR and including pain in the morning, at the end of the day or when standing, was greater among the patients than among the controls, with median scores of 51.4 versus 15, respectively. Dysfunction and pain generate disability, which in this study was also higher among the patients than among the controls. Foot dysfunction measured using the FFI-BR shows individuals' limitations regarding going out from their homes, along with their need to use orthopedic devices and their loss of independence in relation to simple day-to-day activities.

Table 3. Correlation between scores from the Brazilian versions of the Foot Function Index (FFI-BR) and Foot Health Status Questionnaire (FHSQ-BR) and the inflammatory disease activity measured from the Disease Activity Score using 28 joints and erythrocyte sedimentation rate (DAS-28-ESR)

	Rho (Spearman)	95% CI	P
FFI-BR			
Total score	0.45	0.27-0.60	< 0.0001
Median incapacity	0.46	0.28-0.61	< 0.0001
Median difficulty	0.42	0.23-0.57	< 0.0001
Median pain	0.37	0.17-0.53	0.0002
FHSQ			
Foot pain	-0.40	-0.5 to -0.21	< 0.0001
Foot function	-0.34	-0.51 to -0.15	0.0005
Shoes	-0.19	-0.38 to +0.01	0.06
General foot health	-0.26	-0.44 to -0.05	0.009
General health	-0.29	-0.47 to -0.09	0.03
Physical activity	-0.44	-0.60 to -0.26	< 0.0001
Social capacity	-0.15	-0.35 to +0.005	0.13
Vigor	-0.33	-0.50 to -0.13	0.001

CI = confidence interval.

A similar trend was found regarding foot function measured using the FHSQ-BR questionnaire. Although impaired, the function of our patients' feet proved to be better than was seen in Ferreira's sample,[9] among patients from a reference outpatient clinic in São Paulo. In that study, the score was 48, while in the current study it was 68.7.[9] Use of new drugs for treating RA and more aggressive treatment strategies that enable better control over the inflammatory activity of RA may explain this difference.

Impairment of RA patients' motor capacity can compromise their lifestyle, through mechanical difficulties and insecurity, thereby depriving them of their daily activities, social living and ability to access different places.[10] Additionally, this impairment leads to increasing dependence on family support and consequent frustration and anger, which promote social isolation, emotional instability and depression.[11] Anxiety and depression are associated with reduced physical activity and sedentary lifestyle, and thus their presence ends up worsening these individuals' general health in the long run.[12,13] This was reflected, in our study, in the social capacity assessed through the FHSQ-BR questionnaire, which showed worse scores in the group of patients than among the controls. In comparison with Ferreira's sample, our patients had better results regarding sociability, thus showing that those with worse foot health also had worse social performance.[9]

In evaluating the shoes domain of the FHSQ-BR questionnaire, RA patients reported that they had twice as much difficulty in finding adequate shoes, compared with the control group, and that they could not use all types of shoes. The complaints about shoes were even worse in Ferreira's sample, in which the patients scored 5.8, i.e. around five times lower than in the present study.[9] This difference may have been related to fewer footwear options at that time or to less knowledge about the subject.

Some of the difficulties found by RA patients regarding shoes probably resulted from the fact that the shoe industry focuses on esthetics rather than comfort and functionality, thus leaving the population with special needs without any choice. The alternative is to search for orthopedic shoes and insoles that are customized for patients, even though their cost is much higher than that of ordinary shoes. Moreover, the greatest challenge in relation to adherence to orthopedic shoes is patients' own resistance to them, due to their unattractive appearance.[14] Many RA patients find it difficult to tie shoe laces because of hand deformities: for these individuals, footwear with Velcro fastenings or elastic openings are options of greater interest. Non-slip soles can also help in fall prevention. In addition, feet also have esthetic importance; deformities may contribute towards greater personal dissatisfaction, especially among women, who are more affected by this rheumatic disease.[11]

It was evident from both questionnaires in our study that disease activity, as evaluated using DAS-28-ESR, affected foot health. Thus, strict control over disease activity is a way to avoid loss of foot function, and this needs to be implemented from the time of the initial diagnosis.

Body mass index (BMI) usually affects foot function. An excess of mechanical loading on the knee, ankle and foot joints worsens local pain.[15] Additionally, obese patients' response to treatment is worse, with lower likelihood of remission from the disease.[16] Therefore, stimulating weight loss among obese or overweight patients contributes towards their physical and emotional well-being and demonstrates care for the patient's health as a whole.[16] Interestingly, in our study, only the pain assessed according to the FHSQ-BR questionnaire correlated with the BMI value. One possible hypothesis for explaining this is that the degree of inflammation had such an important influence that it caused foot dysfunction even in individuals with low BMI.

This study had limitations and may not have accurately represented patients with RA in general because it was conducted in an outpatient clinic at a tertiary-care hospital, where patients have access to specialized and effective treatment. Greater severity of disease due to uncontrolled disease activity may be seen outside this sample. The Health Assessment Questionnaire (HAQ), an instrument that is used to study functional status in rheumatic diseases,[17] was not used in the present analysis and this was therefore a limitation of this study. Another limitation was the subjectivity of the questionnaires, since ascertaining the dimensions of patients' pain depended directly on the patients' understanding of the questions and on their capacity to understand scales.

Negligence of the impact of foot health on patients' quality of life occurs because feet dysfunction is often interpreted as being natural to the aging process and to the disease. This leads to omission of treatment and delays to it, in this segment of the body.[13]

A holistic approach with multidisciplinary treatment, social support and public health policies combating architectural barriers may help RA patients to ensure their autonomy and ability to function as citizens through ensuring their mobility and independence.

CONCLUSION

We concluded that patients with RA had worse scores in the FFI-BR and FHSQ-BR foot health questionnaires, compared with individuals who did not have the disease. Inflammatory activity is a major determinant of the health of this body segment and it needs to be brought under control in order to improve foot health.

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Address for correspondence:
Elinah Narumi Inoue
Faculdade Evangélica do Paraná (FEPAR)
Rua Padre Anchieta, 2.690
CEP 80730-000
Curitiba (PR) — Brasil
E-mail: elinahn.inoue@gmail.com

Expression of M30 and M65 in celiac disease. Analytical cross-sectional study

Evrim Kahramanoğlu Aksoy[I], Gülçin Güler Şimşek[II], Murat Torgutalp[III], Ferdane Pirinççi Sapmaz[IV], Muhammet Yener Akpınar[V], Metin Uzman[VI], Yaşar Nazlıgül[VII]

Keçiören Training and Research Hospital, Ankara, Turkey

[I]MD. Gastroenterologist, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0001-8887-3428
[II]MD. Associate Professor and Pathologist, Department of Pathology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0001-7710-4631
[III]MD. Rheumatological Researcher, Department Rheumatology, Ankara University Faculty of Medicine, Ankara, Turkey.
orcid.org/0000-0003-4600-9484
[IV]MD. Associate Professor and Gastroenterologist, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0003-1278-110X
[V]MD. Gastroenterologist, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0003-0903-4664
[VI]MD. Associate Professor and Gastroenterologist, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0002-5412-8523
[VII]MD. Professor and Gastroenterologist, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0003-1926-4594

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M65 antigen, human.

ABSTRACT

BACKGROUND: The role of villous atrophy in apoptosis, a distinctive feature of celiac disease, is a matter of controversy. The aim of this study was to determine the apoptosis rate through immunohistochemical staining for M30 and M65 in celiac disease cases.

DESIGN AND SETTING: Analytical cross-sectional study in a tertiary-level center.

METHODS: Duodenal biopsies from 28 treatment-naive patients with celiac disease, 16 patients with potential celiac disease, 10 patients with a gluten-free diet and 8 controls were subjected to immunohistochemical staining for the end-apoptotic marker M30 and the total cell death marker M65. *H*-scores were compared. Several laboratory parameters were recorded concomitantly, and at the one-year follow-up for celiac disease and potential celiac disease patients.

RESULTS: There was a significant difference in *H*-score for M30 expression between the celiac disease, potential celiac disease and gluten-free diet groups (P = 0.009). There was no significant difference in *H*-score for M65 expression. There was a positive correlation between the *H*-score for M30 expression and the anti-tissue transglutaminase immunoglobulin A (anti-tTgIgA) and anti-tissue transglutaminase immunoglobulin G (anti-tTgIgG) levels (R = 0.285, P = 0.036; and R = 0.307, P = 0.024, respectively); and between the *H*-score for M65 expression and the anti-tTgIgA and anti-tTgIgG levels (R = 0.265, P = 0.053; and R = 0.314, P = 0.021, respectively). There was no difference between celiac disease and potential celiac disease patients regarding the laboratory parameters selected.

CONCLUSION: The rates of apoptosis and nutritional deficiencies in patients with potential celiac disease were similar to those in patients with celiac disease.

INTRODUCTION

Celiac disease is an immune-mediated disease triggered by gluten exposure in genetically susceptible people.[1] The clinical symptoms are variable, ranging from classical malabsorption symptoms to atypical presentations and asymptomatic forms that are detected incidentally during serological screening.[2,3] Serum anti-tissue transglutaminase (anti-tTg) immunoglobulin A (anti-tTgIgA), serum anti-tissue transglutaminase immunoglobulin G (anti-tTgIgG) and anti-endomysial antibodies (EmA) are widely used for serological screening.[4] Whereas the diagnosis of celiac disease can be made among children in the presence of very high levels of anti-tTg antibodies (i.e. > 10 times above the upper normal limit), a duodenal biopsy is essential for this diagnosis among adults. Human leukocyte antigen (HLA) typing needs to be done and histological changes after a gluten-free diet need to be seen in order to confirm the diagnosis in patients who are seronegative for celiac disease.[5,6]

While mucosal villous atrophy with crypt hyperplasia (Marsh 3) is defined as celiac disease, normal mucosa (Marsh 0), intraepithelial lymphocytosis (Marsh 1) and intraepithelial lymphocytosis with crypt hyperplasia (Marsh 2) in seropositive and genetically predisposed patients are defined as cases of potential celiac disease.[7] Although vitamin and mineral deficiencies are seen both in patients with celiac disease and in those with potential celiac disease, most clinicians only recommend a gluten-free diet for patients with celiac disease. Kurppa et al.[8-10] showed the favorable effects of a gluten-free diet in terms of improvement of clinical signs, mucosal healing and antibody titer reduction.

Villous atrophy is the hallmark of celiac disease. It results from increased enterocyte destruction and deficient epithelial cell regeneration. Several studies have mentioned that apoptosis has a

role in villous atrophy.[11,12] While higher rates of apoptosis in celiac disease cases were shown in several studies, Augustin et al. were unable to demonstrate higher apoptotic activity.[11,13,14] Das et al. indicated that the rates of apoptosis in patients with mild enteropathy and advanced enteropathy were similar.[15]

M30 is a caspase-cleaved keratin 18 (CK-18) cytoskeletal protein that is known to be a marker for the end of apoptosis. M65 comprises both cleaved and uncleaved CK-18 and is used as a marker for total cell death in situations of both necrosis and apoptosis.[16]

OBJECTIVE

In this study, we aimed to investigate the apoptosis rate as a marker of disease severity through immunohistochemical staining for M30 and M65 in the duodenal mucosa of patients with potential celiac disease and patients with a gluten-free diet, in comparison with patients with celiac disease; and to investigate whether there might be any difference between the groups in terms of nutritional deficiency.

METHODS

Design and setting

This was an analytical cross-sectional study and data were collected from the laboratory and pathology archives of Keçiören Training and Research Hospital. Newly diagnosed celiac disease patients, patients with a gluten-free diet and patients with potential celiac disease who came for consultations at our gastroenterology outpatient clinic between 2010 and 2017 were included the study and compared regarding apoptosis rate in the gut. All patients were followed up for at least one year. Some selected laboratory parameters (hemoglobin, iron, ferritin, folate, vitamin B12, 25-hydroxy-vitamin D3, albumin, calcium, magnesium and phosphorus) were compared at diagnosis and after one year of follow-up among newly diagnosed celiac disease patients and patients with potential celiac disease.

Ethical considerations

This study was approved by an internal review board on June 9, 2017, under the approval number 43278876-929-441-3137, and informed consent was obtained from all patients.

Participants

The sample size was calculated using 90% power and 1% margin of error, as described in the study by Shalimar et al., which used 3 patients for villus evaluation and 28 patients for crypt evaluation.[11] A total of 54 participants were included: 28 treatment-naive patients with celiac disease, 16 patients with potential celiac disease and 10 patients who had been using a gluten-free diet.

The diagnosis of celiac disease was made according to the presence of clinical symptoms with positive results for anti-tTgIgA or anti-tTgIgG and presence of villous atrophy (Marsh 3). All of these patients had previously been receiving a gluten-free diet since receiving their diagnoses. The diagnosis of potential celiac disease was made according to the presence of antibody seropositivity and Marsh 0, 1 or 2 criteria in pathological examinations. The study also included 10 previously diagnosed celiac disease patients who had been using a gluten-free diet for 2-8 years. Six of these patients had duodenitis and four of them had a normal appearance in endoscopic examinations. Three of these patients had borderline positive antibody serological findings and Marsh 0 appearance in pathological examinations. Patients with refractory celiac disease, coexistent systemic diseases, human immunodeficiency virus seropositivity or positive stool tests for parasitic infections were excluded.

Eight patients with dyspepsia who underwent esophagogastroduodenoscopy were recruited as controls. The individuals included in this control group were firstly matched for age and gender. They all presented negative results from endoscopic examinations. Multiple duodenal biopsy samples were obtained from the bulbus and the second part of the duodenum. All the controls were negative for anti-tTgIgA and anti-tTgIgG. Their laboratory parameters were normal and their duodenal biopsy specimens showed normal morphology. They were diagnosed as having functional dyspepsia and it was confirmed that they were not using a gluten-free diet.

Variables

Demographic data, serological (serum anti-tTgIgA and anti-tTgIgG levels), biochemical and hematological parameters and clinical and pathological data were obtained from the file records. Serum anti-tTgIgA and anti-tTgIgG levels were measured by means of the enzyme-linked immunosorbent assay (ELISA) (ImmuLisa, Immco, USA). Anti-tTgIgA or anti-tTgIgG antibody levels > 18 U/ml were accepted as positive and levels between 12 and 18 U/ml were accepted as borderline. Upper gastrointestinal endoscopic views of the duodenal folds were recorded (normal, attenuated and scalloped mucosal folds).

Histological examination

Mucosal biopsies were processed in an automated tissue processor and were set into paraffinized tissue blocks. Hematoxylin and eosin (H&E)-stained slides were prepared from the biopsy samples. Biopsy specimens were considered to be sufficient if at least 3-4 crypts were seen, arranged on the muscularis mucosae. All the duodenal biopsy specimens were evaluated by the same pathologist, who was experienced in celiac disease and was unaware of the patients' clinical data. The modified Marsh grading system described by Oberhuber was used to grade the

mucosal changes for both the end of apoptosis (M30) and total cell death, i.e. apoptosis and necrosis (M65).[17]

Immunohistochemistry

Poly-l-lysine coated slides were prepared from 4-5 µm sections from the paraffin-embedded blocks and were processed for immunohistochemical (IHC) evaluation. All of these slides were subjected to IHC staining for: 1) the end-apoptosis marker protein M30 (CytoDEATH antibody) for detection of caspase-cleaved cytokeratin 18 neo-epitope M30 (PEVIVA, Sweden; 1:100); and 2) the end-apoptosis and necrosis marker protein M65 for detection of uncleaved cytokeratin 18 (LifeSpan Biosciences, Inc., Seattle, WA, USA). A standard overnight IHC staining protocol was applied and the universal secondary antibody (REAL EnVision System, DAKO, Glostrup, Denmark) was used. 3,3-diaminobenzidine and hydrogen peroxide were used for immunostaining.

The IHC-stained sections were described in terms of the distribution and intensity of the staining of the villi in the mucosal biopsies. The intensity of marker staining was graded as follows: grade 1, pale stain expression; grade 2, moderate stain expression; or grade 3, robust stain expression. The distribution of marker staining was graded as follows: grade 0, from no staining to < 10% mucosal area positivity; grade 1, 11%-25% area positivity; grade 2, 26%-60% area positivity; or grade 3, ≥ 61% area positivity. The *H*-scores for villi were calculated by multiplying together the stain distribution grade and intensity grade in a single biopsy that contained both villi and crypt epithelium. All the biopsies were examined by one experienced pathologist.

Statistical analyses

Statistical analyses were performed using the computer software Statistical Package for the Social Sciences (SPSS), version 22.0 (IBM, Armonk, NY, USA). Normally distributed continuous variables were expressed as the mean and standard deviation, while non-normally distributed variables were expressed as the median and interquartile range (IQR). Comparisons were made using the Mann-Whitney U test or Kruskal-Wallis test if the distribution of the variables was not normal; and the t test or one-way ANOVA were used if the distribution of the variables was normal. Categorical data were presented as proportions, and the chi-square test or Fisher's exact test was used to compare proportions in different groups. P-values < 0.05 were taken to be statistically significant.

RESULTS

Baseline characteristics of participants

Mucosal biopsies were obtained from 28 patients with celiac disease, 16 patients with potential celiac disease and 10 patients with a gluten-free diet; and from eight patients with dyspepsia who formed a control group for the study. The demographic data and data from some selected laboratory parameters among the patients (including those with celiac disease, potential celiac disease and gluten-free diet) are summarized in **Table 1**. The endoscopic findings and Marsh scores are shown in **Table 2**.

Table 1. Demographic properties and some selected laboratory parameters of the patients and control group

	Patients (n = 54)	Control (n = 8)	P
Age, years	30.4 ± 9.1	28.6 ± 5.4	0.59
Female sex, %	35 (64.8%)	5 (62.5%)	1.0
Height, cm	162.2 ± 7.5	165 ± 7.0	0.23
Weight, kg	59.4 ± 8.2	65.9 ± 10.1	0.12
BMI, kg/m^2	22.5 ± 1.7	23.9 ± 2.1	0.09
Anti-tTgIgA (IQR) (U/ml)	147.5 (235)	2.5 (1.68)	< 0.001
Anti-tTgIgG (IQR) (U/ml)	90 (96.25)	3.34 (2.03)	< 0.001
Hemoglobin, g/dl	12.4 ± 2.1	14.4 ± 0.5	0.009
Ferritin (IQR), ng/ml	11.3 (15.6)	46.5 (14.5)	0.001
Iron, µg/dl	53.3 ± 38.9	88.6 ± 15.7	< 0.001
Vitamin B12, pg/ml	220.6 ± 85.2	270.5 ± 50.4	0.113
25OHD3, ng/ml	13.3 ± 7.2	30.7 ± 5.8	< 0.001
Folate, ng/ml	4.7 ± 2.1	7.1 ± 0.8	< 0.001
Albumin, g/dl	3.9 ± 0.3	4.2 ± 0.3	0.11
Calcium, mg/dl	8.7 ± 0.6	9.0 ± 0.3	0.037
Magnesium, mg/dl	1.9 ± 0.2	2.1 ± 0.1	0.31
Phosphorus, mg/dl	2.9 ± 0.6	2.8 ± 0.4	0.74

BMI = body mass index; Anti-tTgIgA = anti-transglutaminase immunoglobulin A antibodies; IQR = interquartile range; Anti-tTgIgG = anti-transglutaminase immunoglobulin G antibodies; 25OHD3 = 25-hydroxy-vitamin D3.

Table 2. Numbers of participants according to endoscopic findings and Marsh grade (P = 0.053; and R = 0.314, P = 0.021, respectively)

Numbers of participants	CD (n = 28)	PCD (n = 16)	GFD (n = 10)	Controls (n = 8)
Endoscopic findings				
Duodenitis	4	5	6	5
Attenuated duodenal folds	11	7		
Scalloping of duodenal folds	9			
Normal duodenal folds	4	4	4	3
Marsh grade				
Marsh 0		2	10	
Marsh 1		8		
Marsh 2		6		
Marsh 3a	9			
Marsh 3b	7			
Marsh 3c	12			

CD = celiac disease; PCD = potential celiac disease; GFD = gluten-free diet.

Expression of M30 and M65 markers

There was no stain expression in the mucosal biopsies of the control group (**Table 3**). Altogether, there was a significant difference in *H*-score for M30 expression between the celiac disease, potential celiac disease and gluten-free diet groups (P = 0.009). However, there was no significant difference specifically between the celiac disease and potential celiac disease groups (P = 0.25). The statistical difference found in the overall group comparison was due to differences between the celiac disease and gluten-free diet groups (P = 0.001) and between the potential celiac disease and gluten-free diet groups (P < 0.001). Although there was a difference in *H*-score for M65 expression, this was not statistically significant (P = 0.053). There was only a significant difference between the potential celiac disease and gluten-free diet group (P = 0.04) (**Table 4**; **Figures 1** and **2**).

Laboratory parameters

The mean serum levels of ferritin, iron, vitamin B12, 25-hydroxy-vitamin D3, folate and calcium, and the interquartile range (IQR) levels of anti-transglutaminase antibodies for IgA and IgG, were recorded at the time when the mucosal biopsy samples were taken. There were statistically significant differences in these parameters except for the serum vitamin B12 levels between the celiac disease, potential celiac disease and gluten-free diet groups (**Table 5**). In comparing some selected laboratory parameters between the time when the mucosal biopsy samples were taken and one year after that time, for the celiac disease and gluten-free diet groups, there was no significant difference. There were significant differences in hemoglobin and vitamin B12 levels over the first year of follow-up (P = 0.006 and P < 0.001 respectively) (**Table 6**).

There was a positive correlation between the *H*-score for M30 expression and the anti-tTg antibody levels for IgA and IgG (R = 0.285, P = 0.036; and R = 0.307, P = 0.024, respectively). Again, there was a positive correlation between the *H*-score for M65 expression and the anti-tTg antibody levels for IgA and IgG (R = 0.265, P = 0.053; and R = 0.314, P = 0.021, respectively) (**Figures 3** and **4**). There was no correlation between the *H*-scores for M30 and M65 expression and the levels of some laboratory parameters.

Table 3. *H*-scores for M30 and M65 expression in participants

	Patients (n = 54)	Controls (n = 8)	P
H-score for M30 expression	3.8 ± 2.1	0	< 0.001
H-score for M65 expression	4.1 ± 2.0	0	< 0.001

Table 4. Comparison of *H*-scores for M30 and M65 expression between patient groups

	CD (n = 28)	PCD (n = 16)	GFD (n = 10)	P
H-score for M30 expression	3.9 ± 2.6	4.7 ± 1.6	2.2 ± 0.9	0.009
CD-PCD (P = 0.25) / CD-GFD (P = 0.001) / PCD-GFD (P < 0.001)				
H-score for M65 expression	3.9 ± 1.9	5.1 ± 2.4	3.2 ± 1.5	0.053
CD-PCD (P = 0.08) / CD-GFD (P = 0.30) / PCD-GFD (P = 0.04)				

CD = celiac disease; PCD = potential celiac disease; GFD = gluten-free diet.

DISCUSSION

In the present study, we observed higher expression of the markers for both the end of apoptosis (M30) and total cell death, i.e. apoptosis and necrosis (M65), in patients with celiac disease and potential celiac disease in comparison with controls and patients with a gluten-free diet. Moreover, we did not find any significant difference in the severity of apoptosis and necrosis between patients with celiac disease and those with potential celiac disease.

There was a significant difference in nutrient deficiency levels between the patients and the control group. Comparison between the patient groups did not show any significant difference between patients with celiac disease and those with potential celiac disease regarding nutritional deficiencies. In addition, we found a positive correlation between the *H*-scores for M30 and M65 expression and the serum anti-tTg antibody levels.

Similar mucosal injuries and nutritional deficiencies were detected objectively in the patients with celiac disease and potential

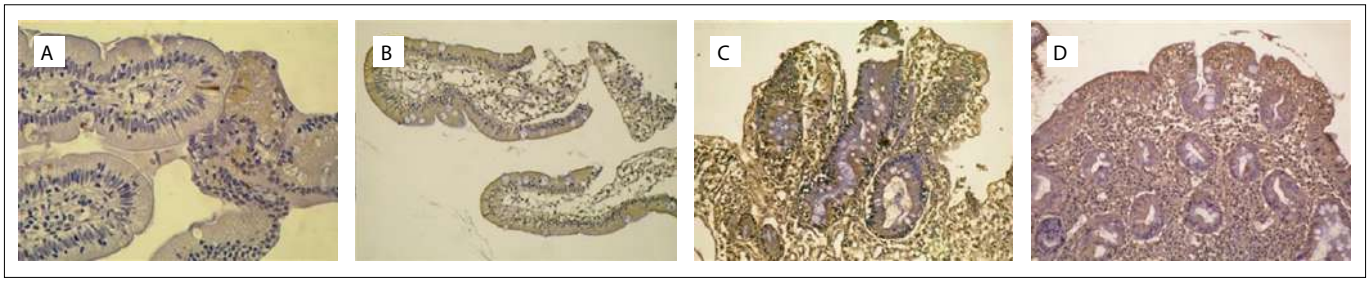

Figure 1. M30 staining, showing positivity of epithelial cytoplasm in the duodenal mucosa of the following patients: a) with a gluten-free diet (Marsh 0) [100 X]; b) with potential celiac disease (Marsh 1) [40 X]; c) with potential celiac disease (Marsh 2) [100 X]; and d) with celiac disease (Marsh 3c) [100 X].

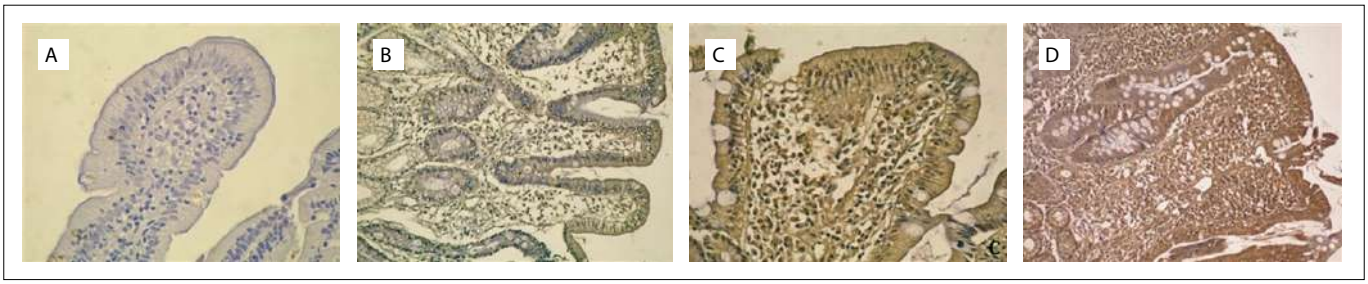

Figure 2. M65 staining, showing positivity of epithelial cytoplasm in the duodenal mucosa of the following patients: a) with a gluten-free diet (Marsh 0) [100 X]; b) with potential celiac disease (Marsh 1) [100 X]; c) with potential celiac disease (Marsh 2) [100 X]; and d) with celiac disease (Marsh 3c) [100 X].

Table 5. Differences in selected laboratory parameters between patient groups

	CD (n = 28)	PCD (n = 16)	GFD (n = 10)	P
Ferritin, ng/ml	16.9 ± 17.2	18.5 ± 12.1	40.7 ± 28.2	0.003
CD-PCD (P = 0.96) / CD-GFD (P = 0.003) / PCD-GFD (P = 0.012)				
Iron, µg/dl	42.0 ± 32.1	51.4 ± 38.8	88 ± 40	0.004
CD-PCD (P = 0.68) / CD- GFD (P = 0.003) / PCD-GFD (P = 0.037)				
25OHD3, ng/ml	11.7 ± 5.1	12.3 ± 7.9	19.2 ± 8.4	0.013
CD-PCD (P = 0.68) / CD-GFD (P = 0.003) / PCD-GFD (P = 0.039)				
Folate, ng/ml	4.1 ± 1.6	4.3 ± 2.0	6.6 ± 2.3	0.003
CD-PCD (P = 0.97) / CD-GFD (P = 0.003) / PCD-GFD (P = 0.009)				
Calcium, mg/dl	8.6 ± 0.6	8.6 ± 0.6	9.39 ± 0.6	0.007
CD-PCD (P = 0.99) / CD-GFD (P = 0.017) / PCD-GFD (P = 0.008)				
Anti-tTgIgA (IQR)	300 (148)	125 (133)	45 (45)	< 0.001
CD-PCD (P = 0.007) / CD-GFD (P < 0.001) / PCD-GFD (P < 0.001)				
Anti-tTgIgG (IQR)	104 (160)	103 (78)	34 (29)	0.001
CD-PCD (P = 0.80) / CD-GFD (P < 0.001) / PCD-GFD (P < 0.001)				
Vitamin B12, pg/ml	241.2 ± 99.6	184.2 ± 57.6	184.2 ± 57.6	0.10

CD = celiac disease; PCD = potential celiac disease; GFD = gluten-free diet; 25OHD3 = 25-hydroxy-vitamin D3; anti-tTgIgA = anti-transglutaminase immunoglobulin A antibodies; IQR = interquartile range; anti-tTgIgG = anti-transglutaminase immunoglobulin G antibodies.

Table 6. Differences between celiac disease and potential celiac disease patients regarding some selected laboratory parameters, at the time of diagnosis and after one year of follow-up

	CD		PCD		P*	P**
	At diagnosis	After one year of follow-up	At diagnosis	After one year of follow-up		
Hgb, g/dl	12.4 ± 2.1	13.4 ± 1.5	11.4 ± 2.6	11.7 ± 1.9	0.178	0.006
Iron, µg/dl	42.0 ± 32.1	67.2 ± 35.4	51.4 ± 38.8	79.5 ± 51.3	0.418	0.402
Ferritin, ng/ml	16.9 ± 17.2	28.9 ± 23.7	18.5 ± 12.1	30.3 ± 10.1	0.746	0.799
Folate, ng/ml	4.1 ± 1.6	4.9 ± 2.1	4.3 ± 2.0	4.5 ± 1.1	0.817	0.415
Vitamin B12, pg/ml	241.2 ± 99.6	341.9 ± 69.9	184.2 ± 57.6	252.6 ± 58.3	0.043	< 0.001
25OHD3, ng/ml	11.7 ± 5.1	18.9 ± 8.9	12.3 ± 7.9	21.9 ± 7.7	0.755	0.281
†Albumin, g/dl	3.9 ± 0.4		3.8 ± 0.6		0.804	
†Calcium, mg/dl	8.6 ± 0.6		8.6 ± 0.6		0.933	
†Magnesium, mg/dl	1.9 ± 0.2		1.9 ± 0.2		0.845	
†Phosphorus, mg/dl	2.9 ± 0.6		3.0 ± 0.3		0.756	

CD = celiac disease; PCD = potential celiac disease; P* = difference between CD and PCD at the time of diagnosis; P** = difference between CD and PCD after one year of follow-up; †differential assessment was not made because of missing data at one-year follow-up.

celiac disease in this study. The degree of micronutrient deficiencies did not correlate with the degree of apoptosis. This result was compatible with the findings of Deora et al., who showed that the degree of micronutrient deficiencies in children with celiac disease did not correlate with the degree of villous atrophy or with the serum titers of anti-tTgIgA antibodies.[17] Zanini et al.[18] demonstrated similar prevalences of anemia, folate deficiency, hypocholesterolemia and hypocalcemia between a group with villous atrophy and another group with mild enteropathy. Kurppa et al.[9,10] found that symptomatic, serological and sometimes histological recovery of diseased mucosa was achieved in patients with mild enteropathy when they started using a gluten-free diet. Imperatore et al.[19] demonstrated that asymptomatic potential celiac disease patients who continued to follow a diet containing gluten were at higher risk of developing villous atrophy and immune-mediated disorders.

It seems that potential celiac disease patients with high antibody levels (anti-tTg) present dynamic changes at the cellular level beyond what is seen through examination of the intestinal mucosa using optical microscopy. This situation may be the reason why nutritional deficiencies do not correlate with the degree of villous atrophy. Gluten-induced immune-mediated changes can be evaluated by means of apoptotic markers, even without

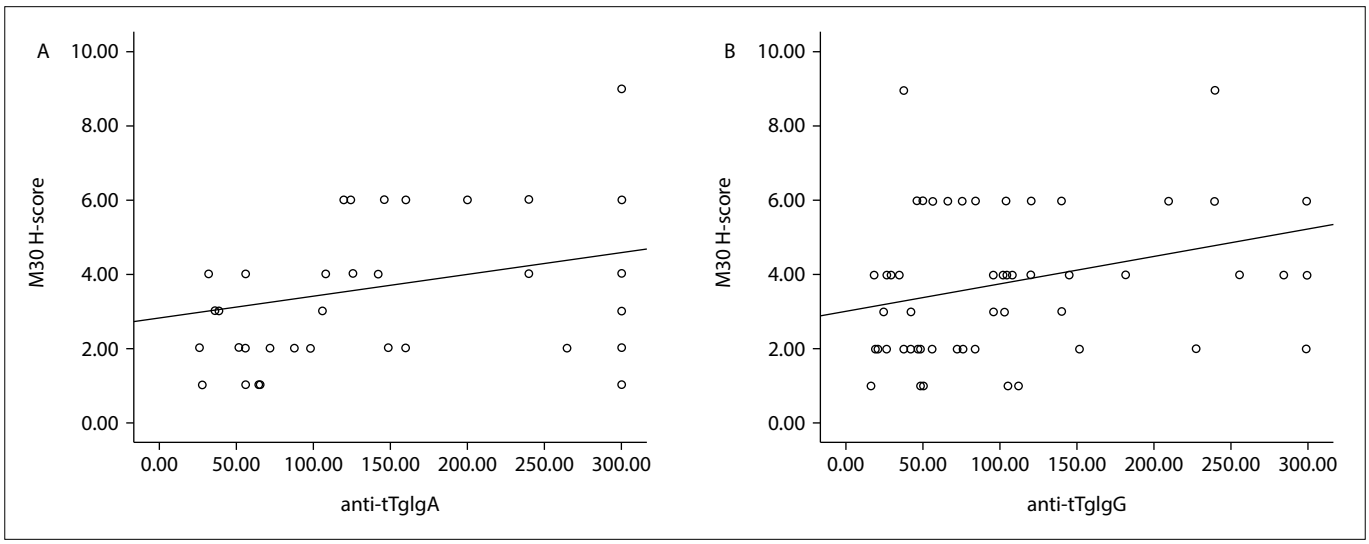

Figure 3. There were positive correlations between the H-score for M30 expression and: a) anti-tissue transglutaminase immunoglobulin A (anti-tTgIgA); b) anti-tissue transglutaminase immunoglobulin G (anti-tTgIgG) (R = 0.285, P = 0.036; and R = 0.307, P = 0.024, respectively).

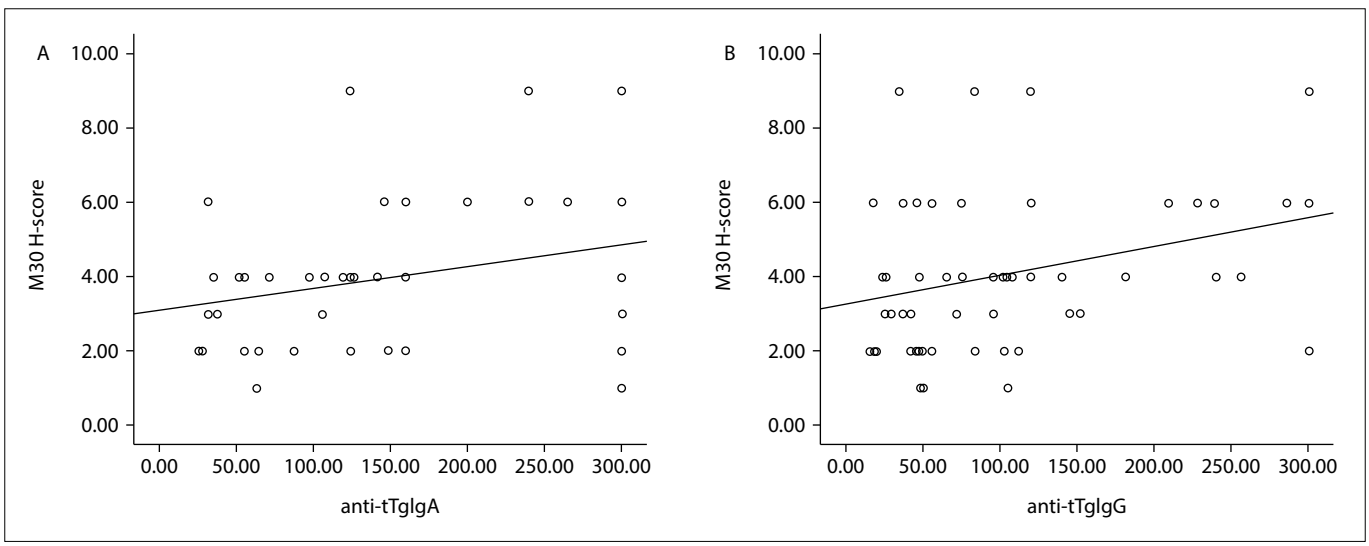

Figure 4. There were positive correlations between the H-score for M65 expression and: a) anti-tissue transglutaminase immunoglobulin A (anti-tTgIgA); b) anti-tissue transglutaminase immunoglobulin G (anti-tTgIgG) (R = 0.265, P = 0.053; and R = 0.314, P = 0.021, respectively).

the villous atrophy that is characteristic of celiac disease and the requirement to start using a gluten-free diet. Presence of these changes can also be used as a supportive diagnostic marker. Patients presenting such changes may need to start using a gluten-free diet.

Several previous studies have emphasized the importance of a gluten-free diet for achieving clinical and histological improvements in patients with mild enteropathy. Our findings revealed the importance of a gluten-free diet through showing that there was no difference between celiac disease and potential celiac disease patients and that there was a significant difference between patients with potential celiac disease and patients with a gluten-free diet, in terms of some selected laboratory parameters. Interestingly, the hemoglobin (13.4 ± 1.5 g/dl and 11.7 ± 1.9 g/dl, respectively/ $P = 0.006$) and vitamin B12 levels (341.9 ± 69.9 and 252.6 ± 58.3 pg/ml, respectively/ $P < 0.001$) of the potential celiac disease patients were significantly lower than those of the celiac disease patients at the one-year follow-up. This may have been due to the large amount of time that was spent on challenge testing and genetic testing to ensure that an accurate diagnosis of celiac disease was made before beginning a gluten-free diet. This may also be another reason why clinicians do not emphasize the importance of a gluten-free diet to patients with potential celiac disease as much as they emphasize it to patients with celiac disease.

The most important limitation of our study was the retrospective format. Another limitation of our study was that, although there were patients with a gluten-free diet, there was no evaluation of apoptosis in mucosal biopsy specimens from the same patients after follow-up with a gluten-free diet to evaluate the changes in apoptosis and necrosis. There were no previous studies validating the use of M30 and M65 in patients with celiac disease or intestinal disease that followed a course leading to villous atrophy. Multicenter prospective studies involving large numbers of patients with celiac disease and diseases with villous atrophy other than celiac disease are needed.

CONCLUSION

The rates of apoptosis and nutritional deficiencies in patients with potential celiac disease were similar to those in patients with celiac disease. Moreover, the apoptosis rate correlated with the anti-tTg levels. The apoptosis markers M30 and M65 may be candidate markers for celiac disease, especially for clinical follow-ups on celiac disease patients presenting histological remission.

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Address for correspondence:
Evrim Kahramanoğlu Aksoy
Department of Gastroenterology, Keçiören Training and Research Hospital
Pınarbaşı Mah., Sanatoryum Caddesi Ardahan Sokak D:25
Ankara — Turkey
Tel. 00905332121579
E-mail: evrims1979@yahoo.com

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Practice of exclusive breastfeeding and its associated factors in a suburban area in Angola: a cross-sectional study

Susana Valéria Dalcastagnê[I], Elsa Regina Justo Giugliani[II], Luciana Neves Nunes[III], Lisiane Hauser[IV], Camila Giugliani[V]

Postgraduate Program on Epidemiology, Federal University of Rio Grande do Sul, Porto Alegre (RS), Brazil

[I]MD, MSc. Physician, Community Health Service, Hospital Nossa Senhora da Conceição, Porto Alegre (RS), Brazil.
orcid.org/0000-0001-5150-7715

[II]MD, PhD. Physician, Assistant Professor, Department of Pediatrics, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre (RS), Brazil.
orcid.org/0000-0001-6569-6473

[III]PhD. Statistician and Associate Professor, Department of Statistics and Postgraduate Program on Epidemiology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre (RS), Brazil.
orcid.org/0000-0003-0151-1876

[IV]PhD. Statistician and Statistical Consultant, Telessaude-RS Scientific Technical Nucleus; and Professor, São Francisco de Assis College, Porto Alegre (RS), Brazil.
orcid.org/0000-0003-3324-5533

[V]MD, PhD. Physician and Assistant Professor, Department of Social Medicine and Postgraduate Program on Epidemiology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre (RS), Brazil.
orcid.org/0000-0002-2652-5214

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Breast feeding.
Infant nutrition disorders.
Angola.

ABSTRACT

BACKGROUND: Exclusive breastfeeding for six months is one of the measures with highest impact on prevention of child deaths. The determinants of breastfeeding practices are complex and differ between populations. This study aimed to identify factors associated with the prevalence of exclusive breastfeeding in a suburban area in Angola.

DESIGN AND SETTING: Population-based cross-sectional study in the municipality of Cacuaco, Luanda.

METHODS: A random sample of children under two years of age and their mothers was included. Prevalence ratios (PR) were estimated using Poisson regression based on a hierarchical model.

RESULTS: 749 children and their mothers were surveyed, including 274 children under six months. The prevalence of exclusive breastfeeding among children under six months was 51.5% (95% confidence interval, CI, 46.3-56.6%). Four variables were positively associated with exclusive breastfeeding at ages of under six months: number of prenatal visits (PR 1.11 for each visit after the first one; 95% CI 1.04-1.18), maternal occupation (other occupations versus self-employed) (PR 1.54; 95% CI 1.05-2.26), younger child age (PR 0.77 for each month; 95% CI 0.71-0.84) and female child (PR 1.34; 95% CI 1.02-1.76).

CONCLUSIONS: Our findings showed that the prevalence of exclusive breastfeeding at six months was satisfactory, according to international recommendations. Factors associated with exclusive breastfeeding practices that had never been surveyed before in Angola were identified through this study. These data are particularly relevant in the context of high infant mortality and may be useful in planning actions aimed at improving child health through promotion of exclusive breastfeeding, in Angola and other countries.

INTRODUCTION

Breastfeeding has proven to be an effective practice for preventing child deaths.[1,2] It has been estimated that scaling up breastfeeding to a near universal level could prevent 823,000 child deaths, along with 20,000 maternal deaths from breast cancer annually.[1] Breastfeeding also has a significant impact on reducing morbidity from infectious diseases, especially gastrointestinal and respiratory diseases. There is evidence showing that it has a protective effect against a variety of illnesses over the short and long terms, and showing that it promotes cognitive development.[1,3]

Over the last two decades, since the World Health Organization (WHO) issued its recommendation of exclusive breastfeeding (EBF) for the first six months of life, followed by introduction of complementary feeding but with continued breastfeeding until at least two years of age,[4] there has been a trend toward increased prevalence and duration of breastfeeding worldwide. However, the distribution of this trend has differed between different locations. The prevalence of exclusive breastfeeding in low-income and middle-income countries has increased by an average of 0.5% per year (from 24.9% in 1993 to 35.7% in 2013), and more sharply among more economically advantaged women.[1]

Breastfeeding practices can be influenced by historical, demographic, socioeconomic, cultural and individual factors. The success of breastfeeding depends on understanding these variables and on interventions at different levels.[5]

In Angola, a country still suffering the consequences of a civil war that lasted 27 years, there are few data on breastfeeding. Despite showing signs of socioeconomic recovery, this country has an under-five mortality rate of 167.4 deaths per 1000 live births: the highest rate in the world.[6,7]

Data from a national survey conducted in 2001 (United Nations Children's Fund [UNICEF] Multiple Indicator Cluster Survey [MICS]) showed that the prevalence of EBF was only 13.6% among children under four months of age.[8] However, no studies involving surveys of the determinants of exclusive breastfeeding in this country have been conducted.

To fill this gap, the present study aimed to identify factors associated with the practice of exclusive breastfeeding among children under six months of age in a municipality in the metropolitan area of Luanda, Angola. In addition, we estimated the prevalences of exclusive breastfeeding among children under 6 months, of breastfeeding among children under 24 months, and of continued breastfeeding from 12 to 24 months.

OBJECTIVE

The aim of this study was to identify factors associated with the prevalence of exclusive breastfeeding in a suburban area in Angola.

METHODS

This was a population-based cross-sectional study that was linked to a broader project entitled "Developing primary healthcare services in Angola: a proposal for assessment of the community health workers program," in which data were collected from August 1 to September 26, 2010.

The study was conducted in Cacuaco (700,000 inhabitants at the time of data collection),[9] which is a municipality in the metropolitan area of Luanda, the capital of Angola (total population of 25 million).[10] This location was chosen because it was the first municipality to implement the community health workers program.

The eligible participants were all children aged 0 to 23 months whose mothers lived in the survey area. If more than one child under two years of age lived in the same household, only the older child was included. In the case of twins, only the firstborn was included. Mothers who had lived in the survey area for less than one year or who did not live with their children were excluded.

Participants were considered lost to follow-up if their mothers were not found at home after at least three visits by interviewers to the household, on different days and at different times. They were considered to be refusals when their mothers refused to participate in the study.

For the original project, a sample of 700 children was calculated based on the estimated prevalence of some of the main outcomes under study (low body mass index-for-age and low height-for-age). Variation from 10% to 40% was assumed, at a precision level of 5% and considering a cluster design effect of 1.5. For the purposes of the present study, the prevalence of exclusive breastfeeding was assumed to depend on determinants that had previously been investigated in other countries in Africa, i.e. maternal education, family income, maternal occupation and child age.[11,12,13] Using the same parameters (precision level of 5% and cluster design effect of 1.5), a sample size of 72 to 486 participants was found to be necessary (the large variation is due to the large number of determinants tested).

Participants were recruited from four districts, which were selected based on the following criteria: availability of neighborhood maps, authorization by residents' committees and researcher safety. The districts were divided into microareas of approximately 100 households each. One household was randomly selected in each microarea as a starting point for the survey, and every third house to the right of the index house was then visited by the interviewers.

The Angolan interviewers underwent five days of intensive training, after which four teams were assembled, each consisting of a field coordinator, four interviewers and an area supervisor. A structured questionnaire was applied to the mother and additional data were obtained from pregnancy and child health cards. Standardized anthropometric measurements were obtained by properly trained field coordinators, using Tanita digital scales and custom-made wooden stadiometers. All questionnaires were coded, scanned and entered into a database using the Teleform software.

Exclusive breastfeeding was assessed among children under six months of age and breastfeeding among children under 24 months of age. In addition, breastfeeding was assessed among children aged 12 to 15.9 months (continued breastfeeding at 12 months) and among children aged 20 to 24 months (continued breastfeeding at 24 months). The information on breastfeeding that was obtained referred to the child's feeding habits on the day of the interview (current status). The indicators were calculated in accordance with the WHO references,[14] except for the prevalence of breastfeeding among children under 24 months of age, for which we thought it would be useful to describe the total prevalence in the sample.

The independent variables investigated are shown in **Figure 1**.

Economic status was assessed indirectly by means of a score that was obtained based on a previous study conducted in Ghana.[15] Through this score, the participating families were stratified into different economic levels. The score was used in this study as a continuous variable, ranging from 0 to 10.

Quantitative variables were expressed as medians and quartiles (Q1 and Q3), and categorical variables were expressed as frequencies and proportions with 95% confidence intervals (95% CI). The frequencies of the independent variables were also examined in the group of children under six months of age, stratified according to the rate of occurrence of exclusive breastfeeding.

Poisson regression with robust variance was used to estimate prevalence ratios (PR) for exclusive breastfeeding as an outcome, with the respective 95% CI. Variables were included in a multivariable model based on a hierarchical model (**Figure 1**) in which the exposure variables were classified into levels considering their proximity to the dependent variable, according to the conceptual

basis for possible interrelationships involving the factors under study.[16,17] At each step of the analysis, the variables were adjusted for others at the same level of the hierarchical model and for those showing statistically significant associations at a P-value of up to 0.20 at previous levels. In the final model, $P < 0.05$ was considered significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 18.0, and using Stata 9.

The present study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul (Universidade Federal do Rio Grande do Sul, UFRGS), under protocol no. 1025941, dated April 16, 2015. In this study, we analyzed secondary data from the database of the original project, which had been approved by the Research Ethics Committee of UFRGS under protocol no. 2008045, dated May 8, 2009. Data collection was authorized by the Health Department of Luanda Province, Angola. All participating mothers provided written informed consent to be interviewed for the study.

RESULTS

A total of 1,360 households were visited, in 49 microareas of four selected districts. Among the 911 eligible children in these households, 162 were excluded; 42 (5.7%) for the reasons described in the flowchart in **Figure 2**, 110 (15.0%) were lost to follow-up and in 10 cases (1.4%) the mothers refused to participate. The final sample consisted of 749 children and their mothers. **Table 1** shows the characteristics of the total sample (n = 749). **Table 2** describes the features of the children under six months of age according to whether exclusive breastfeeding was practiced (n = 269). **Figure 2** shows the flow of study subjects.

Out of the total of 274 children under six months of age, 141 were being exclusively breastfed at the time of the interview. Thus, the prevalence of exclusive breastfeeding among children under six months was 51.5% (95% CI 46.3-56.6%). Out of the 749 children in the total sample, 638 were breastfed, regardless of exclusivity, thus resulting in a prevalence of breastfeeding among children under 24 months of age of 85.2% (95% CI 82.4-87.7%). The prevalences of continued breastfeeding at 12 and 24 months were 88.5% (95% CI 81.7-93.4%) and 45.8% (95% CI 36.7-55.2%), respectively.

Table 3 shows the prevalence ratio for the practice of exclusive breastfeeding among children under six months of age adjusted for predictors, based on the hierarchical model. At the proximal level, the following variables were positively associated with the practice of exclusive breastfeeding: number of prenatal visits, maternal occupation (not being a housewife or self-employed), younger child age and female child.

DISCUSSION

The prevalence of exclusive breastfeeding among children under six months of age in our study population was 51.5%, which is considered a satisfactory rate according to the criteria adopted by WHO (50 to 89%).[18] This rate was considerably higher than the exclusive breastfeeding rate of only 13.6% among children under 4 months of age that was identified in 2001, in a national survey using data collected from 18 provinces in Angola.[8] It was also higher than the overall world prevalence (38%) and the mean prevalences that have been found in countries in the East Asia and Pacific region (30%), Latin America and Caribbean (32%), South Asia (47%), Sub-Saharan Africa (36%) and Central Africa (25%), the region in which Angola is located.[6]

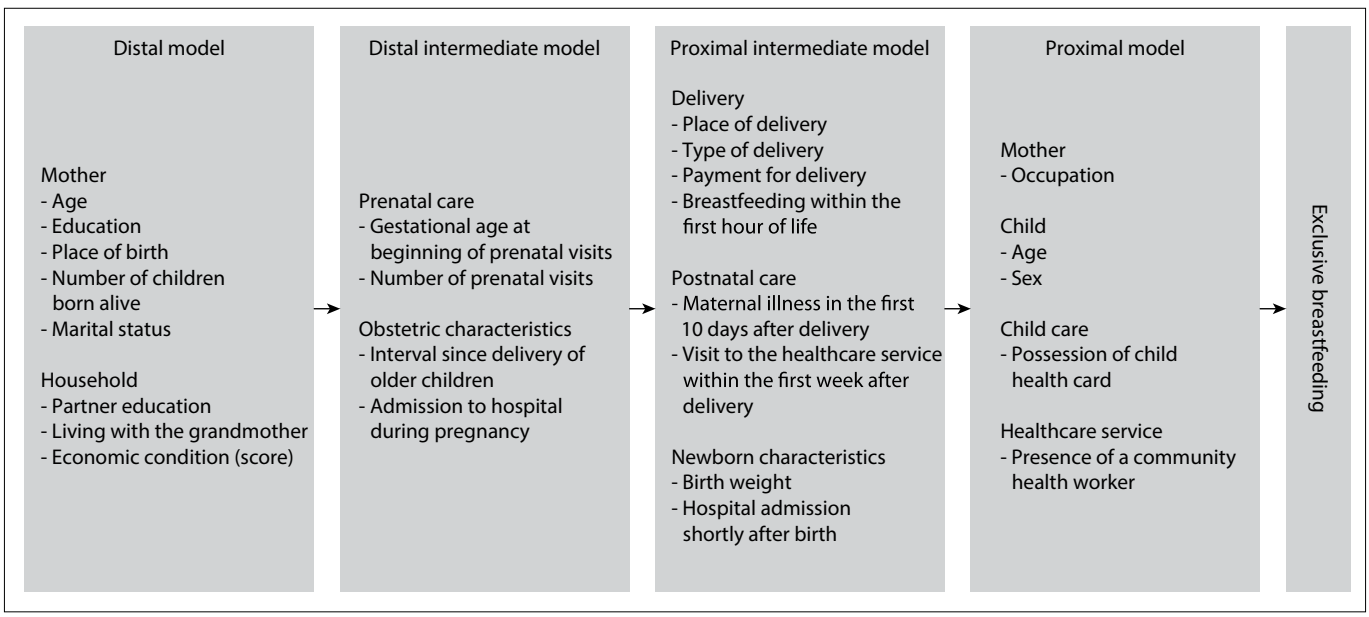

Figure 1. Hierarchical model of the determinants of exclusive breastfeeding.

In Africa, there is considerable heterogeneity in the prevalence of exclusive breastfeeding among countries. Based on the present results, in our study population, the prevalence of exclusive breastfeeding among children under 6 months of age was higher than that reported for Nigeria (17%), Congo (21%), Kenya (32%) and Mozambique (43%); comparable to that reported for Ethiopia (52%); and lower than that reported for Togo (62%), Uganda (63%) and Rwanda (85%).[6] The prevalence found in our setting may reflect post-war improvements in social and economic conditions as well as in healthcare services, especially within the scope of primary healthcare. The municipality where the present study was conducted was the first to implement the community health workers (CHW) program as part of the process of revitalizing municipal healthcare services in Angola. This program was developed in 2006 with the purpose of reducing maternal and child morbidity and mortality.[19] Investment in primary healthcare and improved access to health services are believed to have contributed towards dissemination of information on the importance of exclusive breastfeeding and on the harm done through early introduction of complementary foods, such as liquids, which is a common and culturally accepted practice.

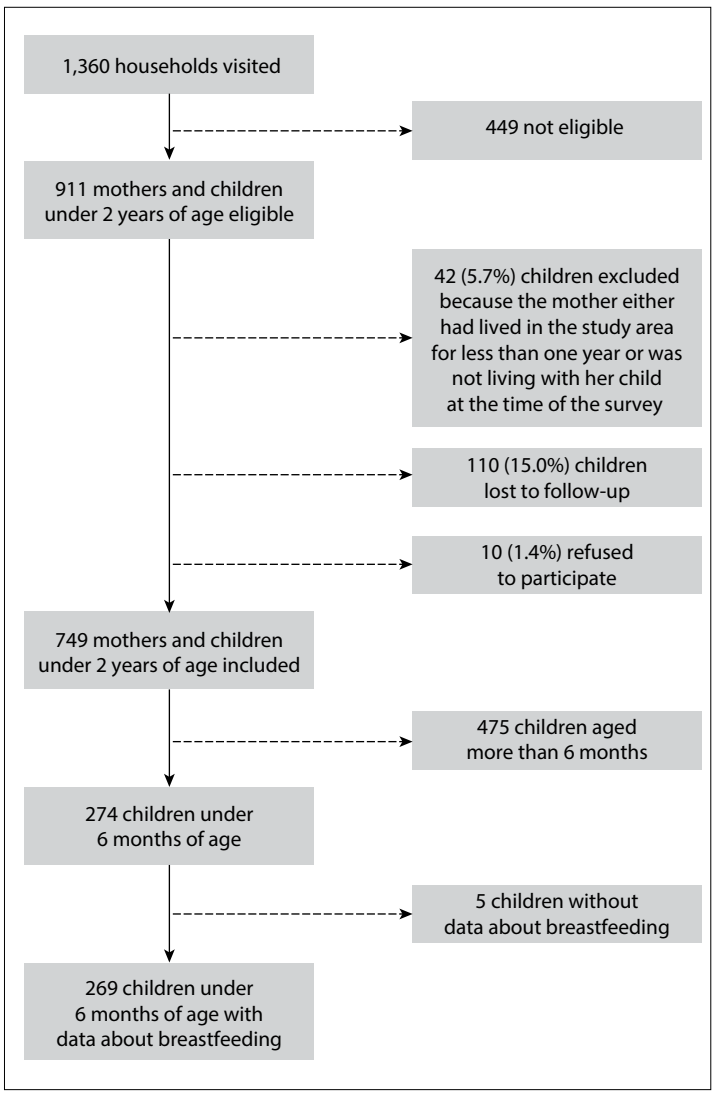

Figure 2. Flow diagram of study population.

The prevalence of continued breastfeeding at 24 months is also heterogeneous among African countries: 82% in Ethiopia, 54% in Kenya, 50% in Nigeria, 37% in Ghana, 31% in South Africa and only 17% in Congo.[6] Maintenance of breastfeeding for longer periods in Luanda may be explained, at least in part, by cultural issues and the positive effect of recent investments in healthcare. It is worth noting that, in Angola and other countries with high poverty rates, low household purchasing power makes it costly or even unfeasible for families to purchase other types of milk, which may contribute towards the high prevalence of breastfeeding.

In the present study, each prenatal visit after the first one was associated with an increase of 11% in the prevalence of exclusive breastfeeding among children under 6 months of age. There is evidence that interventions during pregnancy aimed at promoting breastfeeding have a positive impact on its prevalence, especially among primiparous women.[20] In a study conducted in Nigeria using data from a population survey involving more than 7,000 mothers, attending four or more prenatal visits was also significantly associated with higher prevalence of exclusive breastfeeding (OR 2.7; 95% CI 1.04-7.01).[12] Wishing to breastfeed, which is a strong determinant of successful breastfeeding, is a feeling generally developed during pregnancy, especially in the third trimester. This may therefore be influenced by information received during pregnancy. It has also been demonstrated that interventions within healthcare services, including prenatal counseling, are effective in promoting breastfeeding.[5] The finding of the present study is important because it supports the number of prenatal visits as a predictor that, in addition to being an indicator of quality of care, has a direct impact on exclusive breastfeeding rates.

In families with children under six months of age whose mothers had formal employment (mostly working in the public or private sector), the prevalence of exclusive breastfeeding was observed to be 54% higher than among mothers who were self-employed. There is evidence that maternal employment may negatively affect breastfeeding, especially when the mother returns to work.[21] However, work-related interventions to promote breastfeeding (such as maternity leave policies and support for breastfeeding at the workplace) appear to have a positive impact on breastfeeding indicators.[5] In the present study, there was no difference between housewives and self-employed workers (the latter category was defined as a reference for comparison).

Conversely, having formal employment was a protective factor, compared with being self-employed. While mothers with formal employment are protected by law such that their

income is guaranteed in the first months after birth[23] and thus are more available to provide breastfeeding, mothers who are self-employed and have no right to social security benefits tend to return to work earlier, especially in scenarios of high socioeconomic vulnerability. Thus, these latter mothers, with or without their babies, are prematurely exposed to situations of insecurity and precariousness, which can negatively affect the practice of exclusive breastfeeding. Moreover, mothers with formal employment tend to have a more structured daily life and a more organized routine, including a support network that provides help with household chores.

Within the category "other occupations", there was a significant number of students. The higher prevalence of exclusive breastfeeding in this group may also have been due to a stronger support network and the possibility of being absent from classes during the first months of the infant's life, which thus would make these mothers more available to provide breastfeeding.

There was an association between older child age and lower prevalence of exclusive breastfeeding, with an estimated reduction of 33% for each extra month of life. This was an expected association that had already been widely reported.[11,12,13,17,24] This child age-related decrease in the prevalence of exclusive breastfeeding may be influenced by unawareness of the optimal duration of exclusive breastfeeding and by the belief that breast milk alone is not sufficient for child nutrition as the infant approaches six months of life.

Table 1. Characteristics of the total sample in the municipality of Cacuaco, Angola, 2010 (n = 749)

Variable (n when continuous variable)	Median or n (%)	Q1-Q3 or 95% confidence interval
Maternal age (n = 744)	25	21-30
Maternal education (years of schooling) (n = 657)	6	4-8
Maternal place of birth		
Born in Luanda	76 (10.2)	(8.1-12.6)
Born in another province	669 (89.8)	(87.4-91.9)
Number of children born alive from the mother (n = 749)	3	2-5
Marital status		
Living with a partner	639 (85.3)	(82.6-87.7)
Not living with a partner	110 (14.7)	(12.2-17.4)
Partner education (years of schooling) (n = 490)	9	7-11
Living with the grandmother		
Yes	122 (16.3)	(13.7-19.1)
No	627 (83.7)	(80.9-86.3)
Economic condition (score) (n = 749)	7	5-7
Gestational age at beginning of prenatal visits*		
20 or more weeks	155 (46.1)	(40.7-51.6)
Less than 20 weeks	181 (53.9)	(48.4-59.3)
Number of prenatal visits (n = 369)*	4	3-5
Interval since delivery of older children (months) (n = 572)	33	(24.0-46.8)
Admission to hospital during pregnancy		
Yes	34 (4.6)	(3.2-6.3)
No	713 (95.4)	(93.7-96.8)
Place of delivery		
Home	228 (30.5)	(27.2-33.9)
Healthcare service	520 (69.5)	(66.1-72.8)
Type of delivery		
Cesarean	38 (5.1)	(3.6-6.9)
Vaginal	711 (94.9)	(93.1-96.4)
Payment for delivery		
No or did not recall	478 (64.1)	(60.5-67.5)
Yes	268 (35.9)	(32.5-39.5)
Maternal illness in the first 10 days after delivery		
Yes	141 (18.9)	(16.6-21.9)
No or did not recall	605 (81.1)	(78.1-83.9)
Breastfeeding within the first hour of life		
No	176 (23.5)	(20.5-26.7)
Yes	573 (76.5)	(73.3-79.5)
Visit to the healthcare service within the first week after delivery		
No or did not recall	453 (60.5)	(56.9-64.0)
Yes	296 (39.5)	(36.0-43.1)
Birth weight (kg) (n = 457)*	3.3	3.0-3.6
Child hospital admission shortly after birth		
Yes	18 (2.5)	(1.5-38.9)
No	715 (97.5)	(96.2-98.5)
Maternal occupation		
Self-employed	327 (43.9)	(40.3-47.5)
Housewife	316 (42.4)	(38.8-46.0)
Other	102 (13.7)	(11.3-16.3)
Child age (months) (n = 748)	10.1	4.2-16.0
Child age group (months)		
0 < 1	43 (5.7)	(4.2-7.7)
1 < 2	39 (5.2)	(3.7-7.1)
2 < 3	46 (6.2)	(4.5-8.1)
3 < 4	52 (7.0)	(5.2-9.0)
4 < 5	50 (6.7)	(5.0-8.70)
5 < 6	44 (5.9)	(4.3-7.8)
6 < 12	151 (20.2)	(17.4-23.3)
12 < 24	323 (43.2)	(39.6-46.8)
Child sex		
Male	361 (49.2)	(45.5-52.9)
Female	373 (50.8)	(47.1-54.5)
Possession of child health card		
No	103 (13.9)	(11.5-16.6)
Yes	639 (86.1)	(83.4-88.5)
Presence of a community health worker		
No	378 (50.7)	(46.7-54.1)
Yes	368 (49.3)	(45.5-52.8)

n = number of subjects; CI = confidence interval; Q1 = first quartile; Q3 = third quartile.

*Variables with reduced n, because these data were collected from the child's or pregnant woman's health card.

Table 2. Characteristics of children under six months according to use of the practice of exclusive breastfeeding (n = 269)

Variable	Exclusive breastfeeding at under six months of age			
	Yes		No	
	n (%) or median	CI or Q1-Q3	n (%) or median	CI or Q1-Q3
Maternal age (n = 266)	26.5	22.0-30.0	24.0	19.3-30.0
Maternal education (years of schooling) (n = 232)	6.0	4.0-8.0	6.0	4.0-7.5
Maternal place of birth				
Born in Luanda province	17 (12.1)	(7.18-18.60)	17 (13.3)	(7.93-20.41)
Born in another province	124 (87.9)	(81.40-92.82)	111 (86.7)	(79.59-92.07)
Number of children born alive from the mother (n = 269)	3.0	2.0-5.0	3.0	1.0-5.0
Marital status				
Living with a partner	15 (10.6)	(6.08-16.94)	22 (17.2)	(11.10-24.86)
Not living with a partner	126 (89.4)	(83.06-93.92)	106 (82.8)	(75.14-88.90)
Partner education (years of schooling) (n = 172)	8.0	7.0-12.0	8.0	6.8-10.0
Living with the grandmother				
Yes	18 (12.8)	(7.74-19.42)	20 (15.6)	(9.81-23.09)
No	123 (87.2)	(80.58-92.26)	108 (84.4)	(76.91-90.19)
Economic condition (score) (n = 269)	7.0	5.0-7.0	5.0	5.0-7.0
Gestational age at beginning of prenatal visits				
20 or more week	41 (43.2)	(33.03-53.72)	42 (56)	(44.06-67.45)
Less than 20 weeks	54(56.8)	(46.28-66.97)	33 (44)	(32.55-55.94)
Number of prenatal visits (n = 186)	4.0	3.0-6.0	3.0	2.0-5.0
Interval since delivery of older children (months) (n = 206)	36.0	26.3-47.8	34.0	24.0-43.3
Admission to the hospital during pregnancy				
Yes	3 (2.1)	(4.40-6.09)	8 (6.3)	(2.74-11.94)
No	138 (97.9)	(93.91-99.56)	120 (93.7)	(88.06-97.26)
Place of delivery				
Home	37 (26.2)	(19.20-34.31)	41 (32.0)	(24.06-40.85)
Healthcare service	104 (73.8)	(65.69-80.80)	87 (68.0)	(59.15-75.94)
Type of delivery				
Cesarean	8 (5.7)	(2.46-10.80)	4 (3.1)	(0.86-7.81)
Vaginal	133 (94.3)	(89.13-97.52)	124 (96.9)	(92.19-99.14)
Payment for delivery				
No or did not recall	90 (64.3)	(55.75-72.20)	81 (63.8)	(54.78-72.12)
Yes	50 (35.7)	(27.80-44.25)	46 (36.2)	(27.88-45.22)
Maternal illness in the first 10 days after delivery				
Yes	29 (20.9)	(14.44-28.57)	26 (20.3)	(13.72-28.33)
No or did not recall	110 (79.1)	(71.43-85.56)	102 (79.7)	(71.67-86.28)
Breastfeeding within the first hour of life				
No	33 (23.4)	(16.69-31.27)	33 (25.8)	(18.46-34.26)
Yes	108 (76.6)	6(8.73-83.31)	95 (74.2)	(65.74-81.54)
Visit to the healthcare service within the first week after delivery				
No or did not recall	78 (55.3)	(46.72-63.69)	91 (71.1)	(62.42-78.76)
Yes	63 (44.7)	(36.31-53.28)	37 (28.9)	(21.24-37.58)
Birth weight (kg) (n = 186)	3.3	3.0-3.6	3.3	2.9-3.6
Child hospital admission shortly after birth				
Yes	3 (2.2)	(0.45-6.27)	1 (0.8)	(0.02-4.38)
No	134 (97.8)	(93.73-99.55)	125 (99.2)	(95.66-99.98)
Maternal occupation				
Self-employed	47 (33.3)	(25.63-41.76)	52 (41.3)	(32.58-50.38)
Housewife	68 (48.2)	(39.74-56.79)	62 (49.2)	(40.19-58.26)
Other	26 (18.5)	(12.41-25.84)	12 (9.5)	(5.02-16.05)
Child age (months) (n = 186)	2.1	1.0-3.5	4.2	2.8-5.1
Child sex				
Male	53 (41.1)	(32.50-50.09)	68 (53.1)	(44.11-62.00)
Female	76 (58.9)	(49.91-67.50)	60 (46.9)	(38.00-55.89)
Possession of child health card				
No	17 (12.1)	(7.18-18.60)	5 (3.9)	(12.80-8.88)
Yes	124 (87.9)	(81.40-92.82)	123 (96.1)	(91.12-98.72)
Presence of a community health worker				
No	70 (49.7)	(41.12-58.18)	66 (52.0)	(42.93-60.91)
Yes	71 (50.3)	(41.82-58.88)	61 (48.0)	(39.09-57.07)

n = number of subjects; CI = confidence interval; Q1 = first quartile; Q3 = third quartile.

Table 3. Multivariable analysis using a hierarchical model for exclusive breastfeeding at under six months of age (n = 269)

Variables	Distal level		Intermediate distal level		Intermediate proximal level		Proximal level	
	PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value
Maternal age (n = 266)	1.00 (0.95-1.05)	0.867	–	–	–	–	–	–
Maternal education (years of schooling) (n = 232)	1.03 (0.96-1.11)	0.367	–	–	–	–	–	–
Maternal place of birth								
Born in Luanda	1.00	0.400						
Born in another province	1.22 (0.77-1.95)		–	–	–	–	–	–
Number of children born alive from the mother (n = 269)	1.03 (0.90-1.18)	0.670	–	–	–	–	–	–
Marital status								
Living with a partner	1.00	0.432						
Not living with a partner	0.76 (0.38-1.52)		–	–	–	–	–	–
Partner education (years of schooling) (n = 172)	1.04 (0.97-1.19)	0.269	–	–	–	–	–	–
Living with the grandmother								
Yes	1.00	0.474						
No	1.24 (0.69-2.24)		-	-	-	-	-	-
Economic condition (score) (n = 269)	1.02 (0.93-1.12)	0.653	-	-	-	-	-	-
Gestational age at beginning of prenatal visits								
20 or more weeks			1.00					
Less than 20 weeks	–	–	1.18 (0.85-1.62)	0.325	–	–	–	–
Number of prenatal visits (n = 186)	–	–	1.07 (0.98-1.17)	0.152	1.11 (1.00-1.22)	0.040	1.11 (1.04-1.18)	0.002
Interval since delivery of older children (months) (n = 206)	–	–	1.00 (1.00-1.01)	0.431	–	–	–	–
Admission to hospital during pregnancy								
Yes			1.00	0.499				
No	–	–	1.43 (0.51-4.04)		–	–	–	–
Place of delivery								
Home					1.00	0.938		
Healthcare service	–	–	–	–	0.98 (0.62-1.57)		–	–
Type of delivery								
Cesarean					1.00	0.252		
Vaginal	–	–	–	–	1.36 (0.62-1.56)		–	–
Payment for delivery								
No or did not recall					1.00	0.243		
Yes	–	–	–	–	1.22 (0.88-1.69)		–	–
Maternal illness in the first 10 days after delivery								
Yes					1.00	0.565		
No or did not recall	–	–	–	–	1.14 (0.73-1.79)		–	–
Breastfeeding within the first hour of life								
No					1.00	0.344		
Yes	–	–	–	–	0.84 (0.58-1.21)		–	–

Continue...

Table 3. Continuation

Variables	Distal level		Intermediate distal level		Intermediate proximal level		Proximal level	
	PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value	PR (95% CI)	P-value
Visit to the healthcare service within the first week after delivery								
No or did not recall					1.00	0.372		
Yes	–	–	–	–	1.15 (0.84-1.57)		–	–
Birth weight (kg) (n = 186)	–	–	–	–	0.94 (0.67-1.33)	0.741	–	–
Child hospital admission shortly after birth								
Yes					1.00	0.640		
No	–	–	–	–	0.81 (0.32-2.00)		–	–
Maternal occupation								
Self-employed							1.00	
Housewife	–	–	–	–	–	–	1.28 (0.96-1.70)	0.097
Other	–	–	–	–	–	–	1.54 (1.05-2.26)	0.027
Child age (months) (n = 186)	–	–	–	–	–	–	0.77 (0.71-0.84)	< 0.001
Child sex								
Male							1.00	
Female	–	–	–	–	–	–	1.34 (1.02-1.76)	0.033
Possession of child health card								
Yes							1.00	
No	–	–	–	–	–	–	1.13 (0.80-1.58)	0.491
Presence of a community health worker								
No							1.00	
Yes	–	–	–	–	–	–	1.09 (0.85-1.39)	0.499

PR = prevalence ratio; CI = confidence interval.

An association between the sex of the child and the practice of exclusive breastfeeding was also observed, with a 34% higher prevalence of exclusive breastfeeding among female children under six months of age. In a study conducted in Nigeria, female infants were twice as likely to be exclusively breastfed as were male infants (odds ratio, OR, 2.13; 95% CI, 1.03-4.39).[12] This was also found among children surveyed in all Brazilian state capital cities.[26] Although commonly observed, there is no consensus regarding the explanation for this result.[17] Some cultural factors, such as the belief that boys have a more voracious appetite and need higher energy intake than girls, which would be met through earlier introduction of complementary foods, may help explain this phenomenon. However, further studies are required in order to elucidate this association.

Higher maternal education levels are often associated with improved breastfeeding indicators.[17,27,28] In our sample, although the number of years of education was not very low (half of the women had had more than six years of education), the overall low quality of education offered in Angola may not have allowed any differences in education level among the mothers to be impactful enough to change maternal behavior in relation to breastfeeding.

Family income is another variable that is often identified as a determinant of breastfeeding practices.[11,12,27,28] According to Victora et al., the influence of this variable differs from one country to another, depending on the economic context of the region under study.[1] In the present study, no association was found between family income and the prevalence of exclusive breastfeeding. We believe that the considerable homogeneity of the economic status of the families surveyed in our study might explain this result.

There is evidence showing that home visits by CHWs have a positive impact on maternal and child health outcomes, including breastfeeding practices.[29,30] However, this was not confirmed in the present study. This may be related to the fact that, in the area surveyed, CHWs were working in an incipient manner that was relatively unstructured.

The present study had some limitations. Among these, we can highlight that the small number of events relating to certain

independent variables may have negatively affected the statistical analysis (e.g. only three cases of twins and 38 cases of cesarean section). Therefore, the variable "twin pregnancy", which was initially included in the analysis, had to be excluded from the final model. Also, the sample may have been too small to provide significant differences in some variables.

It was not possible to determine the cause-effect relationship between the variables in the associations that were found in the present study because of the study design. However, the results suggest that fewer prenatal visits are associated with lower likelihood that women will be able to care for their own health and their child's health, and consequently lower likelihood of breastfeeding. The same applies to early return to informal work, as in the case of the mothers who were self-employed. Thus, our findings highlight the importance of providing high-quality prenatal care and adopting a broader view of breastfeeding. This implies taking into account women's biopsychosocial context, in order to increase adherence to prenatal care and healthcare guidelines, especially those relating to breastfeeding.

CONCLUSION

Based on the present results, the study population of Luanda had satisfactory indicators for exclusive breastfeeding practices, although lagging behind international targets. The results point towards the importance of high-quality prenatal care for exclusive breastfeeding promotion. Prenatal visits and other occasions of contact that pregnant women and mothers have with healthcare services should also be used to share knowledge and practices regarding child nutrition in an attempt to delay the introduction of complementary foods during the first six months of life, especially in relation to male infants. Moreover, the protective factors of formal employment and provision of social security benefits regarding maintenance of exclusive breastfeeding were also noteworthy. These findings may contribute towards current knowledge through indicating paths to be followed in order to improve the health of women and children, in Angola and other low-income countries.

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Address for correspondence:
Susana Valéria Dalcastagnê
Universidade Federal do Rio Grande do Sul (UFRGS)
Avenida Getúlio Vargas, 1.026/403
Porto Alegre (RS) – Brasil
CEP 90150-002
Cel. (+55 51) 98260-3043
E-mail: su_vd@hotmail.com

Vascular endothelial growth factor, endostatin levels and clinical features among patients with ulcerative colitis and irritable bowel syndrome and among healthy controls: a cross-sectional analytical study

Evrim Kahramanoğlu Aksoy[I], Hülya Çetinkaya[II], Berna Savaş[III], Arzu Ensari[IV], Murat Torgutalp[V], Cumali Efe[VI]

Ankara Üniversitesi Tıp Fakültesi, Ankara, Turkey

[I]MD. Physician, Department of Gastroenterology, Keçiören Training and Research Hospital, Ankara, Turkey.
orcid.org/0000-0001-8887-3428
[II]MD. Professor, Department of Gastroenterology, Ankara University Faculty of Medicine, Ankara, Turkey.
orcid.org/0000-0001-5788-1011
[III]MD. Professor, Department of Pathology, Ankara University Faculty of Medicine, Ankara, Turkey
orcid.org/0000-0003-3971-1419
[IV]MD. Professor, Department of Pathology, Ankara University Faculty of Medicine, Ankara, Turkey.
orcid.org/0000-0001-7036-4457
[V]MD. Physician, Department of Rheumatology, Ankara University Faculty of Medicine, Ankara, Turkey.
orcid.org/0000-0003-4600-9484
[VI]MD. Associate Professor, Department of Gastroenterology, Gazi Yaşargil Training And Research Hospital, Diyarbakır, Turkey.
orcid.org/0000-0001-6593-5702

KEY WORDS:
Colitis, ulcerative.
Vascular endothelial growth factors.
Endostatins.

ABSTRACT

BACKGROUND: Increased angiogenetic activity in inflammatory bowel disease (IBD) has been shown in previous studies. The aim of this study was to evaluate the relationship of serum vascular endothelial growth factor (VEGF) and endostatin levels with clinical features and mucosal expression in patients with ulcerative colitis (UC).

DESIGN AND SETTING: Cross-sectional analytical study conducted in a tertiary-level public hospital.

METHODS: Serum VEGF and endostatin levels were determined in 82 individuals: 39 with UC, 28 with irritable bowel syndrome (IBS) and 15 healthy controls (HCs), using enzyme-linked immunosorbent assays (ELISA). VEGF and endostatin expressions were studied using immunohistochemistry (IHC).

RESULTS: Mean serum VEGF and endostatin levels were significantly higher in patients with UC than in patients with IBS and in HCs (511.9 ± 377.5 pg/ml, 305.0 ± 121.42 pg/ml and 36.1 ± 40.6 pg/ml; P = 0.001 for VEGF; and 155.50 ± 59.8 ng/ml, 116.9 ± 23.8 ng/ml and 102.2 ± 22.4 ng/ml; P < 0.001 for endostatin, respectively). There was a positive correlation between serum VEGF and endostatin levels (r = 0.422; P < 0.01). Mean H-scores for VEGF expression were higher in the active UC group than in the inactive UC and IBS groups, in the stroma, endothelium and epithelium. Mean H-scores for endostatin expression were higher in the active UC group than in the inactive UC and IBS groups, in the stroma and endothelium. There was no endostatin expression in the epithelium.

CONCLUSION: Increased endostatin appears to be a defensive reaction to increased VEGF in patients with UC.

INTRODUCTION

Ulcerative colitis is characterized by chronic inflammation and ulceration of the colonic mucosa. Several environmental and genetic factors are responsible for chronic inflammation.[1] Angiogenesis has been defined as growing of new blood vessels from the preexisting ones. In addition to playing a role in physiological events such as wound healing and growth, it is also seen in tumor development, metastasis, and chronic inflammation.[2]

In inflammatory bowel disease (IBD), although angiogenesis is necessary for ulcer healing and tissue regeneration through providing oxygen and nutrients to the healing zone, it turns into a pathological process through inflow of inflammatory cells and cytokines. Under the influence of inflammatory mediators such as cytokines, growth factors and proteases that come to the healing site, angiogenesis increases further and the microvascular bed enlarges. In this manner, this condition becomes a vicious circle that attracts more inflammatory mediators to the center. It thus leads to chronic inflammation.[3,4]

Previous studies have shown the existence of several angiogenic factors that may become elevated, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), angiogenin and angiopoietin-2.[5-8] VEGF is defined as the potent proangiogenic factor that is secreted by parenchymal, endothelial and activated immune cells.[9] Elevated serum and tissue levels of VEGF in patients with IBD have been shown in many studies.[5-7,9,10] Kanazawa et al. and Kapsoritakis et al. found elevated serum VEGF-A

levels in IBD patients with active disease. Griga et al. showed that there was increased expression of VEGF in the inflamed intestinal mucosa of patients with active IBD.[5,6,11] Chidlow et al. and Ardelean et al. demonstrated the effect of anti-angiogenic treatment through decreased angiogenic and histopathological activity in the inflamed mucosa.[3,4,12]

Endostatin is a 20-kDa fragment of collagen-18 that is generated by proteinases such as matrix metalloproteinases (MMPs), especially MMP-9. It acts as an endogenous angiogenesis inhibitor through inhibiting proliferation and inducing apoptosis of endothelial cells.[13] Endostatin downregulates many angiogenic factors, such as VEGF, bFGF, hepatocyte growth factor (HGF), hypoxia-induced-factor-1α (HIF-1α) and tumor necrosis factor-α (TNF-α) and it upregulates anti-angiogenic genes such as thrombospondin-1, HIF-1α-inhibitor, etc.[14]

Studies on the effects of endostatin in cases of ulcerative colitis have produced contradictory results. Sandor et al. reported the presence of increased levels of endostatin and angiostatin in the colonic mucosa, rather than increased levels of VEGF, in a study on experimental colitis. They speculated that the reason for the chronicity of the disease, decreased healing of mucosal lesions and lack of increased VEGF levels was the increased levels of endostatin and angiostatin.[15] In another study, they reported that the effect of 5-aminosalicylate acid (5-ASA) on the healing of ulcerative colitis (UC) may be related to downregulation of the anti-angiogenic factors endostatin and angiostatin.[16]

In the present study, we aimed to evaluate the relationship between serum VEGF and endostatin levels and the clinical features of patients with ulcerative colitis, and to evaluate VEGF and endostatin expression in the colonic mucosa of these patients.

METHODS

Participants

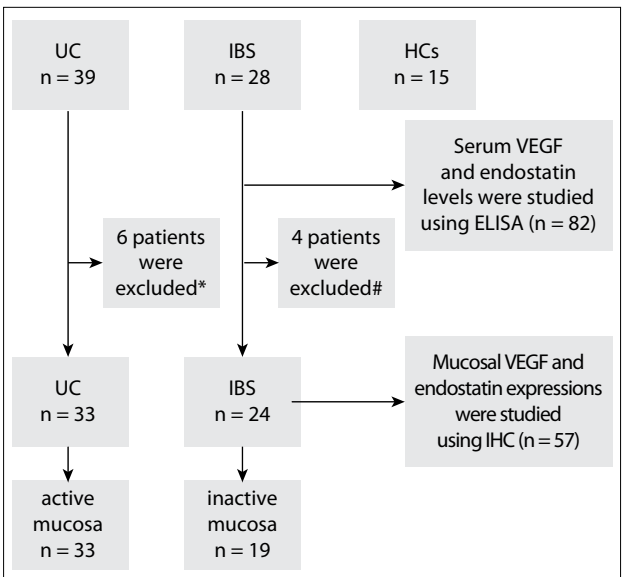
The participants of this study were recruited according to their consecutive admittance to a gastroenterology outpatient clinic: 39 UC patients, 28 patients with irritable bowel syndrome (IBS) and 15 healthy controls (HCs) who came for consultations between September 2007 and July 2008 were included in this study. The sample size was calculated with a 5% error margin and 80% power, in accordance with the prescriptions of Liou et al.,[17] yielding an ideal sample of 24 participants for the study group (21 for studying VEGF and 13 for studying endostatin) and 15 for the control group.

The UC clinical activity index (UCAI) was calculated as described by Seo et al.[18] The Rachmilewitz endoscopic activity index (EAI) was also calculated for the UC group.[19] All of the IBS patients met the Rome III criteria for their diagnosis. HCs were selected from among individuals who had been admitted because of dyspeptic symptoms and who had normal endoscopic examinations and laboratory tests. The IBS patients and HCs had normal erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels and no parasitic infections in their stool tests. All the patients were between 18 and 65 years of age.

Patients who presented the following conditions were excluded: chronic kidney disease; chronic liver disease; oncological diseases; pregnancy or breastfeeding; use of nonsteroidal anti-inflammatory drugs, anticoagulants or antithrombotic drugs; or previous abdominal surgery.

Serum VEGF and endostatin levels were measured in all groups. All UC patients and 24 IBS patients underwent colonoscopic examination, and colonic biopsy samples were taken from all of these patients. Four patients who did not undergo colonoscopic examination also met the Rome III criteria for IBS. They had normal ESR and CRP levels and no parasitic infection in their stool tests, and they did not want to undergo colonoscopy. The biopsy specimens from six patients with UC could not be evaluated because of insufficient material. VEGF and endostatin expressions were studied by means of immunohistochemical analysis using 33 active mucosal samples and 19 inactive mucosal samples from 33 patients with UC, and using 24 normal mucosal samples from 24 patients with IBS (**Figure 1**).

This study was approved by the Medical Ethics Committee of Ankara University Faculty of Medicine (June 16, 2008; approval no.



UC = ulcerative colitis; IBS = irritable bowel syndrome; HCs = healthy controls; VEGF = vascular endothelial growth factor; ELISA = enzyme-linked immunosorbent assay; IHC = immunohistochemistry. *The biopsy specimens from 6 patients with UC could not be evaluated because of insufficient material. #Four patients did not undergo colonoscopic examination but also met the Rome III criteria for IBS.

Figure 1. Flowchart of the study.

132-3784) and was conducted in accordance with the revised Helsinki Declaration. Informed consent was obtained from all patients.

Serum VEGF and endostatin levels

Venous blood samples were taken after overnight fasting and were centrifuged at 10,000 rpm for 10 minutes. The resultant serum was collected and kept at -80 °C until the examination date. Serum VEGF levels and endostatin levels were measured in duplicate by means of enzyme-linked immunosorbent assays (ELISA) using commercial kits in accordance with the manufacturers' instructions (for VEGF: Biosource International, California, USA; and for endostatin: R&D Systems, Minneapolis, MN, USA).

Immunohistochemical analysis

Paraffin-embedded tissue material from the patients with UC (n = 33) and IBS (n = 24) was retrieved from the pathology archives. Tissue sections of 4 μm were cut and mounted on poly-L-lysin coated slides. The sections were deparaffinized and rehydrated through a graded ethanol series and were then incubated in methanol containing 0.3% H_2O_2 to inhibit endogenous peroxidase. Immunostaining was performed using the avidin-biotin-peroxidase complex technique, using 3,3'-diaminobenzidine as the chromogen.

The primary antibodies that were used were recombinant human VEGF (Thermo Fisher Scientific, Fremont, CA, USA; 1/100 dilution) and human monoclonal endostatin (Hycult Biotechnology, Netherlands; 1/15 dilution). Colonic mucosal tissue was used as the positive control for VEGF and prostate tissue was used as the positive control for endostatin.

VEGF and endostatin expressions in the epithelium, stroma and vascular endothelium were evaluated. Intracytoplasmic staining was positive in the cells. The distribution of staining was categorized as follows: - no staining; + staining in a few cells; ++ staining approximately half of the cells; and +++ staining in the majority of cells. The intensity of staining was categorized as follows: + poor cytoplasmic staining; ++ significant cytoplasmic staining; and +++ severe cytoplasmic staining. The assessments were all made by the same pathologist, who was experienced in IBD. The H-scores were calculated by multiplying the overall stain distribution and intensity, in biopsies from the stroma, endothelium and epithelium separately (**Figure 2**).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0 (SPSS Inc, Chicago, IL, USA), was used for statistical analysis. The results were given as the mean ± standard deviation or as the median with minimum-maximum. The normality of the distributions was checked.

To compare pairs of groups, the t test was used if the distribution was normal and the Mann-Whitney U test was used if the distribution was not normal. To make comparisons between more than two groups, analysis of variance (ANOVA) was used if the distribution was normal and the Kruskal-Wallis test was used if the distribution was not normal. The Mann-Whitney U test was used to compare pairs of subgroups. If the P value found in the test result was significant, multiple comparison tests were used to find out which group the difference originated from. P-values < 0.05 were considered statistically significant.

RESULTS

There were no significant differences among the three groups in terms of age and gender. The demographic characteristics of the study population and the drugs used by the patients with UC during the serum sampling are presented in **Tables 1** and **2**. There was also no significant difference in immunohistochemical expression among the patients, in terms of age and gender. For UC patients (n = 33), the mean age was 45.4 ± 11.4 years and there were 13 females; while for IBS patients (n = 24), the mean age was 46.8 ± 12.1 years and there were 15 females.

A statistically significant difference between the groups was found in terms of serum VEGF and serum endostatin levels (P = 0.001 and P < 0.001, respectively) (**Table 1**). The difference was due to the UC group. There was no statistically significant difference between the patients with IBS and the healthy controls in terms of serum VEGF and endostatin levels (P = 0.709 and P = 0.562, respectively) (**Figures 3** and **4**).

Comparison between the mean serum VEGF and serum endostatin levels according to the clinical activity of the UC patients showed statistically significant differences (P = 0.004 and P = 0.011, respectively) (**Table 3**). There were significant differences between the groups with severe and moderate disease (P = 0.024) and between the groups with severe and mild disease (P = 0.04), but there was no significant difference between the groups with mild and moderate disease (P = 0.343), in terms of serum VEGF levels. There were statistically significant differences between the groups with severe and mild disease (P = 0.015) and between the groups with moderate and mild disease (P = 0.030), but there was no difference between the groups with severe and moderate disease (P = 0.625), in terms of serum endostatin levels.

Comparison between the mean serum VEGF and endostatin values according to the disease involvement site in patients with UC did not show any significant differences (P = 0.826 and P = 0.867, respectively). The mean serum VEGF level in the proctitis group was 430.0 ± 340.0 pg/ml; in the group with left colon involvement, 529.12 ± 375.1 pg/ml; and in the pancolitis group, 530.5 ± 412.7 pg/ml. The mean serum endostatin level in the proctitis group was 157.6 ± 54.1 ng/ml; in the group with left colon

involvement, 160.7 ± 66.5 ng/ml; and in the pancolitis group, 149.4 ± 58.3 ng/ml.

Grouping of the patients with UC according to endoscopic activity index, i.e. as active (n = 19) or inactive (n = 20), showed significant differences in terms of mean serum VEGF levels (660.2 ± 416.5 pg/ml and 371.0 ± 278.9 pg/ml respectively; P = 0.011) and mean serum endostatin levels (179.8 ± 65.1 ng/ml and 132.4 ± 44.6 ng/ml respectively; P = 0.012)

There were statistically significant positive correlations between the serum VEGF and serum endostatin levels and the UCAI, ESR, CRP level and platelet count (**Table 4**).

There were statistically significant differences in the stroma, endothelium and epithelium between the active UC, inactive UC and IBS groups in terms of H-scores of VEGF expression (P < 0.001). This difference was based on the active group (**Table 5**).

Endostatin expression was not viewed in the epithelium in any group. There were statistically significant differences in terms of H-score for endostatin expression in the stroma between the active UC, inactive UC and IBS groups (P < 0.001); whereas there was only a statistically significant difference in terms of H-score for endostatin expression in the endothelium between the active UC and IBS groups (P = 0.043).

DISCUSSION

In the present study, we demonstrated for the first time that higher colonic mucosal expression of the potent angiogenic factor VEGF and the potent anti-angiogenic factor endostatin occurred, rather

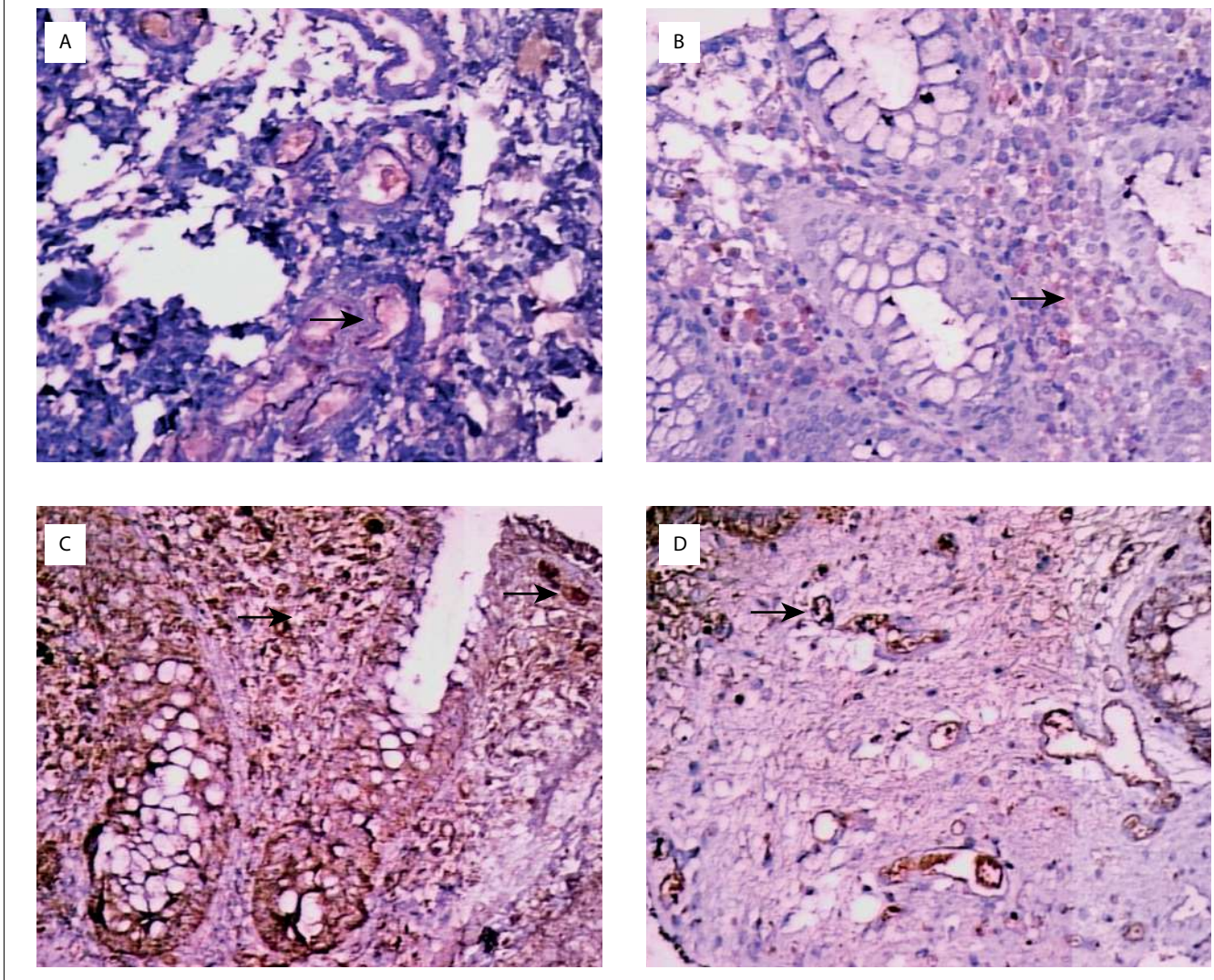


Figure 2. Immunohistochemical expression of vascular endothelial growth factor (VEGF) and endostatin in the colonic mucosa of ulcerative colitis patients. A: endothelial endostatin expression, x 400; B: stromal endostatin expression, x 400; C: stromal and epithelial VEGF expression, x 400; D: endothelial VEGF expression, x 400.

than elevated serum levels, in patients with UC. Comparison of serum VEGF and endostatin levels according to UCAI showed that the most notable difference was between the groups with severe and mild disease. In addition, there was a significant difference between the active and inactive groups, in terms of both serum VEGF and endostatin, through evaluation according to EAI. There was no difference between the groups through evaluation according to disease extent. There were positive correlations between UCAI and the VEGF, endostatin, ESR, CRP and platelet levels. Mucosal VEGF and endostatin expressions were higher in the active UC group than in the inactive UC group and IBS group. There was no difference in mucosal VEGF and endostatin expressions between the inactive UC group and the IBS group.

The role of angiogenesis in IBD is double-sided. While it is necessary for wound healing and tissue repair, it also promotes

Table 1. Baseline characteristics of the group with ulcerative colitis

Characteristic	n (%)
Disease duration, months (IQR)	72 (88)
Disease involvement site, n (%)	
Proctitis	7 (17.9)
Left colon site	16 (41)
Pancolitis	16 (41)
Disease activity, n (%)	
Mild	9 (23.1)
Moderate	22 (56.4)
Severe	8 (20.5)
Endoscopic activity, n (%)	
Inactive	20 (51.3)
Active	19 (48.7)
Types of drugs, n (%)	
ASA	32 (82.1)
Steroid	8 (20.5)
Metronidazole/ciprofloxacin/ ampicillin	5 (12.8)
Azathioprine	5 (12.8)
Cyclosporine	1 (2.6)
None	5 (12.8)

IQR = interquartie range; ASA = aminosalicylic acid.

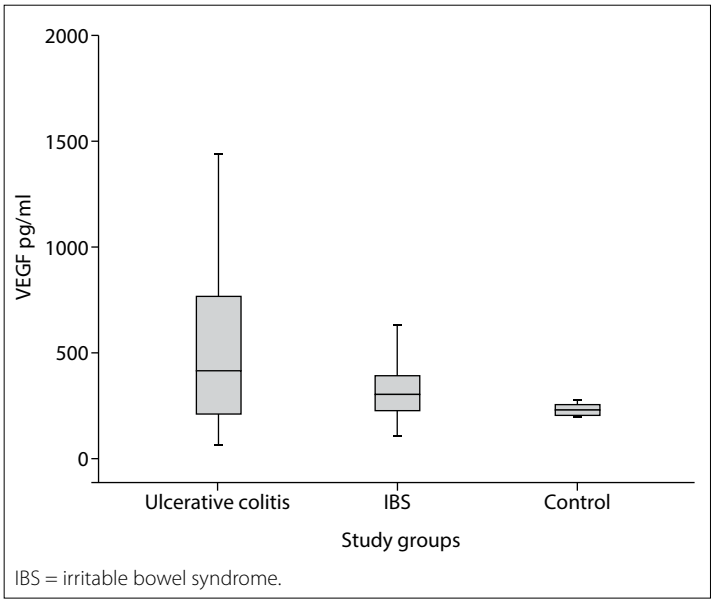


Figure 3. Serum vascular endothelial growth factor (VEGF) levels of the groups.

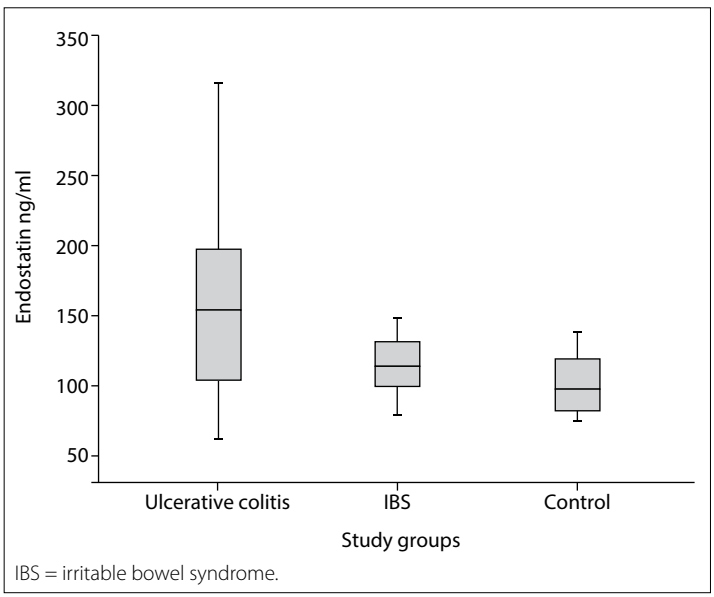


Figure 4. Serum endostatin levels of the groups.

Table 2. Comparison of baseline characteristics of the study groups

	UC (n = 39)	IBS (n = 28)	HC (n = 15)	P-value	P-value: UC versus IBS	P-value: UC versus HC	P-value: IBS versus HC
Age, years	46.1 ± 12.6	48.2 ± 11.7	41.4 ± 12.6	0.232			
Female, n (%)	15 (38.5)	18 (64.5)	7 (46.7)	0.112			
VEGF (pg/ml)	511.9 ± 377.5	305.0 ± 121.4	236.1 ± 40.6	0.001*	0.007	≤ 0.001	0.709
VEGF, median (range)	420.8 (59.2-1700.2)	309.3 (108.6-638.1)	230.4 (190.5-340.4)	0.001**	0.032	0.019	0.044
Endostatin (ng/ml)	155.50 ± 59.8	116.9 ± 23.8	102.2 ± 22.4	< 0.001*	0.002	0.001	0.562
Endostatin, median (range)	156.1 (62.1-318.4)	115.3 (80.8-194.1)	98.5 (75.0-140.6)	0.001**	0.009	0.003	0.053

UC = ulcerative colitis; IBS = irritable bowel syndrome; HC = healthy control; VEGF = vascular endothelial growth factor; IQR = interquartile range; ASA = aminosalicylic acid. *This was studied using one-way analysis of variance. The homogeneity of the variances was tested by means of the Levene test. The post-hoc Tamhane test was used because the variances were not homogeneous. ** This was studied using the Kruskal-Wallis test. The Mann-Whitney U test was used in association with post-hoc analysis using the Bonferroni correction, and $P < 0.017$ was considered significant.

inflammation as a consequence of expansion of the tissue microvascular bed and the increased inflow and production of inflammatory cells and cytokines. In other words, angiogenesis contributes greatly towards the chronicity of inflammation.[20]

UC is a chronic inflammatory disease characterized by inflammation, ulceration and regeneration of the colonic mucosa.[1] It has long been known that vascular changes occur in IBD. Abnormal vasculature, increased permeability and increased microvessel density have been reported previously. Danese et al. reported that the microvessel density was greater, along with the numbers of αVβ3-positive angiogenic vessels, in the mucosa of IBD patients. They indicated that the dense inflammation was due to local microvascular changes.[20]

Several proangiogenic molecules are involved in angiogenesis in IBD cases. VEGF is a potent angiogenic molecule. Several studies have now shown that serum and tissue VEGF concentrations become greater in IBD patients. It has also been shown that there is a correlation between disease activity and serum VEGF level in patients with IBD. Our results are similar to those from previous studies in terms of higher serum VEGF levels and higher mucosal VEGF expression, and a positive correlation of VEGF levels with disease activity.[5-7,9-11]

Angiogenesis is sustained by the balance between proangiogenic and antiangiogenic factors. Sandor et al. were the first to demonstrate the presence of increased expression of the antiangiogenic molecules endostatin and angiostatin instead of increased expression of VEGF, in an experimental model for colitis.[15] They speculated that the antiangiogenic factors inhibited ulcer healing despite the presence of increased angiogenic factors and that this might be the reason for chronicity of colitis. Deng et al. demonstrated

Table 3. Serum vascular endothelial growth factor (VEGF) and endostatin levels in patients with ulcerative colitis (UC)

	Mild (n = 9)	Moderate (n = 22)	Severe (n = 8)	P-value	P-value: severe versus moderate	P-value: severe versus mild	P-value: moderate versus mild
VEGF (pg/ml)	290.3 ± 209.9	477.2 ± 283.7	856.8 ± 528	0.004*	0.024	0.04	0.343
VEGF, median (range)	229.9 (63.8-755.5)	425.0 (59.2-1037.3)	837.4 (75.4-1700.2)	0.018**	0.063	0.011	0.078
Endostatin (ng/ml)	107.2 ± 33.5	164.5 ± 60.0	185.3 ± 55.40	0.011*	0.625	0.015	0.030
Endostatin, median (range)	97.0 (74.9-182.2)	156.6 (62.1-318.4)	199.3 (75.7-271.0)	0.008**	0.344	0.011	0.004

*This was studied using one-way analysis of variance. The homogeneity of the variances was tested by means of the Levene test. The post-hoc Tukey test was used because the variances were homogeneous **This was studied using the Kruskal-Wallis test. The Mann-Whitney U test was used in association with post-hoc analysis using the Bonferroni correction, and P < 0.017 was considered significant.

Table 4. Spearman correlations for ulcerative colitis patients

	VEGF	Endostatin	ESR	CRP	Platelet	Albumin
UCAI	0.459**	0.475**	0.564***	0.564***	0.542***	-0.457**
VEGF		0.422**	0.703***	0.416**	0.542***	-0.401*
Endostatin			0.379*	0.398*	0.291	-0.272
ESR				0.727***	0.703***	-0.637***
CRP					0.669***	-0.429**
Platelet						-0.517***

UCAI = ulcerative colitis clinical activity index; VEGF = vascular endothelial growth factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.
* P < 0.05; ** P < 0.01; *** irritable bowel syndrome, P < 0.001.

Table 5. H-scores for vascular endothelial growth factor (VEGF) and endostatin expressions in the mucosa of the active ulcerative colitis (UC), inactive UC and irritable bowel syndrome (IBS) groups

	Active UC (N = 33)	Inactive UC (N = 19)	IBS (N = 24)	P-value
H_VEGF_stroma	6.51 ± 3.49	2.38 ± 2.83	2.86 ± 1.58	< 0.001*
Median (range)	9 (0-9)	0 (0-9)	4 (0-4)	< 0.001*
H_VEGF_endothelium	5.26 ± 3.02	2.26 ± 2.92	2.50 ± 1.82	< 0.001*
Median (range)	6 (0-9)	0 (0-9)	2 (0-6)	< 0.001*
H_VEGF_epithelium	6.51 ± 3.34	2.64 ± 3.15	4.14 ± 2.99	< 0.001*
Median (range)	9 (0-9)	2 (0-9)	4 (0-9)	< 0.001*
H_endostatin_stroma	4.05 ± 3.05	1.87 ± 2.55	1.82 ± 1.52	< 0.001*
Median (range)	4 (0-9)	1 (0-9)	1 (0-4)	0.001*
H_endostatin_endothelium	2.08 ± 2.21	1.41 ± 2.12	0.86 ± 1.18	0.043**
Median (range)	1 (0-9)	0 (0-6)	0 (0-4)	0.059

*This difference was based on the active UC group (no difference between inactive and IBS). **There was a significant difference only between the active UC group and IBS group.

that mesalazine treatment reduced the expression of endostatin and angiostatin through restoring the balance between MMM-2 and MMM-9 via TNF-α inhibition.[16] They did not demonstrate any effect of reducing the increase in VEGF expression.

Tolstonova et al. showed that there were concomitant increases in the levels of endostatin and VEGF.[21] They found a correlation between colonic lesion size and endostatin and VEGF levels. Our study is compatible with their study in terms of the positive correlation between serum VEGF and endostatin levels. They stated that increased endostatin levels were a defensive response to the increased VEGF in UC. They also suggested that endostatin might be an alternative treatment for UC.

Konstatinos et al. found elevated levels of the proangiogenic molecules angiogenin and angiopoietin-2, along with higher serum levels of endostatin in UC patients with extensive colitis.[8] We did not find any correlation between serum VEGF and endostatin levels and the extent of the disease. Salem et al. reported that the levels of endostatin, angiostatin, VEGF and TNF-α were reduced through niacin treatment.[22]

The complexity and range of pathways within the pathophysiology of chronic diseases have led to proposals for a number of options for treatments. The contribution of increased and unregulated angiogenesis in IBD, towards chronic inflammation, has long been known. It has been demonstrated that antiangiogenic therapy is effective against chronic inflammatory diseases such as arthritis, psoriasis and retinal neovascularization, and this suggests that it may also be effective for treating IBD.

The greatest limitations of our study were its cross-sectional nature and the small number of patients. Another limitation was that we were unable to evaluate mucosal expression in all of the patients whose serum levels of VEGF and endostatin we assessed. There is a need for prospective studies to show whether the serum or tissue endostatin level increase or decrease through treatment. Further experimental studies are also needed, to show the mucosal effect of local or systemic endostatin treatment.

CONCLUSIONS

The serum and tissue VEGF and endostatin levels were found to be higher in patients with UC, and especially in those with active disease. This finding may explain the chronicity of the disease and may also form the basis of an idea for a new treatment option for UC patients.

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Address for correspondence:
Evrim Kahramanoğlu Aksoy
Keçiören Training And Research Hospital,
Department of Gastroenterology,
Pınarbaşı Mah, Sanatoryum Caddesi Ardahan Sokak D:25,
06280 Keçiören/Ankara, Türkiye
Tel. 00905332121579
E-mail: evrims1979@yahoo.com

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Patient satisfaction with breast reconstruction using musculocutaneous flap from latissimus dorsi versus from rectus abdominis: a cross-sectional study

Lilian Baldan Záccaro Augustinho[I], Miguel Sabino Neto[II], Daniela Francescato Veiga[III], Luiz Eduardo Felipe Abla[IV], Yara Juliano[V], Lydia Masako Ferreira[VI]

Breast Surgery Outpatient Clinic, Hospital São Paulo (HSP) and Hospital Pérola Byington, São Paulo (SP), Brazil

[I]MSc. Physiotherapist, Faculdade de Filosofia Ciências e Letras de Catanduva (FAFICA), Catanduva (SP), Brazil
orcid.org/0000-0001-6336-4416

[II]MD, PhD. Physician, Adjunct Professor and Coordinator of the Postgraduate Program on Translational Surgery, Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil.
orcid.org/0000-0003-2847-9386

[III]MD, PhD. Physician and Professor, Postgraduate Program on Translational Surgery, Universidade Federal de São Paulo (UNIFESP), São Paulo; and Pro-Rector of Postgraduate Studies, Universidade do Vale do Sapucaí (UNIVÁS), Pouso Alegre (MG), Brazil.
orcid.org/0000-0002-8713-2940

[IV]MD, PhD. Physician and Director, Discipline of Plastic Surgery, Hospital Pérola Byington, São Paulo (SP), Brazil.
orcid.org/0000-0002-2791-5652

[V]PhD. Business Administrator and Full Professor, Discipline of Collective Health, School of Medicine, Universidade de Santo Amaro (UNISA), São Paulo (SP), Brazil.
orcid.org/0000-0002-8563-8622

[VI]MD, PhD. Physician and Titular Professor, Discipline of Plastic Surgery, Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil.
orcid.org/0000-0003-4587-509X

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ABSTRACT

BACKGROUND: Breast cancer is the second most frequent type of cancer worldwide and the most common type among women. The treatment for this condition has evolved over recent decades with therapeutic and technological advances. Breast reconstruction techniques using musculocutaneous flaps from the latissimus dorsi and rectus abdominis have aroused interest regarding patients' quality of life. Our goal here was to compare patients' satisfaction scores after they underwent breast reconstruction using musculocutaneous flaps from either the latissimus dorsi or the rectus abdominis.

DESIGN AND SETTING: Primary, clinical, analytical, observational and cross-sectional study conducted in a federal university and a public hospital.

METHODS: Demographic and clinical data were collected. The Mini-Mental State Examination was then applied, with testing for specificity and sensitivity. Lastly, a breast evaluation questionnaire was applied to evaluate breast satisfaction among 90 women, who were divided into three groups: mastectomy (control; n = 30); breast reconstruction using flap from the latissimus dorsi (n = 30); and reconstruction using flap from the rectus abdominis (n = 30).

RESULTS: The groups were homogeneous regarding the main demographic data and the questionnaire responses ($P < 0.05$). Compared with the control group, the reconstruction groups showed significant improvement in satisfaction ($P < 0.0002$) after one year.

CONCLUSION: Within our sample, women who underwent breast reconstruction with flaps from either the latissimus dorsi or the rectus abdominis had similar satisfaction scores.

INTRODUCTION

Breast cancer is the second most frequent type of cancer in the world and the most common type among women. Overall, it accounts for 28% of new cases of cancer.[1] In developed countries, the five-year survival rate is about 85%, whereas in developing countries, it remains between 50 and 60%.[1] It has been estimated that there will be 59,700 new cases of breast cancer in Brazil in 2018, i.e. 56.33 cases per 100,000 women.[1]

Therapeutic and technological advances relating to breast cancer have allowed patients to attain greater life spans, and this has drawn attention to the quality of life of these women.[2-4] Mastectomy is considered to be one of the most devastating types of cancer treatment from a psychological point of view, in that it affects the self-esteem, femininity and body image of these patients.[5,6] However, breast reconstruction can be undertaken immediately after mastectomy or as a delayed procedure. Breast reconstruction procedures have positive impacts on all aspects of quality of life, including body image, particularly among younger women.[2,7]

There are several types of breast reconstruction. The autologous reconstruction methods that are most used are breast reconstruction using a transverse rectus abdominis musculocutaneous flap and breast reconstruction using a latissimus dorsi flap, which provide safety and satisfaction for patients and improve their quality of life.[2,8,9,10]

Anderson et al. developed a specific questionnaire for patients with breast diseases (named the Breast Evaluation Questionnaire) that has proven to be reliable and valid. It contains 55 questions and was developed to assess patients' satisfaction with their breasts and their contentment

with their general appearance and the appearance of their breasts. This questionnaire thus reveals changes in the quality of life of patients who have undergone breast surgery.[11] This instrument has been translated and validated for use in the Portuguese language.[12]

There is a gap in the literature concerning comparison of the results from these two reconstruction techniques, in relation to the patients' quality of life. This is what motivated the present study.

OBJECTIVE

Our objective was to compare patients' satisfaction scores after they underwent breast reconstruction using musculocutaneous flaps from either the latissimus dorsi or the rectus abdominis.

METHODS

Design, setting and ethics

This was a primary, clinical, analytical, observational and cross-sectional study. It was conducted at a federal university and a public hospital.

It was approved by the Ethics Committee of the Federal University of São Paulo on October 26, 2012, under the approval number 131.769. Patients signed informed consent forms for their inclusion in the study.

Participants

The patients were recruited from the breast surgery outpatient clinic of São Paulo Hospital (which is linked to the discipline of plastic surgery within the Federal University of São Paulo) and from the breast surgery outpatient clinic of Perola Byington Hospital. The patients in the control group were selected from the plastic surgery outpatient clinic and went through the same scheduling and data collection process.

The sample was formed by 90 women aged between 30 and 55 years, who were divided into three homogeneous groups according to the surgical technique used: 30 women who had undergone mastectomy without reconstruction (control group); 30 women who had undergone immediate or delayed breast reconstruction using a musculocutaneous latissimus dorsi flap (LD group); and 30 women who had undergone immediate or delayed reconstruction using a transverse rectus abdominis musculocutaneous flap (TRAM group).

The following inclusion criteria were taken into account in selecting the patients:

- Age between 30 and 55 years;
- Mastectomy (control group) with immediate breast reconstruction or breast reconstruction that had been delayed for up to one year (LD and TRAM groups);
- Completed adjuvant treatment for at least six months; and
- Score greater than or equal to 18 in the Mini-Mental State Examination. This test forms a practical method for evaluating cognitive function and tracking states of dementia. It has different cutoff scores: 13 points for illiterate people; 18 points for individuals with one to seven years of schooling; and 26 points for people with eight years or more of schooling. Scores greater than 18 refer to people who are literate and able to understand and answer questions.[13]

The following were exclusion criteria:

- Neoadjuvant chemotherapy or adjuvant radiotherapy;
- Breast disease occurring during the study;
- Illiteracy;
- Recurrences or metastases.

Breast evaluation instrument (breast evaluation questionnaire)

The Breast Evaluation Questionnaire (BEQ) evaluates patients' satisfaction with their breasts, regarding breast size, shape, firmness and overall appearance, along with the appearance of their breasts when wearing clothes or swimsuits and when naked. Moreover, this questionnaire enables assessment of the importance of breast appearance for the patient and for other people.[11,12]

It comprises 55 items for which the patient selects one answer. These are grouped into 11 sections with five items in each section. Each item can be scored from one to five, as follows: very dissatisfied (score = 1); slightly dissatisfied (score = 2); neither satisfied nor dissatisfied (score = 3); reasonably satisfied (score = 4); or very satisfied (score = 5). The total score for the questionnaire is obtained by summing the scores given for each item in each section.

The data from the questionnaire were analyzed after standardization as percentages, because the score which each item receives can vary and, consequently, change the total score.[11]

$$\text{Score: } \frac{(\text{total score} - \text{lowest score}) \times 100}{\text{Possible variation}}$$

Data collection procedure

The interviews for data-gathering were scheduled to take place at the time of the patients' return visits to the outpatient clinic (i.e. in the cases of the LD and TRAM groups). At these meetings, the patients were oriented and were invited to take part in the study. If they agreed, they would sign an informed consent form.

Initially, sociodemographic and clinical data were gathered. Following this, the Mini-Mental State Examination and then the Breast Evaluation Questionnaire (BEQ-55) were applied. All questionnaires were self-administered in a reserved room, immediately after the patient's medical consultation.

Statistical analysis

To analyze the results, the BioEstat 5.0 software was used.

The following tests were applied:

- Kruskal-Wallis variance analysis, to compare the three study groups concerning the quantitative variables. When differences between the groups were significant, the analysis was complemented with multiple comparison testing to determine which group(s) differed from the other(s).[13]
- Chi-square test, to study associations between the groups and the characteristics observed.[13]

In all tests, the rejection level for the null hypothesis was set at 5%.

RESULTS

Table 1 shows the absolute and relative frequencies for the categorical demographic variables (marital status, skin color, schooling and occupation) that were obtained from the patients in each study group, and the comparisons between the groups (chi-square test).

Table 2 presents the data relating to numerical sociodemographic variables (BMI and age), and the comparisons between the groups (Kruskal-Wallis test).

The analysis on individuals' data obtained from application of the BEQ in the control-mastectomy group, the group with breast reconstruction using a musculocutaneous flap from the latissimus dorsi and the group with breast reconstruction using a musculocutaneous flap from the rectus abdominis is presented in **Table 3**.

To complement the Kruskal-Wallis analysis, a multiple comparison test was conducted. This showed that the total score from the BEQ in the control-mastectomy group was 48.4%, which was significantly smaller than the scores in the breast reconstruction groups. The analysis of LD versus TRAM showed that these two groups were statistically similar. The TRAM group presented a total BEQ score of 59.2%, while the LD group presented 62.6%.[14]

DISCUSSION

Mastectomy directly affects patients' self-esteem, femininity and body image. Therefore, identifying these women's degree of satisfaction and the impact of their treatment on their quality of life is of utmost interest.[2,3]

We reviewed the literature to search for specific instruments for assessing breast surgery. We found studies using seven different validated instruments that assessed the results from esthetic and reconstructive breast surgery.

One of these instruments is the Breast Evaluation Questionnaire (BEQ), which was previously validated among 1,244 women who

Table 3. Total scores from the Breast Evaluation Questionnaire (BEQ) among the women who underwent breast reconstruction

	%			Kruskal-Wallis test: P
	Control	LD	TRAM	
Range	14.0-88.6	40.0-91.0	22.0-87.0	
Median	50	65	61.2	0.0002
Average	48.4	62.6	59.2	

LD = reconstruction using musculocutaneous flap from the latissimus dorsi; TRAM = reconstruction using musculocutaneous flap from the rectus abdominis.

Table 1. Distribution of the women who underwent breast reconstruction, according to sociodemographic characteristics

		Control	%	LD	%	TRAM	%	Total	%	Chi-square test: P
Marital status	With partner	17	56.7	22	73.3	13	43.3	52	57.8	0.0621
	Without partner	13	43.3	8	26.7	17	56.7	38	42.2	
Skin color	Caucasian	20	66.7	19	63.3	19	63.3	58	64.4	0.9527
	Non-Caucasian	10	33.3	11	36.7	11	36.7	32	35.6	
Occupation	Housewife	6	20	6	20	8	26.7	20	22.2	0.6717
	Working outside the home	24	80	24	80	22	73.3	70	77.8	
Schooling	Elementary school	5	16.7	4	13.3	5	16.7	14	15.6	0.8315
	High school	16	53.3	20	66.7	19	63.3	55	61.1	
	Higher education	9	30	6	20	6	20	21	23.3	
Total		**30**	**100**	**30**	**100**	**30**	**100**	**90**	**100**	

LD = reconstruction using musculocutaneous flap from the latissimus dorsi; TRAM = reconstruction using musculocutaneous flap from the rectus abdominis.

Table 2. Age and body mass index (BMI) among the women who underwent breast reconstruction

		Control	LD	TRAM	Kruskal-Wallis test: P
BMI (kg/m^2)	Range	20.1-25.9	20.3-27.2	20.4-26.9	
	Median	24.1	23.7	23.4	0.4066
	Average	23.9	23.3	23.6	
Age (years)	Range	30.0-52.0	34.0-55.0	36.00-55.0	
	Median	47	47	48	0.4907
	Average	45.4	46.7	46.9	

LD = reconstruction using musculocutaneous flap from the latissimus dorsi; TRAM = reconstruction using musculocutaneous flap from the rectus abdominis.

had undergone breast augmentation.[11] The BEQ was chosen to evaluate satisfaction with overall appearance and breast appearance for the present study because, at the time when this study was designed, it was the only validated instrument available for breast evaluation in Brazil.[12] In addition, it offers the advantage of being self-administered, thus providing greater freedom for patients to express their perceptions and minimizing any constraints that the patients might feel regarding speaking to the surgical team, especially about scars and recurrence.

The age group from 30 to 55 years was considered because, according to the Brazilian National Cancer Institute (Instituto Nacional do Câncer, INCA), the incidence of breast cancer among women grows rapidly and progressively over this age range. The maximum age considered was 55 years in order not to include patients in the perimenopause period, since these women present distinctive hormonal changes that possibly would interfere with the results from the study.[1, 15,16]

Furthermore, demographic data such as body mass index (BMI) and age were collected to verify whether these factors might interfere in the results regarding patients' satisfaction concerning their breasts.

Regarding age, the average for the control group was 45.4 years, whereas it was 46.7 years in the group with reconstruction using a latissimus dorsi (LD) musculocutaneous flap and 46.9 years in the transverse rectus abdominis musculocutaneous (TRAM) group. Thus, there was consistency between the three groups and the age factor was considered a low risk of interference in patients' satisfaction regarding their breasts. These data closely match those found in the literature.[17-19]

The same observation can be made regarding BMI, since there was no significant difference between the groups. The average BMI was 23.9 kg/m^2 in the control group, while in the reconstruction groups with musculocutaneous flaps from the LD and from the TRAM, the BMI was 23.3 kg/m^2 and 23.6 kg/m^2, respectively. These values are slightly below the average observed in the literature.[20,21]

Data about the level of education were collected, and these showed that the control group and the reconstruction groups all presented higher numbers of patients who had completed high school, while smaller numbers of them had only attended elementary school. This made it easier to apply the BEQ, since this is a self-administered instrument.[12]

Moreover, there were no significant differences between the groups with regard to marital status, skin color or occupation. Thus, these factors contributed towards greater homogeneity among the groups and lower risk of interference in the results obtained. The same was noted in the literature.[22]

Patients' surgical results can be evaluated through their satisfaction with these results. This analysis is subjective and therefore questionnaires have been applied in an attempt to obtain an objective analysis and enable data measurement and comparison between patients.[19,23]

The BEQ was applied one year after all surgical procedures had been completed. Thus, the patients had already gone through the stage of surgical recovery and had returned to their routine. It has been shown that the results may be influenced by elation after surgery, but that six months after surgery, patients' feelings regarding their operations (such as helplessness, isolation, fear of death, pain and mutilation) had stabilized.[21,24-26]

The limitation of this study was the difficulty in contacting the patients. According to the requirements of the BEQ, they need to have completed all surgical procedures before application of the questionnaire, so that the result from the survey is not affected.

The total scores from the BEQ showed that the women who had undergone breast reconstruction using LD and TRAM flaps were satisfied with their breasts. Thus, these findings were consistent with the results from previous studies evaluating satisfaction.[8,16,17,27-29]

According to some authors, mastectomy can cause low self-esteem and body image issues. Therefore, reconstruction is indicated for patients requiring mastectomy. These authors observed that patients' opinions regarding their surgical results and hospital care influenced their quality of life.[24] A scale of satisfaction applied to patients and surgeons showed that the esthetic results were better and the level of satisfaction was higher according to the patients than according to the surgeons.[28]

It was found in previous studies that reconstructions with LD and TRAM flaps resulted in symmetry with the contralateral breast. Therefore, it was concluded that both methods produced good esthetic results and improved quality of life.[2,8,17,27] These findings corroborate the results obtained from the present study, which identified a greater level of satisfaction with reconstructed breasts, in comparison with patients without reconstruction.

Communication between doctors and patients is important because, through the guidelines given by doctors, patients can decide what kind of surgery is best for them. This increases the chances of obtaining the expected results in relation to body image and satisfaction with breasts.[30,31]

It has been seen that the numbers of indications of breast reconstruction for women who have undergone mastectomy is increasing. Therefore, there is a need for further research and interventions to ensure that patients have fair access to this important component of multidisciplinary breast cancer treatment. Reconstructions are crucial for mastectomized women, and the importance of such indications was corroborated by the results from the present study: all the patients who underwent breast tissue reconstruction, irrespective of whether this was with LD or TRAM musculocutaneous flaps, were satisfied with the results.[32]

CONCLUSION

Within our sample, the women who underwent breast reconstruction using flaps from either the latissimus dorsi or the rectus abdominis had similar satisfaction scores.

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Address for correspondence:
Lilian Baldan Záccaro Augustinho
Rua Alice Garcia Vega, 415
Freguesia do Ó — São Paulo (SP) — Brasil
CEP: 02737-050
Tel. +55 11 98188-2069
E-mail: lilian.baldan@bol.com.br

Safety assessment of omeprazole use: a review

Marcela Forgerini[I], Stephania Mieli[II], Patrícia de Carvalho Mastroianni[III]

Universidade Estadual Paulista (UNESP), Araraquara (SP), Brazil

[I]BSc. Pharmacist and Master's Student in the Postgraduate Program on Pharmaceutical Sciences, Universidade Estadual Paulista (UNESP), Araraquara (SP), Brazil.
orcid.org/0000-0002-2905-8519

[II]Undergraduate Pharmacy Student, Universidade Estadual Paulista (UNESP), Araraquara (SP), Brazil.
orcid.org/0000-0001-5002-966X

[III]PhD. Pharmacist and Adjunct Professor, Department of Drugs and Medicines, Universidade Estadual Paulista (UNESP), Araraquara (SP), Brazil.
orcid.org/0000-0001-8467-7278

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Drug interactions.
Treatment outcome.

ABSTRACT

BACKGROUND: Risks regarding hospital admission due to adverse drug reactions and drug interactions from use of omeprazole have been reported. The question guiding the present review was "Which adverse events occur in patients using omeprazole in a Food and Drug Administration-approved and/or off-label manner?" It was also proposed to evaluate the safety of use of omeprazole.

DESIGN AND SETTING: Qualitative narrative review with critical evaluation, in a public university.

METHODS: The PubMed, SCOPUS, LILACS, SciELO, EMBASE and EBSCO databases were searched on July 31, 2018. Studies evaluating adverse events were screened.

RESULTS: 72 articles were included, among which 58 reported on adverse drug events (47, adverse drug reactions; 5, drug interactions; and 6, situations of ineffectiveness). 28 adverse drug reactions not described in compendia and drug leaflets were described in these studies: myocardial infarction (6); stroke (2); spontaneous abortion (1); proliferative changes (1); chills (1); heart failure (1); thrombosis (2); and dementia (1), among others. Severe adverse reactions, for instance cardiac problems, Steven-Johnson syndrome and proliferative changes, were identified. The antiplatelet effects of drugs such as clopidogrel, in patients who underwent heart-related surgery, increased the risk of developing cardiac problems, such as cardiovascular death, myocardial infarction and stroke. In newly transplanted patients, decreased absorption of mycophenolate mofetil occurred, thus leading to rejection of transplanted organs.

CONCLUSION: Use of omeprazole should be monitored primarily in patients with heart disorders using antiplatelet agents concomitantly, and in newly transplanted patients using mycophenolic acid, in order to avoid serious adverse reactions.

INTRODUCTION

Proton-pump inhibitors (PPIs) such as omeprazole are one of the most widely prescribed classes of drugs worldwide. PPIs are indicated for treatment of ulcers with or without *Helicobacter pylori* infection; for treatment of gastroesophageal reflux, Zollinger-Ellison disease, dyspepsia, esophagitis and gastritis; and for prevention of peptic ulcers in patients receiving nonsteroidal inflammatory agents (NSAIDs) and in patients with upper gastrointestinal bleeding.[1] Therefore, they are medications that are ever-present in gastroenterologists' practice.[2]

Omeprazole is effective and safe most of the time.[1] However, Mastroianni et al.[3] found that omeprazole was the drug most commonly associated with hospital admission, in a survey on the prevalence of hospitalizations due to adverse drug reactions. In addition, the safety of a drug may change over time through increased use and according to patients' characteristics. Therefore, risk assessment is required.[4]

This context can be elucidated from reports on abusive use of omeprazole and irrational prescription of this drug.[4] Thus, there have been studies reporting on the risks (adverse events) of use of omeprazole, such as: (a) gastric proliferative changes;[5] (b) increased creatinine and urea levels, leading to acute interstitial nephritis[6-8] and increased risk of developing chronic kidney disease;[9] (c) increased risk of asthma concomitant with gastroesophageal reflux;[10] (d) increased risk of infection by *Clostridium difficile*;[11-13] (e) decreased absorption of vitamin B;[14] (f) steatorrhea caused by cystic fibrosis;[15] (g) fracture with decreased calcium absorption;[16,17] (h) gynecomastia;[18] (i) hypomagnesemia;[19] (j) hyponatremia;[20] (k) spontaneous bacterial peritonitis;[21] l) pneumonia;[22] (m) anaphylactic reactions to omeprazole;[23] and (n) risk of celiac disease.[24]

In addition, studies that evaluated the prevalence of hospital admission due to adverse drug events have cited omeprazole among the drugs that were possibly related to hospitalization, thus also suggesting that off-label use of omeprazole occurs frequently.[23,24] Off-label use of drugs consists of their use for unapproved indications and usually occurs among polymedicated patients

and as prophylactic gastric protection for use of some drugs, such as antimicrobials and nonsteroidal anti-inflammatory drugs.[25-27] These off-label indications are for long-term use and are widespread and commonly prescribed in some countries,[28] such as Brazil.

OBJECTIVE

The purpose of this review was to evaluate the adverse outcomes relating to omeprazole use in clinical practice.

METHODS

Study design

We conducted a qualitative narrative review with critical evaluation, to answer the following guiding question: "Which adverse events occur in patients using omeprazole in a Food and Drug Administration (FDA)-approved and/or off-label manner?" Thus, we aimed to gather, organize and critically review articles on these topics, to include the highest level of scientific evidence.

1. Search of the literature and inclusion criteria

The search for studies was performed using the MEDLINE (via PubMed), LILACS, EMBASE (via Ovid), SciELO and SCOPUS databases and was conducted on July 31, 2018. During the search and selection process, there was no limitation on the time when articles were published. The languages were restricted to Portuguese, English and Spanish.

The following search strategies were used: ("Omeprazole" OR "Proton Pump Inhibitors") AND ("Adverse Drug Reaction Reporting Systems" OR "Pharmacovigilance" OR "Drug-Related Side Effects and Adverse Reactions" OR "Risk Assessment" OR "Treatment Outcome" OR "Off-Label Use"). All descriptors used in these search strategies are Medical Subject Headings (MeSH terms). We included randomized clinical trials, phases I and II clinical trials, case-control studies, cohort studies, cross-sectional and quasi-experimental studies (clinical trials in which there was no comparator group for the intervention) evaluating adverse events from therapeutic or prophylactic use of omeprazole among individuals in all age groups whose health status was well defined and who were using omeprazole in an FDA-approved and/or off-label manner.

We excluded review articles, dissertations and theses, case reports, abstracts published in annals of events, editorials, letters to the editor, news and comments.

2. Selection process and data extraction

Types of participant

The target population comprised patients of any kind whose health status was well defined and who were using omeprazole in an FDA-approved and/or off-label manner. There was no age limitation.

Types of intervention

The interventions considered comprised use of omeprazole from the outset of treatment to clinical outcome, without restrictions on doses, therapeutic regimens or duration of use. In addition, it was proposed to include both preventive use and therapeutic use.

Types of outcome

The outcomes considered comprised any safety-related outcome, including adverse events, withdrawal due to adverse events, mortality and therapeutic ineffectiveness, i.e. adverse events in which the medicine used did not present any therapeutic response or its therapeutic response was lower than expected. Safety-related outcomes of all causes and omeprazole-related causes were considered.

After selecting potential articles in the databases, the titles and abstracts were reviewed by verifying patient exposure to omeprazole. The following variables were defined during the screening of articles: indication of use; study design; patient's clinical condition; clinical outcomes, including all types of adverse events relating to use of omeprazole; recommendations; author; and year of publication.

The severity of adverse events was classified as described by the World Health Organization. In this definition, severe adverse reactions are harmful effects that occur during drug treatment and which can result in death, be life-threatening or lead to persistent or significant disability, congenital anomaly, clinically important effects, hospitalization or prolongation of hospitalization. Non-serious adverse reactions also fall within the concept of severe adverse reactions.[29]

The search for studies, selection of studies and extraction of data were performed by three authors, in triplicate independently, to avoid the presence of bias in the selection and exclusion of articles. In addition, the kappa function was applied to analyze the agreement rate.

3. Risk of bias assessment

For randomized clinical trials, risk of bias was evaluated using the Cochrane collaboration tool (RoB 1),[30] which is based on seven domains: random sequence generation, concealment of allocation, blinding of participants and professionals, blinding of outcome assessors, outcome completeness, selective reporting of outcomes and other sources of bias. Each domain is judged as presenting low risk of bias, uncertain risk of bias or high risk of bias.

For case-control and cohort studies, we used the Newcastle-Ottawa tool. This provides evaluations in three domains: selection, comparability and outcome for cohort studies; and selection, comparability and exposure for case-control studies. Each item that is identified as presenting low risk of bias is given a "'star". There is a maximum of one "star" for each item within the "selection" and

"exposure/outcome" categories; and a maximum of two "stars" for "comparability". Therefore, each study can be classified with a maximum of nine "stars", which corresponds to a low risk of bias.[31]

The cross-sectional and quasi-experimental studies included in this review were not evaluated, since there are no validated tools for analysis on these study designs.

RESULTS

A total of 5,500 potentially relevant studies were identified. After reading the titles and/or abstracts, 4,746 studies were excluded because they did not meet the inclusion criteria. Another 218 were duplicates, and thus 536 studies were examined further.

It was not possible to access 2 of these 546 studies, because one of them is no longer indexed in the database and the other does not provide for the option to purchase and access the article. Our attempts to contact the authors of these two studies were unsuccessful. After screening the remaining articles, 191 studies were found to be eligible for complete text reading. After reading in full, 119 were excluded because they did not meet the inclusion criteria. Thus, 72 articles were considered eligible for the safety assessment on use of omeprazole, since they included all the variables that were being analyzed (**Figure 1**).

The proportion of overall agreement (kappa) observed in relation to making final decisions (inclusion and exclusion) from the database that included the screened articles was 0.807 (confidence interval, CI: 0.658-0.957).

Among these 72 studies, 58 reported on adverse drug events (ADEs): 47 studies on adverse drug reactions (ADRs), 5 studies on drug interactions (DIs) and 6 studies on therapeutic ineffectiveness (Table 1).[13,14,32-48, 60, 65-67] The duration of use of omeprazole ranged from 5 days to 11 years in these studies. Only one study evaluated the off-label use of omeprazole.[41]

A relationship was observed between use of omeprazole and increased risk of severe adverse events, such as development of coronary disorders that might lead to death.[32,39,43,62,88,94,96,101]

Regarding the clinical outcomes of the studies, the safety (ADRs and DIs) and the therapeutic ineffectiveness can be correlated. Among the 62 studies included, 39 studies reported on ADRs, with 28 potential events that were identified during the post-marketing phase in relation to omeprazole (spontaneous abortion, proliferative changes and chills, among others); 6 studies demonstrated the drug interactions between omeprazole and clopidogrel or mycophenolate mofetil; and 5 studies described the therapeutic ineffectiveness that occurred with omeprazole (Table 2).[5,13,16,32-34,38-39,40-42,44-99]

Among the 40 clinical trials included in the review, after risk-of-bias analysis, it was found that eight were classified as presenting low risk of bias, 14 as having high risk of bias and 17 as having uncertain risk of bias. The 17 studies analyzed using the Newcastle-Ottawa scale had low risk of bias (Table 3).[13,16,28,32,33,35-39, 43-97]

DISCUSSION

This review allowed us to identify and update the most severe and prevalent ADEs relating to use of omeprazole, and our findings corroborate similar results found in other studies.[3,4] Severe ADEs occurred in patients who underwent heart-related surgery or drug interventions, such as in situations of acute coronary syndromes or percutaneous coronary intervention,[75,78,96] or in cases of concomitant use of such medications.[76] These events were associated with concomitant use of omeprazole and clopidogrel, which caused inhibition of the antiplatelet effect of omeprazole,[83] due to competitive inhibition of CYP2C19.[32]

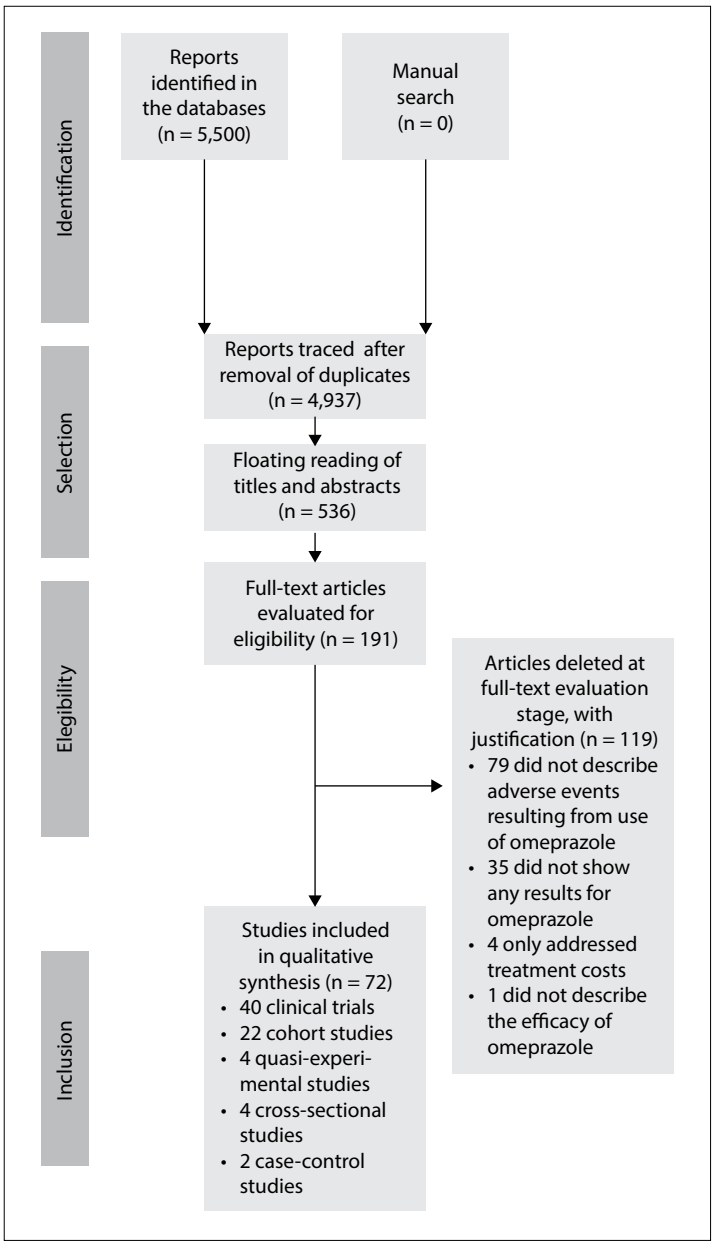

Figure 1. Flowchart of the stages of selection, skim-reading and full reading of the eligible articles.

Table 1. Frequencies of adverse events resulting from indication of omeprazole that were reported in studies published up to 2016

Assessment	Type of ADE	Frequency	Description
Safety	ADR (n = 47)	Common reaction (≥ 1% and < 10%)	Headache, constipation, diarrhea, abdominal pain, back pain, flatulence, respiratory tract infection and maculopapular rash.[32-37]
		Uncommon reaction (≥ 0.1% and < 1%)	Eczematous eruption, insomnia, somnolence, urticaria, urticaria vasculitis and vertigo.[38]
		Rare reaction (≥ 0.01% and < 0.1%)	Angioedema, arthralgia, muscle pain, erythema multiforme, weakness, metallic taste in the mouth, allergic reaction, Steven-Johnson's syndrome and thirst.[38]
		Post-marketing experience	Unstable angina, increased risk of fractures, cancer, cystitis, ulcerative colitis, stomatitis, abnormal renal function, hypergastrinemia, decreased levels of vitamin B12, increased creatinine levels, hypomagnesemia.[14,39-43]
		Potential events not described in omeprazole monograph (n = 28)	Miscarriage, proliferative changes, increased levels of chromogranin A, increased levels of fibroblast growth factor 2, chills, cardiovascular events (myocardial infarction, heart failure, stroke, ischemic stroke, pulmonary embolism and thrombosis), scarlet fever, hyperglycemia, mononucleosis infection, gastrointestinal bleeding, nasopharyngitis, otitis media, loss of libido, rhinitis, dementia, metabolic syndrome and hepatic steatosis, low sperm motility, increased risk of fibrosis progression, cirrhosis, hepatic decompensation and development of hepatocellular carcinoma.[44-48,60,65-67]
	DI (n = 6)	Omeprazole and clopidogrel: cardiovascular death, myocardial infarction, inhibition of the effect of clopidogrel, increased leukocyte and platelet levels and increased brain adverse events.[75,76] Omeprazole and acenocoumarol: increased anticoagulant effect of acenocoumarol.[77] Omeprazole and mycophenolate mofetil: reduced absorption of mycophenolic acid.[49]	
Efficacy	TI (n = 5)	Some patients did not respond to omeprazole therapy and continued with colitis symptoms and gastrointestinal discomforts. Omeprazole failed to control the gastric acidity of some patients.[13]	

ADE = adverse drug event; ADR = adverse drug reaction; DI = drug interaction; TI = therapeutic ineffectiveness.
The frequency of adverse reactions was classified according to the leaflet of the reference drug product, except for the 28 studies for which there was no information on the leaflet.

Table 2. Adverse events from approved use of omeprazole that were reported in the studies analyzed, published from 1994 to July 2018

	Adverse events	Participants (n)	Author, year
Clinical trials (28)	ADR: Diarrhea, vomiting and circulatory problems	Patients with reflux esophagitis (193)	Bate et al., 1995[50]
	ADR: Dyspepsia, flatulence, abdominal pain and diarrhea	Patients with active duodenal ulcer (180)	Marzio et al., 1995[51]
	ADR: Abdominal pain, diarrhea, nausea, headache and respiratory tract infection	Patients with gastric ulcer (520)	Valenzuela et al., 1996[52]
	ADR: Diarrhea, headache, melena, chills and mononucleosis infection plus allergic reaction	Patients with duodenal ulcer (381)	Labenz et al., 1997[53]
	ADR: Stroke, cancer, pulmonary embolism and gastrointestinal bleeding/ perforation	Patients with peptic ulcer with bleeding (274)	Muckadell et al., 1997[39]
	ADR: Cardiovascular events such as myocardial infarction, heart failure, stroke, pulmonary embolism, gastrointestinal bleeding and cancer	Patients with peptic ulcer in the stomach or duodenum (333)	Hasselgren et al., 1997[54]
	ADR: Diarrhea, stomatitis, metallic taste in the mouth and abdominal pain	Patients with active gastric or duodenal ulcer (78)	Annibale et al., 1997[55]
	ADR: Epigastric pain, facial erythema and loss of libido	Patients with erosive or ulcerative esophagitis, grade 2 or 3 (231)	Annibale et al., 1998[56]
	ADR: Dizziness, fatigue and aphthous stomatitis	Outpatients with symptoms of reflux esophagitis (70)	Ladas et al., 2000[57]
	TI: Omeprazole failed to control the gastric acidity of some patients	Patients with gastroesophageal reflux disease (88)	Leite et al., 1998[58]
	ADR: Diarrhea, taste disorder, increased levels of liver enzymes and cholecystitis	Patients diagnosed with at least one duodenal ulcer and with a test for *H. pylori* (539)	Lind et al., 1999[61]
	ADR: Death due to cardiovascular problems	Patients with persistent reflux esophagitis and who did not respond to treatment with H2 receptor antagonists (230)	Klinkenberg-Knol et al., 2000[60]
	ADR: Diarrhea, nausea, headache, cold, vomiting and fever	Patients with gastroesophageal reflux without erosive esophagitis (359)	Richter et al., 2000[59]

Continue...

Table 2. Continuation

	Adverse events	Participants (n)	Author, year
Clinical trials (28)	TI/ADR: Worsening of symptoms; taste disorder and scarlet fever	Patients with chronic functional dyspepsia with or without gastritis due to *H. pylori* (974)	Blum et al., 2000[62]
	ADR: Diarrhea, pericarditis and chest pain	Patients with erosive gastroesophageal reflux cured within 90 days (243)	Thjodleifsson et al., 2000[63]
	ADR: Diarrhea, abdominal pain and headache	Patients with dyspeptic symptoms (73)	Gottrand et al., 2001[33]
	TI: Some patients did not respond to treatment with omeprazole	Patients with dyspepsia (514)	Rabeneck et al., 2002[64]
	ADR: Increased fibroblast growth factor 2	Patients with gastric neoplasm (16)	Esaki et al., 2002[65]
	ADR: Myocardial infarction, ventral hernia, deep vein thrombosis, miscarriage, headache, respiratory infection, diarrhea and abdominal pain	Patients who suffered with burning in the stomach for at least three months (390)	Armstrong et al., 2005[66]
	ADR: Diarrhea, taste disorders and dyspepsia	Patients infected with *H. pylori* with abdominal disorders (323)	Manes et al., 2005[38]
	ADR: Nasopharyngitis, upper respiratory tract inflammation, diarrhea, headache, arthralgia, back pain, insomnia, cystitis, abdominal pain and hyperglycemia	Japanese patients with recurrent reflux esophagitis (119)	Ohkusa et al., 2005[67]
	ADR: Allergic reaction	Patients with lymphocytic gastritis (51)	Madisch et al., 2006[68]
	ADR/TI: Headache, somnolence and diarrhea	HIV-negative, healthy patients (19)	Schöller-Gyüre et al., 2008[32]
	ADR: Headache and gastrointestinal disorders	Patients with burning in the stomach or reflux (55)	Howden et al., 2009[69]
	ADR: Increased weight, increased ferritin level, increased death related to cardiac disorders and non-fatal heart attack	Patients with esophageal reflux (310)	Lundell et al., 2009[70]
	ADR: Omeprazole reduced antiplatelet effects	Unmedicated male patients (24)	Ferreiro et al., 2010[71]
	DI: Increased levels of leukocytes and platelets and increased incidence of cardiac and cerebral adverse events	Patients with stent implantation (38)	Hudzik et al., 2010[72]
	ADR: Diarrhea, tiredness, dizziness, abdominal pain and headache	Patients with typical symptoms of reflux more than twice a week (200)	Miwa et al., 2011[73]
Cohort studies (17)	ADR: Thrombosis, hyperthyroidism, complete retinal detachment, ulcerative colitis and skin rash	Patients with persistent reflux esophagitis and who did not respond to treatment with H_2 receptor antagonists (178)	Klinkenberg-Knol et al., 1994[74]
	ADR: Death due to cardiovascular, cerebrovascular, respiratory and postoperative problems, carcinomas, urinary tract infections and suicide	Diagnosed with colitis due to *C. difficile* (140)	Cadle et al., 2007[13]
	ADR: Myocardial infarction, stroke, cardiovascular death and unstable angina	Patients using clopidogrel after percutaneous coronary intervention (16,690)	Kreutz et al., 2010[75]
	DI: Inhibition of the effect of clopidogrel	Patients using clopidogrel (18,139)	van Boxel et al., 2010[76]
	DI: Increased anticoagulant effect of acenocoumarol	Patients that used acenocoumarol for at least 42 days in the study period (2,755)	Teichert et al., 2011[77]
	TI: Cardiovascular death, myocardial infarction and stroke	Patients who underwent coronary intervention (13,144)	Kimura et al., 2011[78]
	ADR: Increased levels of chromogranin A	Patients with increased levels of chromogranin A that could not be caused by neuroendocrine tumors (196)	Korse et al., 2011[79]
	ADR: Hypergastrinemia	Patients with moderate to severe peptic esophagitis	Ligumsky et al., 2011[80]
	TI: Omeprazole failed to control the gastric acidity of some patients	Patients who underwent kidney transplantation	David-Neto et al., 2012[49]
	DI: Inhibition of the effect of clopidogrel	Patients with acute coronary syndrome (37,099)	Lin et al., 2012[81]
	ADR: Increased risk of fractures	Patients who underwent medical consultations in the last two years (61,916)	Soriano et al., 2014[16]
	ADR: Increased risk of dementia	Elderly people over 75 years old (73,679)	Gomm et al., 2016[44]
	ADR: Increased risk of first-time ischemic stroke	- (396,296)	Yi et al., 2017[47]
	ADR: Increased serum creatinine levels	Inpatient patients (419)	Varallo et al., 2018[41]
	ADR: Increased risk of metabolic syndrome and hepatic steatosis	Patients with a recent diagnosis of celiac disease (301)	Imperatore et al., 2018[45]
	ADR: Hypomagnesemia	Hospitalized patients with Torsades de pointes (48)	Lazzerini et al., 2018[42]
	ADR: Increased risk of fibrosis progression, cirrhosis, hepatic decompensation and development of hepatocellular carcinoma	Patients with hepatitis C virus (HCV) infection.	Li et al., 2018[48]

Continue...

Table 2. Continuation

	Adverse events	Participants (n)	Author, year
Quasi-experimental studies (4)	ADR: Diarrhea and ringing in the ears	Patients with burning in the stomach, erosive esophagitis or non-erosive reflux disease (108)	Tsuzuki et al., 2011[82]
	ADR: Respiratory infection, otitis media, pharyngitis, change in bowel habit, fever and rhinitis	Patients with cured reflux esophagitis (64)	Hassall et al., 2012[83]
	ADR: Nausea, vomiting, constipation, diarrhea, metallic taste in the mouth, headache, abdominal pain, loss of appetite, drowsiness, weakness, dizziness and dry mouth	Patients with *H. pylori* (134)	Sezgin et al., 2014[34]
	ADR: Myocardial infarction or heart failure with or without consequent death	Patients who were hospitalized due to myocardial infarction within 12 weeks after starting use of proton-pump inhibitors (5,550)	Juurlink et al., 2013[84]
Case-control studies (2)	ADR: Maculopapular rash, angioedema and/or urticaria, Steven-Johnson's syndrome, erythema multiforme, eczematous eruption and urticarial vasculitis	Patients with dyspepsia, gastroesophageal reflux disease and upper gastrointestinal tract bleeding; prevention of ulcers induced by nonsteroidal anti-inflammatory drugs, stress and prednisolone (170)	Chularojanamontri et al., 2012[85]
	ADR: Low sperm motility	Men who were planning to have children (955)	Heijgen et al., 2016[46]
Cross-sectional studies (2)	ADR: Proliferative changes	Patients who underwent endoscopy and who had been using proton-pump inhibitors for at least 2 months (22)	Menegassi et al., 2010[5]
	ADR: Decreased serum levels of vitamin B12	Patients with diagnosis of gastrointestinal disease in the consumption of proton pump inhibitors (109)	Mindiola et al., 2017[40]
No clinical outcomes	**Clinical trials (12)**	Many conditions	Yamamoto et al., 1995[86]; Goh et al., 1995[87]; Soga et al., 1999[37]; Noordzij et al., 2001[88]; Zhou et al., 2002[43]; van Zanten et al., 2005[35]; Fujiwara et al., 2005[89]; Liu et al., 2013[90]; Miner JR et al., 2010[91]; Ummarino et al., 2012[36]; Sakurada et al., 2012[92]; Solana et al., 2013[93]
	Cohort studies (5)	Many conditions	Zairis et al., 2010[94]; Harjai et al., 2011[95]; Chen et al., 2014[28]; Galante et al., 2012[96]; Wang et al., 2017[97]
	Cross-sectional studies (2)	Newborns with hypospadias born to mothers who had used proton-pump inhibitors during pregnancy (430,569)	Erichsen et al., 2014[98]
		Patients with stage 5 chronic kidney disease (CKD) on hemodialysis therapy and chronic use of proton pump inhibitors (37)	Restrepo et al., 2017[99]

ADE = adverse drug event; ADR = adverse drug reaction; DI = drug interaction; TI = therapeutic ineffectiveness.

Table 3. Assessment of risk of bias in clinical trials using the RoB 1.0 tool and evaluation of quality of cohort and control case studies using the Newcastle-Ottawa scale

	Study	Risk of bias						
		Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Clinical trials (n = 39)	Yamamoto et al., 1995[86]	Unclear	High	High	High	Low	Low	Low
	Bate et al., 1995[50]	Unclear	Unclear	Low	Low	Low	Low	Low
	Goh et al., 1995[87]	Unclear	Unclear	Unclear	Low	Unclear	Low	Low
	Marzio et al., 1995[51]	Unclear	Unclear	Low	Low	Low	Low	Low
	Leite et al., 1996[58]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
	Valenzuela et al., 1996[52]	Unclear	Unclear	Low	Low	Low	Low	Low
	Muckadell et al., 1997[39]	Low	Low	Low	Low	Low	Low	Low
	Labenz et al., 1997[53]	Low	Unclear	Low	Low	Low	Low	Low
	Annibale et al., 1997[55]	Unclear	Unclear	Low	Low	Low	Low	Low
	Hasselgren et al., 1997[54]	Unclear	Low	Low	Low	Low	Low	Low
	Lind et al., 1999[61]	Unclear	High	Low	Low	Low	Low	Low
	Soga et al., 1999[37]	Low	Low	Unclear	Unclear	Low	Low	Low
	Klinkenberg-Knol et al., 2000[60]	High	Unclear	Unclear	Low	High	Low	Low
	Ladas et al., 2000[57]	Low	High	Low	High	Low	Low	Low
	Richter et al., 2000[59]	Unclear	Unclear	Low	Unclear	Low	Low	Low
	Blum et al., 2000[62]	Unclear	Low	Low	Low	Low	Low	Low
	Noordzij et al., 2011[88]	Unclear	Low	Low	Low	Low	Low	Low
	Gottrand et al., 2001[33]	Low	Low	Low	Low	Low	Low	Low
	Esaki et al., 2002[65]	Unclear	Unclear	Low	Low	Low	Low	Low
	Rabeneck et al., 2002[64]	Low	Low	Low	Low	Low	Low	Low
	Zhou et al., 2002[43]	Unclear	Unclear	High	High	Low	Low	Low
	Thjodleifsson et al., 2000[63]	Low	Low	Low	Low	Low	Low	Low
	Armstrong et al., 2005[66]	Low	Low	Low	Low	Low	Low	Low
	Fujiwara et al., 2005[89]	Unclear	Unclear	High	High	Low	Low	Low
	Manes et al., 2005[38]	Low	Unclear	Low	High	Low	Low	Low
	Ohkusa et al., 2005[67]	High	High	Unclear	Unclear	Low	Low	Unclear
	Van Zanten et al., 2005[35]	Low	Low	Low	Low	Low	Low	Low
	Madisch et al., 2005[68]	Low	Low	Low	Low	Low	Low	Low
	Schooler et al., 2008[32]	Low	Unclear	Low	High	Low	Low	Low
	Howden et al., 2009[69]	Unclear	Unclear	Unclear	High	Low	Low	Low
	Lundell et al., 2009[70]	Unclear	Unclear	Low	Unclear	Low	Low	Low
	Miner et al., 2010[91]	Low	Low	Low	Low	Low	Low	Low
	Hudzik et al., 2010[72]	High	High	Low	High	Low	Low	Low
	Ferreiro et al., 2010[71]	Unclear	Unclear	Low	Low	Low	Low	Low
	Miwa et al., 2011[73]	Low	Low	Low	Low	Low	Low	Low
	Sakurada et al., 2012[92]	Unclear	Low	Low	High	Low	Low	Low
	Ummarino et al., 2011[36]	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
	Liu et al., 2013[90]	Low	Unclear	Low	High	Low	Low	Low
	Solana et al., 2013[93]	Low	Unclear	Unclear	Unclear	Low	Low	Low

Evaluation of quality of cohort and control case studies using the Newcastle-Ottawa scale				
	Study	Domains		
		Selection (4*)	Comparability (2*)	Outcome (3*)
Cohort studies (22)	Klinkenberg-Knol et al., 1994[74]	4*	1*	3*
	Kreutz et al., 2010[75]	4*	2*	3*
	Van Boxel et al., 2010[76]	4*	2*	3*
	Zairis et al., 2010[94]	4*	2*	3*
	Cadle et al., 2007[13]	4*	2*	3*

Continue...

Table 3. Continuation

	Study	Domains		
		Selection (4*)	Comparability (2*)	Outcome (3*)
Cohort studies (22)	Teichert et al., 2011[77]	4*	2*	3*
	Ligumsky et al., 2011[80]	3*	2*	3*
	Galante et al., 2012[96]	4*	1*	3*
	Lin et al., 2012[81]	4*	2*	3*
	David-Neto et al., 2012[49]	4*	1*	3*
	Soriano et al., 2014[16]	4*	2*	3*
	Chen et al., 2014[28]	4*	2*	3*
	Wang et al., 2017[97]	4*	2*	3*
	Gomm et al., 2016[44]	4*	2*	3*
	Yi et al., 2017[47]	4*	2*	3*
	Varallo et al., 2018[41]	4*	2*	3*
	Imperatore et al., 2018[45]	4*	2*	3*
	Lazzerini et al., 2018[42]	4*	1*	3*
	Li et al., 2018[48]	4*	2*	3*
	Study	**Domains**		
		Selection (4)	**Comparability (2*)**	**Exposure (3*)**
Case-control study (2)	Chularojanamontri et al., 2012[85]	4*	2*	3*
	Heijgen et al., 2016[46]	4*	2*	3*

Several drug interactions relating to omeprazole, especially with antiplatelet agents, are known.[78,94] The non-serious events that have been described are diarrhea, headache and somnolence, relating to use of omeprazole concomitantly with the antiretroviral drug etravirine.[32] The severe adverse events that have been described comprise inhibition of the antiplatelet effects of drugs such as clopidogrel, which increases the risk of developing heart problems that may lead to death; and decreased absorption of mycophenolic acid, which leads to rejection of transplanted organs.[49]

Nevertheless, it is not possible to say with certainty that the adverse events described in these studies occurred due to drug interactions with omeprazole, since some of the studies included did not present statistically significant results.[71,94-96]

In two studies in which omeprazole was added to dual antiplatelet therapy (a combination of clopidogrel and acetylsalicylic acid), it reduced the stomach pain resulting from this therapy and no risk was found in this combination.[95] Nonetheless, it is always necessary to monitor potentially dangerous drug combinations between omeprazole and clopidogrel, acetylsalicylic acid and mycophenolate mofetil, among others.

Regarding drug interactions, all patients may be exposed to their effects, regardless of age or clinical condition. However, some patients are more susceptible, such as those who already have some type of heart disease or the elderly, who commonly use polypharmacy.

Only 12 studies included elderly patients, and these studies reported occurrences of severe adverse events such as dementia, myocardial infarction, cardiovascular death, stroke and pulmonary embolism, among others. In the non-elderly population, the severe adverse events reported included myocardial infarction, stroke, death and pulmonary embolism, but no relationship between the severity or the frequency of events and the patients' age was observed from use of omeprazole. However, other authors have suggested that age is a factor that influences occurrences of adverse events. Varallo et al.[24] observed in a cross-sectional study that the elderly population had fewer ADEs than adults did, probably because doctors provide greater care and attention regarding pharmacotherapeutic management for patients of this age group, since there are other factors that increase the likelihood of ADEs, such as polypharmacy. Beijer and de Blaey[100] reported that the chances that elderly individuals would need to be hospitalized due to adverse drug reactions (ADRs) were four times higher than those of younger people (16.6% versus 4.1%). Additionally, in 2015, the American Geriatrics Society advised through the Beers criteria that unjustified use of PPIs among the elderly for more than eight weeks should be avoided, since exposure to

such drugs increases the risks of infection by *Clostridium difficile*, bone loss and fractures.[13,16,101]

Another factor that may have influenced the appearance of adverse events is the duration of use of omeprazole. Non-serious adverse events such as diarrhea, headache, flatulence and abdominal pain, among others, have been reported among patients taking omeprazole for short periods of time, i.e. from a few days of use to a maximum of two weeks.[32-36,74] Severe adverse events have been reported among patients who used omeprazole for longer times, i.e. more than one month.[35-37, 54,57,60,63,65-67,70,71,74-76,70,95,]

In only one of the studies analyzed here was omeprazole prescribed for off-label use.[41] However, off-label prescription of omeprazole is widespread in many countries and there is a need to assess the safety of this use. We take the view that the duration of exposure is likely to increase the likelihood of adverse events, since polypharmacy alone is a risk factor for occurrences of adverse events.[24]

Outcomes of therapeutic ineffectiveness and symptom worsening were identified. It was noted that some patients did not respond to omeprazole treatment[13,32,64,70] and that for others, their symptoms worsened.[62] The most likely reason for such events would be high concentrations of acid in the stomach, which could cause gastroparesis, decrease absorption and, consequently, decrease the therapeutic effect of omeprazole.

Although most of the adverse events reported were already known, unexpected events such as dementia,[44] low-motility sperm,[46] miscarriage, proliferative changes,[5] increased levels of chromogranin A,[79] increased levels of fibroblast growth factor 2,[72] chills, cardiovascular events (myocardial infarction, heart failure, stroke, ischemic stroke, pulmonary embolism and thrombosis),[47] scarlet fever, hyperglycemia, mononucleosis infection, gastrointestinal bleeding, nasopharyngitis, otitis media, loss of libido and rhinitis have also been identified.[4,65-67,102] Because the associations between these adverse events and use of omeprazole are not fully understood, there is a need to carry out further studies to investigate the relationships between omeprazole and these events. If such associations are verified, they should be described in the package leaflet.

In addition, more recent studies have identified other adverse events, such as decreased vitamin B12 levels,[40] increased levels of creatinine[41] and hypomagnesia.[42]

Use of omeprazole is considered safe in the following situations: when it is not combined with antiplatelet drugs; when it is administered to replace H2 receptor antagonists in patients who are resistant to treatment with drugs of this class; when the most appropriate posology and dosage is established for each condition and patient; and when omeprazole is used in conjunction with a combination of antibiotics to eradicate *H. pylori* and to treat esophagitis, among other situations.[94,102]

Limitations of the present study

No *a priori* design was provided for this review and the languages were restricted to Portuguese, English and Spanish.

Gray literature was not included. However, its inclusion would be unviable and probably would not add to the results found, since this type of literature is characterized by incomplete and poorly constructed data.

No methods were used to assess the homogeneity or heterogeneity between the studies, and the risk of publication bias among the studies included was not assessed. Furthermore, no information regarding potential conflicts of interest in the primary studies included was available.

All the outcomes evaluated related to approved indications for use of omeprazole. Therefore, the data confirm that there is no evidence of clinical outcomes (safety and effectiveness) resulting from unapproved use of omeprazole, such as polypharmacy (although polypharmacy is commonly used). The duration of use of omeprazole influenced occurrences of adverse events. Severe adverse events, such as death, stroke and myocardial infarctions occurred during prolonged treatments (more than one month). Non-serious adverse events occurred over short periods (from a few days to a maximum of two weeks). Use of omeprazole needs to be monitored primarily in patients with heart disorders who are using antiplatelet agents and omeprazole concomitantly and in newly transplanted patients who are using mycophenolic acid as a suppressive agent, in order to avoid severe adverse reactions such as organ transplant rejection, death, stroke and myocardial infarction.

CONCLUSION

Therefore, use of omeprazole can be considered safe in the following situations: when it is not combined with antiplatelet drugs; when it is administered to replace H2 receptor antagonists in patients who are resistant to treatment with drugs of this class; when the posology is well established for each condition and type of patient; and when omeprazole is used to eradicate *H. pylori*, among others. Most of the trials included in this review presented uncertain risk or high risk of bias, which indicates that there is a need for better-designed studies. The high risk of bias related mainly to the blinding of the participants and outcome assessors. It should be noted that if patients and professionals believe that omeprazole is a gastric protector and is risk-free, this may lead to bias in the analysis and to under identification and underreporting of adverse events relating to omeprazole. This may suggest that the existing studies may have underestimated the adverse events.

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Address for correspondence:
Patrícia de Carvalho Mastroianni
São Paulo State University (UNESP), School of Pharmaceutical Sciences
Rodovia Araraquara-Jaú, Km 1, s/nº
Campus Ville — Araraquara (SP) — Brazil
CEP 14800-903
Tel. (+55 16) 3301-6977
E-mail: patriciamastroianni@yahoo.com.br

Teaching skills for medical residents: are these important? A narrative review of the literature

Saadallah Azor Fakhouri Filho[I], Lorena Pinho Feijó[II], Kristopherson Lustosa Augusto[III], Maria do Patrocínio Tenório Nunes[IV]

Universidade Federal de Uberlândia (UFU), Uberlândia (MG), Brazil

[I]MD. Doctoral Student, Universidade Federal de Uberlândia (UFU), Uberlândia (MG), Brazil.
orcid.org/0000-0001-8413-0619

[II]MD. Professional Master's Student, Centro Universitário Christus (UNICHRISTUS), Fortaleza (CE), Brazil.
orcid.org/0000-0001-9638-1992

[III]MD, PhD. Adjunct Professor, Department of Clinical Medicine, Faculdade de Medicina da Universidade Federal do Ceará (FAMED - UFC) and Universidade de Fortaleza (UNIFOR), Fortaleza (CE), Brazil.
orcid.org/0000-0001-9254-9129

[IV]MD, PhD. Associate Professor, Department of Internal Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo (SP), Brazil.
orcid.org/0000-0003-3616-515X

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ABSTRACT

BACKGROUND: There is extensive evidence, mainly from the United States and Canada, that points towards the need to train medical residents in teaching skills. Much of the "informal curriculum", including professional values, is taught by residents when consultants are not around. Furthermore, data from the 1960s show the importance of acquiring these skills, not only for residents but also for all doctors. Teaching moments can be identified in simple daily situations, like discussing a clinical situation with patients and their families, planning patients' care with the healthcare team or teaching peers and medical students. The aim here was to examine the significance of resident teaching courses and estimate the effectiveness of these courses and the state of the art in Brazil.

METHODS: We conducted a review of the literature, using the MEDLINE, PubMed, SciELO and LILACS databases to extract relevant articles describing residents-as-teachers (RaT) programs and the importance of teaching skills for medical residents. This review formed part of the development of a doctoral project on medical education.

RESULTS: Original articles, reviews and systematic reviews were used to produce this paper as part of a doctoral project.

CONCLUSIONS: RaT programs are important in clinical practice and as role models for junior learners. Moreover, these educational programs improve residents' self-assessed teaching behaviors and teaching confidence. On the other hand, RaT program curricula are limited by both the number of studies and their methodologies. In Brazil, there is no such experience, according to the data gathered here, except for one master's thesis.

INTRODUCTION

Learning medicine is not a lonely journey and is no longer a passive act. Doctors at both junior and senior levels participate in this complex process, thereby facilitating learning and consolidating and updating knowledge daily. Santos et al.[1] described facilitators as doctors who are more experienced and who thus help in the professional development of undergraduates, medical residents and their peers. These authors referred to the work of Vygotsky,[2] for whom "the learning process comes from outside sources and is conceived through individual interactions with the world". Furthermore, they postulated that the role of facilitators (preceptors) is to enable some situations in which apprentices' knowledge assimilation and production becomes transformed. The preceptor's main role is therefore to facilitate the acquisition of theory and skills by stimulating his/her pupils to make their own discoveries.

Residency is characterized by in-service learning, i.e. training during practice within a scenario in which residents may become role models, in accordance with statements based on educational strategies for teaching and learning. Therefore, it is crucial to balance teaching, learning and healthcare assistance.

The most remarkable characteristic of medical residency is its in-service training, in which teaching is integrated with practice scenarios, so as to build a model for physicians' ideological, ethical and professional identity. The professional competence that is expected at the end of a medical residency program needs to go beyond technical knowledge. It also encompasses skills and attitudes that show effective team capabilities, leadership, communication skills, empathy, self-control and metacognition.[3] Sternszus et al.[4] investigated the importance of resident role models in the education and career choices of medical students, in a cross-sectional survey-based study. Their study was the first to illustrate that resident role models are perceived by medical

students to be as important as role models formed by attending physicians, for their education.

Medical residents are an essential part of the workforce in most Brazilian hospitals. In teaching centers, because of the humdrum nature of daily tasks, there is an additional responsibility towards helping students and peers, to help them improve their knowledge and technical skills. Several authors have noted that the word 'doctor' comes from the Latin verb *docere*,[5] meaning to teach, with the aim of highlighting how teaching is important for all medical professionals, including those under training.

American researchers have estimated that residents spend almost a quarter of their residency programs teaching others, even though they are undertrained for this purpose.[6] Much of the "informal curriculum", including professional values, is taught by residents.[7] This process focusing on peer-to-peer cooperative learning remains poorly studied.

Development of clinical acumen through good clinical teaching is a key component of medical education. Few residents will come to postgraduate training with well-developed teaching skills or a sense of their relevance to student education, or with knowledge of the principles of adult learning and its theory and practice.[5]

Pioneering studies conducted in the United States and Canada have highlighted the relevance of training medical residents to provide them with teaching skills. These studies demonstrated that 20-25% of the residents' working hours were spent on teaching activities,[6,8,9] and that medical students attributed 30-85% of all the clinical theory that they acquired during their undergraduate programs to teaching given by their residents.[10,11] All of this exchange of information is provided through long working hours and exhaustive periods on call. Despite all the information presented above, doctors, medical students and residents receive little or no formal instruction on how to teach.[10,12,13]

Both the American College of Graduate Medical Education[14] and the Liaison Committee on Medical Education[15] have recommended that formal instruction on teaching skills should be provided for medical residents. These activities have been deemed to be so important that Louie et al.[5] described the development of residents as teachers in terms not only of a necessary personal obligation but also of a national priority.

The aim of the present review was to assess the significance attributed to programs on teaching skills for residents that have been described in the literature. We aimed to compare these results with findings in Brazil, in order to provide up-to-date conclusions and recommendations regarding this topic.

METHODS

We conducted a review of the literature, using MEDLINE, PubMed, SciELO and LILACS databases to extract relevant articles describing residents-as-teachers (RaT) programs and the importance of teaching skills for medical residents. This review formed part of the development of a doctoral project on medical education. We searched the literature between January 2015 and December 2017, covering the period between 1970 and 2017.

The MeSH terms used in the search were "internship and residency", "education" and "education, medical". The key words used in the search included "residents", "residents as teachers", "residents-as-teachers", "residents AND teachers", "residents AND education", "education" and "medical education". The key words used for the search in Portuguese in LILACS and SciELO included "medicos residentes", "medicos residentes AND educacao", "medicos residentes AND ensino", "educacao medica" and "ensino em medicina". The articles identified as containing information regarding teaching skills for medical residency were selected for intensive review and were analyzed by two authors (SAF and MPTN). Searches of primary publications referenced in other articles were also included. These were selected for intensive review and were analyzed by two authors (SAF and MPTN).

RESULTS

The search in LILACS and SciELO using Portuguese key words did not yield any results on the specific topic of formal residents-as-teachers programs, while the search in MEDLINE/PubMed yielded 213 articles, of which 44 were found to contain information regarding teaching skills for medical residency. The references in Portuguese that were included in this paper were extracted from the personal files of one author (MPTN).

1) Selection of relevant articles:

Table 1 describes the numbers of texts extracted from each database. The relevant publications included original articles, systematic reviews, critical reviews, randomized controlled trials, guidelines from medical education experts and material from pioneering authors in this field. **Table 2** shows the most relevant studies included in this review and summarizes their main characteristics.

2) Residents-as-teachers programs from abroad:

Specific agendas for training residents in teaching skills were first developed in the 1960s. Since then, the numbers of residents-as-teachers (RaT) programs around the world has increased and their methodologies have improved. RaT programs are nowadays part of most residency programs in the United States.

Table 1. Databases and numbers of articles extracted

Database	Number of articles
PubMed	68
MEDLINE	95
Other sources*	4

*Personal files of one author.

Table 2. Relevant studies on residents-as-teachers (RaT) programs that were included in this review

Authors	Year	Study type*	Participants (n)	Specialty	Main objectives	Conclusions
Jewett et al.[20]	1982	RCT	55 residents	Pediatrics	To compare residents who received clinical teaching instruction with those who did not.	The intervention group was significantly more confident as teachers and received more positive feedback on their teaching.
Sheets et al.[19]	1991	RL	NA	Surgery	To describe evidence that supported the importance of training surgery residents in teaching skills.	Residency program directors and faculty members within surgery needed to acknowledge that teaching was an important component of residents' daily agenda.
Bordley et al.[22]	2000	NR	NA	Internal medicine	To describe the importance of teaching skills for residents and discuss the costs involved in these activities at different institutions.	The authors strongly supported investment in training residents as teachers.
Morrison et al.[13]	2000	NR	NA	NA	To describe the number of RaT programs in the US and demonstrate evidence for their implementation and evaluation.	Research was needed to identify the most appropriate design for RaT programs and how they affected educational outcomes.
Furney et al.[30]	2001	RCT	57 second- and third-year residents	Internal medicine	To compare residents who received a one-hour intervention based on the One-Minute Preceptor, with a control group.	Intervention group residents reported statistically significant changes in all behaviors after the One-Minute Preceptor.
Morrison et al.[37]	2002	QS	100 medicine students, residents and faculty members	Internal medicine Pediatrics Family medicine	To describe the learning needs of residents for becoming more effective teachers, using 11 focus groups and 4 semi-structured interviews.	Residents filled important roles as practical clinical teachers and role models for junior learners.
Wamsley et al.[26]	2004	RL	14 articles on RaT programs	Multiple	To examine the evaluation methods for resident teaching courses and estimate the effectiveness of those teaching courses.	Resident teaching courses improved resident self-assessed teaching behaviors and teaching confidence. Further studies were needed to elucidate the best format, length, timing and content of these courses and to determine whether they influenced learner performance.
Morrison et al.[23]	2005	QS	21 third-year residents	Internal medicine Family medicine Pediatrics	To compare residents who received a 13-hour training in teaching skills in the previous year, with those who did not, through semi-structured interviews.	Intervention group residents expressed more enthusiasm for teaching, learner-centered learning and self-knowledge about teaching. Control group residents seemed easily frustrated by time constraints and often expressed cynicism and guilt toward learners.
Dewey et al.[33]	2008	SR	13 articles on RaT programs	Multiple	To identify all randomized control trials (RCTs) on residents' teaching skills programs in psychiatry.	Only one trial incorporating psychiatry residents was found to exist.
Busari et al.[21]	2009	QS	18 residents	Pediatrics Obstetrics and gynecology	To extract recommendations from interviews, regarding how a training program for residents could be created.	Enthusiasm and enjoying teaching were good attributes of successful teachers. Reasons for poor teaching were lack of time and absence of support from attending staff.
Post et al.[27]	2009	SR	24 articles on RaT programs	Multiple	To provide an updated systematic review of the literature on RaT program curricula and determine the most evidence-based curriculum and evaluation strategy.	Research on RaT program curricula was limited by both the number of studies and their methodology. The results demonstrated that these curricula can significantly improve residents' teaching skills.

Continue...

Table 2. Continuation

Authors	Year	Study type*	Participants (n)	Specialty	Main objectives	Conclusions
Karani et al.[18]	2014	QS	37 third-year medical students	NA	To describe what students learned from residents and teaching strategies used by excellent resident teachers.	In this study, role modeling was the most frequently classified teaching model. Residents' teaching was critically important for undergraduate students.
Owolabi et al.[38]	2014	QS	20 residents	Internal medicine	To evaluate the clinical teaching skills of internal medicine residents from the perspective of medical students in a tertiary-level teaching institution in Africa.	Residents' clinical teaching skills were suboptimal, particularly regarding their ability to promote understanding and retention.
Dannaway et al.[28]	2016	RL	12 articles on RaT programs	Multiple	To assess the current evidence regarding the efficacy of teaching skills programs for junior medical officers.	The review of the literature demonstrated many positive effects from teaching skills programs, thus supporting their use. Substantial threats of bias were present in most studies.
Ramani et al.[17]	2016	FG	NA	Multiple	To guide medical educators involved in the implementation of RaT programs.	The authors highlighted the importance of congruence between formal and hidden curricula and encouraged evidence-based approaches within education.
Al Achkar et al.[16]	2017	QQS	221 residency program directors	Multiple	To compare the number of RAT programs with data from a previous study in 2000 and ask for feedback about the importance of these activities.	Over 80% of the residency programs surveyed had implemented RaT programs. Program directors had realized that there was a clear need for formative training experiences for residents.
Chokshi et al.[32]	2017	IR	29 second-year residents	Pediatrics	The authors developed and evaluated an intensive one-day RaT program curriculum using a flipped classroom approach.	Residents demonstrated statistically significant improvements in performance between pre- and postworkshop evaluations through objective structural teaching evaluations and attitudinal and self-efficacy questionnaires.

*NR = narrative review; QQS = qualitative and quantitative survey; FG = framework guide; QS = qualitative study; RL = review of the literature; RCT = randomized controlled trial; SR = systematic review; IR = innovation report; NA = not applicable.

Morrison et al.[13] reported that the prevalence of programs for developing residents' teaching skills in the United States was 55%. In 2017, the same authors published new data in which the prevalence was established as 80.54%, i.e. a 26.34% increase (95% confidence interval, CI: 20.39% to 32.29%) over the last 15 years.[16] Ramani et al.[17] described the potential benefits of RaT programs in the Association for Medical Education in Europe (AMEE) 2016 guide 106. Karani et al.[18] reported that being a role model was the tool that residents who were teaching most frequently identified. Acquiring teaching skills was seen to involve complex conscious and unconscious activities, through observation and reflection on behaviors.

3) Impact of residents-as-teachers programs on residents and students:

RaT programs have been particularly successful for several reasons. Medical students like to work with residents and, additionally, appreciate their close supervision. Thus, residents are seen as a positive influence and example of professionalism.[10,19-21] Moreover, residents spend large amounts of time together with medical students. They are close to them in practical activities and have similar ages and professional development processes. Most residents feel more satisfaction with their work while experiencing teaching duties.[22,23] Moreover, these programs are especially attractive because they improve self-confidence in teaching.

Teaching by residents is different from and probably complementary to that of institutions' attending staff and faculty members. Residents tend to teach

1. different things (bedside skills and patient management rather than factual knowledge);
2. in a different way (as near-peer teachers); and
3. at different times (teaching while on-call).[24]

In a qualitative analysis on a teaching initiation module that had been presented, it was concluded that it was possible to develop pedagogical skills at this stage, in a process that would be coherent with changes to health and education policies.[25] According to the same authors, residents needed to learn:

1. to take on leadership and provide a role model;
2. to give guidance to learners;
3. to give feedback,
4. to teach bedside skills;
5. to teach about procedures;
6. to teach about inpatients;
7. to teach about charting; and
8. to give lectures.

Most of the studies on RaT programs have shown that residents achieved significant improvement in teaching skills after some specific training. The first review of the literature on this topic was published in 2004 and analyzed 13 studies with different experimental designs.[26] A review conducted in 2009 assessed 24 studies on residents who were enrolled in several programs and found that in 21 of these studies there was significant enhancement of teaching performance after specific training.[27] A recent assessment on 39 studies, published in 2016, demonstrated that interventions based on providing teaching skills up to level 3 on Kirkpatrick's outcome scale were effective (**Table 3**).[28,29]

The evidence supporting RaT programs appears not to correlate the time spent on training with efficiency in teaching. Even a one-hour intervention showed benefits in a study involving internal medicine residents.[30] A four-week elective course created for senior and family medicine residents paved the way for a paper giving advice on how to create a RaT program.[31] In another study published in 2017, 29 participants were enrolled in a successful one-day RaT program that used a "flipped classroom" approach.[32]

In another systematic review, an efficacy analysis was conducted on residents from a wide spectrum of specialties who participated in single programs.[33] On the whole, the interventions and outcomes measured were heterogeneous and the quality of the methodologies varied. The authors felt that these programs brought the opportunity to advance educational research in this field.[33]

Residents who teach acquire the material that they teach more effectively than they would if they did it through self-study or through attending lectures.[34] Their teaching duties have been linked to greater job satisfaction.[23]

In 2016, a meta-analysis revealed that most studies on residents-as-teachers programs had significant methodological flaws. Nevertheless, it was found that the main impacts of these interventions included improvement of attitudes and positive perceptions toward clinical teaching (Kirkpatrick level 2a); support for modification of knowledge or skills (Kirkpatrick level 2b); development of teaching skills (Kirkpatrick level 3) and some improvement of students' learning after the intervention (Kirkpatrick level 4b). Some studies revealed positive organizational change (Kirkpatrick level 4a).[28]

Residents with better teaching skills seem to have greater knowledge of taught material and better clinical skills. In a retrospective study on senior residents in general surgery (covering the period from 2009 to 2013), technical ability was assessed through their performance in the Fundamentals of Laparoscopic Surgery examination. Teaching ability was assessed through evaluations among medical students on a Likert scale. There was evidence that residents who were better teachers had greater knowledge of taught material and a higher degree of laparoscopic skills.[35]

Snell[24] provided information on the effects of RaT programs on residents. This author showed that those who were teaching had greater enthusiasm for teaching and greater job satisfaction.

Residents with effective teaching skills may also have a positive effect on patient care. Involvement of residents in teaching

Table 3. Kirkpatrick's model[29] for evaluating educational outcomes*

Kirkpatrick level	Evaluation outcome	Explanation
Level 1	Reaction	Participants' views of the learning experience and its organization, presentation, content, teaching methods and quality of instruction
Level 2A	Learning – change in attitudes	Changes in attitudes or perceptions among participant groups towards teaching and learning
Level 2B	Learning – modification of knowledge or skills	For knowledge, this relates to the acquisition of concepts, procedures and principles For skills, this relates to the acquisition of thinking and problem-solving, psychomotor and social skills
Level 3	Behavior – change in behavior	Documents the transfer of learning to the workplace or willingness of learners to apply new knowledge and skills
Level 4A	Results – change in the system or organizational practice	Refers to wider changes in the organization attributable to the educational program
Level 4B	Results – change among the participants: students and peers	Refers to improvement in medical student or peer learning or performance as a direct result of the educational intervention

*Adapted from Kirkpatrick (1994).[29]

activities has been shown to have a positive effect on their communication skills, and good patient communication skills have been associated with better clinical outcomes.

4) Content of current RaT programs:

RaT programs have been delivered using many methods, including lectures, small-group discussion, practice with peers, videotape reflections and role-playing. The total number of hours dedicated to RaT program instruction varies widely. In the USA, RaT programs are more prevalent within pediatrics, family medicine, internal medicine, psychiatry, obstetrics/gynecology and surgery.

A thematic analysis identified five main reasons for implementing RaT programs: (1) teaching is part of residents' role; (2) learners desire formal RaT training; (3) regulatory bodies require RaT training; (4) RaT programs improve residents' education; and (5) RaT programs prepare residents for their current and future roles. There are also five reasons for not implementing RaT training: (1) lack of time and energy; (2) lack of expertise and resources; (3) newness of the program; (4) limited access to students; and (5) RaT instruction is not desired.[16]

5) Brazilian context:

A recent review of the literature only identified one study on RaT programs in Brazil.[36] The authors concluded that there was no description of formal development of teaching skills within medical residency curricula, according to the databases that were searched. Regarding the teaching-learning process (for residents in family medicine), the authors stated that the pedagogical project and teaching plan for RaT programs constituted a social complex that would need a reflective, critical and collaborative approach.

DISCUSSION

This narrative review shows that residents with better teaching skills might have greater knowledge of taught material and better clinical skills, according to some studies, which in turn enhances patient care. Students and senior doctors agree that residents have a critical role in the teaching process.[37] Residents themselves can also recognize their protagonist role in spreading knowledge to students, peers, patients and families.

Around the world, universities set the goals for preparing residents to teach. These objectives have been seen as an effective way of improving the entire educational experience of residents and preparing them for the future.

It is not just in developed countries that the importance of training residents in teaching skills is acknowledged. Some developing nations are also publishing their own experiences in this field. One study in Nigeria has highlighted the importance of bringing medical residents into the teaching scenario in countries with limited resources for hiring teachers.[38]

RaT programs are delivered using many methods and a great variety of lengths of training. However, the resources available, and especially the expertise to lead the instruction, are not distributed equally among residency programs.

Data on training residents as teachers in Brazil is scarce despite the creation of new medical schools and the resulting increase in the number of students, which has brought difficult challenges to the educational system. There is an urgent need for better preparation of educators for teaching. Since 2006, Hospital das Clínicas of the Federal University of Pernambuco (Universidade Federal de Pernambuco, UFPE) has offered a 64-hour training program to medical residents within family medicine, with the aim of transforming this reality.[36]

In the literature, it is stated that residents who teach have greater enthusiasm for teaching, with greater job satisfaction. Residents are also role models for medical students. It is possible that pedagogical development also has a positive effect on patient care, though addition of effects regarding physician communication skills, with better clinical outcomes

In an ongoing study, Brazilian residents at a public university are being trained in the One-Minute Preceptor[39] methodology, in comparison with controls. Our training education program combines theoretical and practical resources with a unique one-hour long dynamic. The partial results are very encouraging, with good validation scores for peer feedback based on the Stanford Faculty Development Program 26 (SFDP-26) instrument.[40] Our study also revealed that significant positive changes to the residents' teaching skills were achieved.

One innovative facet of this study was that it included scarce data on RaT programs in Brazil. We aimed to explore the reasons for implementing RaT instruction, given the fact that most Brazilian programs for residency training currently do not include RaT programs. We face the challenge of teaching pedagogical strategies for medical residents in this country. The positive effects may be of great significance for students, medical residents and patients.

The present study was susceptible to selection bias and reporting bias. We also only explored instruction of RaT program mode that had been introduced. Hence, little can be concluded regarding the effectiveness of any other form of instruction or the relevance of any other targeted skills.

CONCLUSIONS

It is necessary to add pedagogical training to the training for residents and others working in the Brazilian National Health System, regarding ethical, technical and scientific knowledge. Some successful initiatives for developing skills and attitudes within healthcare education have emerged. However, these are still insufficient.

The necessary expansion of medical residency programs and the already inflated number of medical schools in Brazil both require

qualified teachers. Implementing pedagogical training during residency training could improve clinical skills and patients' care.

An accurate diagnosis regarding the state of pedagogical attributions among medical residents in Brazil is urgently necessary. There is also a need to understand what tools are at their disposal to perform the act of teaching with quality and efficacy, cooperatively.

Considering the positive effects that have been demonstrated, it can be argued that all residency programs should require residents to undergo instruction relating to teaching skills.

Along with teacher development programs, training for medical residents as teachers has the capacity to boost the quality of healthcare education in Brazil.

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Address for correspondence:
Saadallah Azor Fakhouri Filho
Av. Pará, 1.720 — Bloco 2H
Departamento de Clínica Médica, Universidade Federal de Uberlândia (UFU)
Campus Umuarama
Uberlândia (MG) — Brasil
CEP 38405-320
Tel. (+55 34) 3225-8621
E-mail: safakhouri@hotmail.com

What do Cochrane systematic reviews say about interventions for insomnia?

Florence de Lucca Melo[I], Juan Fulgencio Welko Mendoza[II], Carolina de Oliveira Cruz Latorraca[III], Rafael Leite Pacheco[IV], Ana Luiza Cabrera Martimbianco[V], Daniela Vianna Pachito[VI], Rachel Riera[VII]

Discipline of Evidence-Based Health, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil. Cochrane Brazil, São Paulo (SP), Brazil.

[I]Undergraduate Medical Student, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil.
orcid.org/0000-0002-5688-4533

[II]Undergraduate Medical Student, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo (SP), Brazil.
orcid.org/0000-0002-1616-0231

[III]MSc. Psychologist; Postgraduate Student, Evidence-Based Health Program, Universidade Federal de São Paulo (UNIFESP); and Assistant Researcher, Cochrane Brazil, São Paulo (SP), Brazil.
orcid.org/0000-0001-9146-4684

[IV]MD. Postgraduate Student, Evidence-Based Health Program, Universidade Federal de São Paulo (UNIFESP), and Assistant Researcher, Cochrane Brazil, São Paulo (SP), Brazil.
orcid.org/0000-0001-7487-8471

[V]MSc, PhD. Physiotherapist; Postdoctoral Student, Evidence-Based Health Program, Universidade Federal de São Paulo (UNIFESP); Professor, Health and Environment Program, Universidade Metropolitana de Santos (UNIMES); and Volunteer Researcher, Cochrane Brazil, São Paulo (SP), Brazil.
orcid.org/0000-0002-4361-4526

[VI]MD, MSc. Neurologist; Postgraduate Student, Evidence-Based Health Program, Universidade Federal de São Paulo (UNIFESP); and Assistant Researcher, Cochrane Brazil, São Paulo (SP), Brazil.
orcid.org/0000-0002-7052-7735

[VII]MD, MSc, PhD. Rheumatologist; Adjunct Professor, Discipline of Evidence-Based Medicine, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP); and Researcher, Cochrane Brazil, São Paulo (SP), Brazil.
orcid.org/0000-0002-9522-1871

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Sleep Initiation and Maintenance Disorders.
Comparative Effectiveness Research.

ABSTRACT

CONTEXT AND OBJECTIVE: Insomnia is a frequent complaint that generates more than five million visits to doctors per year in the United States. This study summarizes all Cochrane systematic reviews (SRs) that evaluated interventions to treat insomnia.

DESIGN AND SETTING: Review of SRs, conducted in the Discipline of Evidence-Based Medicine, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP).

METHODS: A sensitive search was carried out in the Cochrane Database of Systematic Reviews to identify Cochrane SRs that assessed the effects of any type of intervention for people with insomnia. The results, main characteristics of the SRs and the certainty of the evidence obtained from them were synthesized and discussed.

RESULTS: Seven SRs were included. They addressed the benefits and harm of acupuncture (n = 1), behavioral interventions (n = 1), music (n = 1), pharmacotherapy (n = 2), phototherapy (n = 1) and physical exercise (n = 1). The certainty of the evidence ranged from moderate to very low.

CONCLUSION: Acupuncture, music, physical exercise, paroxetine, doxepin, trimipramine and trazodone seem to present some benefit for patients with insomnia. However, the uncertainty around these results means that no robust and definitive recommendations for clinical practice can be made until the benefits and harms from each intervention for patients with insomnia have been confirmed through further studies.

INTRODUCTION

Insomnia is a frequent complaint that generates more than five million visits to doctors per year in the United States.[1] It is considered to be a subjective condition that affects sleep maintenance, onset or early waking.[2] It is also a public health issue because of its impact on people's wellbeing.[3]

Patients with insomnia usually present difficulty in initiating or maintaining sleep and may wake up without the capacity to go back to sleep. This situation may induce development of symptoms during the day, such as sleepiness, mood disturbances and fatigue.[4,5]

Insomnia may precede or appear along with other diagnoses and may occur as a result of different stressors. The following individual factors are commonly associated with the risk of insomnia: female sex, older age (for any sex), previous episodes of insomnia and family history.[6,7]

It is classified as a disorder and may be divided into primary or secondary according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Primary insomnia is more common and secondary insomnia is associated with another disorder.[4,5] Acute or short-term insomnia is considered to consist of at least three nights of difficulty in sleeping for no more than three months. If no treatment is provided, it may progress to chronic insomnia, which is a stage of the disorder that is more difficult to treat.[8]

Insomnia may be a risk for developing hypertension, diabetes, obesity and cardiovascular diseases.[9-12] It may also increase the risk of psychiatric comorbidities and substance abuse and it reduces the quality of life.[13-15]

The treatments available include pharmacological options, psychological interventions and alternative therapies, but all of these may have limitations. Physicians need to take into account the symptoms relating to the insomnia, treatment availability, effectiveness and safety.

OBJECTIVE

The aim of this study was to make a synthesis of the evidence from Cochrane systematic reviews that assessed different therapies for insomnia and to discuss this evidence.

METHODS

Design

Review of Cochrane systematic reviews (SRs).

Setting

Discipline of Evidence-Based Medicine, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP).

Criteria for including reviews

Types of studies

We considered the latest version of Cochrane SRs. We did not include any protocols or any systematic reviews (SRs) that had been withdrawn from the Cochrane Database of Systematic Reviews (CDSR).

Types of participants

We considered participants with insomnia. We excluded SRs in which was not possible to identify insomnia as the type of sleep disturbance presented by the participant. We did not impose any restriction based on age or sex.

Types of intervention

We considered any surgical, pharmacological or non-pharmacological intervention.

Types of outcomes

We considered any outcomes that had been evaluated by the authors of the SRs included. These outcomes included any clinical, social, laboratory or economic outcomes that had been reported.

Search for reviews

We conducted a broad and unrestricted systematic search in the Cochrane Database of Systematic Reviews (via Wiley) on August 1, 2018. The search strategy is presented in **Table 1**.

Selection of systematic reviews

The selection phase consisted of independent reading of all the abstracts retrieved, by two researchers, to check their eligibility in relation to the inclusion criteria. Any disagreement was resolved through reaching a consensus or by consulting a third author.

Presentation of the results

We made a synthesis of all the SRs included and presented the key results and methodological issues using a narrative approach (qualitative synthesis).

The SRs included were summarized based on the following characteristics:

- Inclusion criteria/PICO (population, intervention, comparator and outcomes)
- Methodological issues relating to searching for and coding of studies
- Main results
- Critical assessment of studies included (risk-of-bias assessment)
- Analyses performed (including methods for pooling research through meta-analysis)
- Assessment of the certainty of the evidence using the GRADE approach.[16]
- Applicability

When the SRs included considered multiple interventions, we presented only those that were relevant for this review.

RESULTS

Search results

The search strategy retrieved 80 systematic reviews (SRs). After the screening and selection process, seven SRs were included and a synthesis was produced from them.[17-23]

Results from systematic reviews

The SRs included assessed acupuncture (n = 1), behavioral interventions (n = 1), music (n = 1), pharmacotherapy (n = 2), phototherapy (n = 1) and physical exercise (n = 1). The main findings and the certainty of the evidence (according to GRADE)[16] for all the SRs included are shown in **Table 2**.

Table 1. Search strategy

#1 MeSH descriptor: [Sleep Initiation and Maintenance Disorders] explode all trees
#2 (Disorders of Initiating and Maintaining Sleep) OR (DIMS (Disorders of Initiating and Maintaining Sleep)) OR (Early Awakening) OR (Awakening, Early) OR (Nonorganic Insomnia) OR (Insomnia, Nonorganic) OR (Primary Insomnia) OR (Insomnia, Primary) OR (Transient Insomnia) OR (Insomnia, Transient) OR (Rebound Insomnia) OR (Insomnia, Rebound) OR (Secondary Insomnia) OR (Insomnia, Secondary) OR (Sleep Initiation Dysfunction) OR (Dysfunction, Sleep Initiation) OR (Dysfunctions, Sleep Initiation) OR (Sleep Initiation Dysfunctions) OR (Sleeplessness) OR (Insomnia Disorder) OR (Insomnia Disorders) OR (Insomnia) OR (Insomnias) OR (Chronic Insomnia) OR (Insomnia, Chronic) OR (Psychophysiological Insomnia) OR (Insomnia, Psychophysiological)
#3 #1 OR #2
#4 #3 in Cochrane Reviews

Acupuncture

The aim of this review[17] was to determine the efficacy and safety of acupuncture for insomnia. The authors compared groups receiving the same interventions with or without associated acupuncture, against placebo or sham or no treatment. Randomized controlled trials (RCTs) that compared different acupuncture methods or acupuncture against other treatments were not considered. The review included 33 RCTs (2293 participants, aged 15 to 98 years) that had assessed needle acupuncture, electroacupuncture, acupressure or magnetic acupressure. The main results are presented below.

Acupressure versus no treatment/sham/placebo

Acupressure resulted in a benefit regarding sleep quality, compared with no treatment (odds ratio, OR = 13.08; 95% confidence interval, CI = 1.79 to 95.59; 2 RCTs; 280 participants)

Table 2. Characteristics of interventions for insomnia: comparisons, outcomes and certainty of evidence

Intervention	Comparisons	Population	Main findings	GRADE
Acupuncture[17]	Acupuncture with or without association with other interventions versus placebo or sham or no treatment	Adults	Acupressure versus no treatment/sham/placebo: benefit from acupuncture regarding sleep quality Acupuncture as adjunct to other treatment versus other treatment alone: benefit from acupuncture regarding sleep quality	Not assessed
Antidepressants[18]	Serotonin reuptake inhibitors versus placebo	Adults	**Paroxetine**: benefit regarding sleep quality	Very low
			Fluoxetine: no important difference between groups	Low
	Tricyclic antidepressants versus placebo		**Doxepin** or **trimipramine**: • benefit regarding subjective sleep quality, sleep efficiency and duration of sleep	Moderate
			• no difference regarding sleep latency or adverse events	Moderate/low
	"Other" antidepressants versus placebo		**Trazodone**: • Benefit regarding subjective sleep outcomes	Moderate
			• No benefit regarding sleep efficiency	Low
			• Worse on adverse events	Low
Bright light therapy[19]	Bright light therapy versus any other active intervention, no intervention or sham	Adults aged 60 or above	No study found	---
Cognitive behavioral intervention[20]	Cognitive behavioral intervention versus no intervention, use of placebo or patient on waiting list	Adults aged 60 or above	No difference in sleep onset latency or duration of sleep	Not assessed
Melatonin[22]	Melatonin (2 mg of slow-released melatonin, once daily, before bedtime) versus placebo	Adults with dementia due to Alzheimer's disease	No difference in caregivers' sleep quality or in activities of daily living	Not assessed**
Music[21]	Listening to pre-recorded music versus no intervention	Adults	Benefit regarding subjective sleep quality	Moderate
			No difference in sleep onset latency, total duration of sleep, sleep interruption or sleep efficiency	Low
Physical exercise[23]	16 weeks of moderate-intensity community-based exercise training versus no treatment (patient on waiting list)	Adults aged 60 or above	Benefit regarding sleep quality	Not assessed
Trazodone[22]	Trazodone (50 mg) versus placebo	Adults with dementia due to Alzheimer's disease	Benefit regarding total nocturnal sleep time and sleep efficiency	Low
			No difference in time spent awake after sleep onset, number of nocturnal awakenings, daytime sleep or activities of daily living	Low

*GRADE (Grading of Recommendations Assessment, Development and Evaluation) has the aim of assessing the certainty of the evidence. From this, the results are classified as having high certainty of evidence (high confidence that the estimated effect is close to the true effect); moderate certainty of evidence (likely that the estimated effect is close to the real effect, but there is a possibility that it is not); low certainty of evidence (limited confidence in the effect estimate) or very low certainty of evidence (the true effect is likely to be substantially different from the estimate effect).

**The certainty of the evidence was graded considering all studies on melatonin that were included in the review. However, only one of them was on insomnia (the other studies considered all types of sleep disturbance. Thus, the GRADE that was specific for this study was not assessed.

or sham/placebo (OR = 6.62; 95% CI = 1.78 to 24.55; 2 RCTs; 112 participants). However, in a sensitivity analysis in which an assumption was made that dropouts had worse outcomes, acupuncture ceased to be conclusively beneficial.

Acupuncture as an adjunct to another treatment versus this other treatment alone

Acupuncture as an adjunct to another treatment was found to present the possibility of benefit regarding sleep quality (OR = 3.08; 95% CI = 1.93 to 4.90; 13 RCTs; 883 participants,). In subgroup analyses, needle acupuncture alone was shown to be beneficial, but not electroacupuncture.

All the trials presented high risk of bias and were heterogeneous regarding the definition of insomnia, participant characteristics, acupoints and scheme of treatment. Overall, the effect sizes were small, with wide CIs. A risk of publication bias was detected and adverse events were poorly reported and were rare. For further details, refer to the original abstract, available at: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD005472.pub3/full.

Antidepressants for treating insomnia in adults

The aim of this review[18] was to assess the effects of antidepressants for treating insomnia. Twenty-three RCTs were included (2806 participants), comparing any antidepressant with placebo, other medications for insomnia or a different antidepressant.

Serotonin reuptake inhibitors (SSRIs) versus placebo

- Paroxetine: significant improvements on the Pittsburgh Sleep Quality Index scale at six weeks (2 RCTs; 60 participants; p = 0.03) and 12 weeks (2 RCTs; 27 participants; p < 0.001).
- Fluoxetine: no important difference between groups.

There was also no proper reporting of adverse events. The overall certainty of evidence for the subjective outcomes ranged from low to very low.

Tricyclic antidepressants (TCA) versus placebo

Six RCTs (812 participants) compared tricyclic antidepressants (TCA) with placebo (five used doxepin and one used trimipramine). No trials for amitriptyline were found. The main results are presented below.

- Subjective sleep quality: benefit from TCA (standardized mean difference, SMD = -0.39; 95% CI = -0.56 to -0.21; 5 RCTs; 518 participants).
- Sleep efficiency: benefit from TCA (mean difference, MD = 6.29 percentage points; 95% CI = 3.17 to 9.41; 4 RCTs; 510 participants).
- Duration of sleep: benefit from TCA (MD = 22.88 minutes; 95% CI = 13.17 to 32.59; 4 RCTs; 510 participants).
- Sleep latency (time taken to fall asleep): no difference between groups (MD = 0.27 minutes; 95% CI = -9.01 to 0.48; 4 RCTs; 510 participants).
- Adverse events: no difference between groups (risk ratio, RR = 1.02; 95% CI = 0.86 to 1.21; 6 RCTs; 812 participants).

Other antidepressants versus placebo

Eight RCTs compared other antidepressants with placebo (one used mianserin and seven used trazodone). The main results are presented below.

- Subjective sleep outcomes: benefit from trazodone (SMD = -0.34; 95% CI = -0.66 to -0.02; 3 RCTs; 370 participants).
- Sleep efficiency (measured using polysomnography): no difference between trazodone and placebo (MD = 1.38 percentage points; 95% CI = -2.87 to 5.63; 2 RCTs; 169 participants).
- Adverse effects: more frequent with trazodone than with placebo (no numerical data provided; 2 RCTs).

The authors concluded that the effects of SSRIs were uncertain, compared with placebo. There may have been be a small improvement in sleep quality with short-term use of low-dose doxepin and trazodone. There was no evidence of any effect in relation to use of amitriptyline or long-term antidepressants for treating insomnia. The tolerability and safety of antidepressants for treating insomnia were also uncertain. For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD010753.pub2/full#CD010753-sec1-0017.

Bright light therapy for sleep problems in adults aged 60+

"Bright light therapy" ("phototherapy") involves administration of high-intensity light (frequently, 10,000 lux at the point of impact) for set periods of time with the aim of synchronizing sleep onset to socially acceptable norms. This review[19] aimed to evaluate the effects of bright light therapy on improving sleep quality among adults aged 60 and above. The reviewers did not find any RCTs on which to base conclusions regarding the effectiveness of this treatment. Future RCTs to evaluate this intervention are imperative and, until then, bright light therapy cannot be indicated routinely for treating sleep problems among adults aged 60 years and over. For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD003403/full.

Cognitive behavioral interventions

Cognitive and behavioral approaches towards sleep problems include making changes to poor sleep habits, promoting better sleep hygiene practices and challenging negative thoughts, beliefs and behaviors relating to sleep. These therapeutic

options, if beneficial and safe, could have advantages over pharmacological approaches, which are frequently associated with undesirable events.

The purpose of this review[20] was to assess the efficacy and safety of cognitive-behavioral interventions (CBT) among older people (older than 60 years) with insomnia. All forms of CBT were considered, including sleep hygiene, stimulus control, muscle relaxation, sleep restriction and cognitive therapies alone. CBT was compared with no intervention, waiting-list control and/or placebo ("quasi-desensitization"). Six randomized clinical trials (RCTs) were included (282 participants). The main results are presented below.

- Sleep onset latency (time taken to fall asleep), as reported in participants' diaries: no difference between CBT and control groups immediately post-intervention (mean difference, MD, in time to sleep onset = -3 minutes; 95% confidence interval, CI = -8.92 to 2.92; 3 RCTs; 135 participants; certainty of evidence not assessed) or at 12 months or more after treatment (MD = -11.48 minutes; 95% CI = -23.54 to 0.58; 1 RCT; 74 participants; certainty of evidence not assessed).
- Sleep onset latency (time taken to fall asleep), as measured by polysomnography: no difference between CBT and control groups immediately post-intervention (MD = 4.3 minutes; 95% CI = -13.29 to 4.55; 1 RCT; 24 participants; certainty of evidence not assessed) or at 12 months or more after treatment (MD = 2.49 minutes; 95% CI = -3.24 to 8.22; 1 RCT; 74 participants; certainty of evidence not assessed).
- Sleep duration (total, in minutes), as reported in participants' diaries: no difference between CBT and control groups immediately post-intervention (MD in time to sleep onset = -14.56 minutes; 95% CI = -36.13 to 7.01; 4 RCTs; 153 participants; certainty of evidence not assessed), at 3 months (MD = 14.77 minutes; 95% CI = -38.96 to 68.50; 1 RCT; 26 participants; certainty of evidence not assessed) or at 12 months or more after treatment (MD = -31.49 minutes; 95% CI = -71.11 to 8.13; 1 RCT; 50 participants; certainty of evidence not assessed).

The benefits associated with CBT regarding waking after sleep onset were probably clinically modest. The data were based on a single study with a small-sized sample regarding total time awake. For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD003161/full.

Music for insomnia among adults

This review[21] assessed the effects of listening to music on insomnia among adults. Six RCTs and quasi-randomized controlled trials (qRCTs) were included, comprising 314 participants. The studies compared the effects of listening to music daily at one's own house with no treatment or treatment-as-usual, on sleep improvement among adults with insomnia. The main results are presented below.

- Sleep quality as assessed using the Pittsburgh Sleep Quality Index (scale from 0 to 21 scale, on which lower scores mean better sleep quality): benefit from listening to music (MD = -2.80; 95% CI = -3.42 to -2.17; 5 studies; 264 participants; moderate certainty of evidence).
- Sleep onset latency, total duration of sleep, sleep interruption and sleep efficiency assessed using a questionnaire in the morning, with evaluations on participants using polysomnography. One study (50 participants) reported results from these outcomes but did not provide sufficient numerical data for analysis. The authors of that study only reported that there was no evidence of any effect from the intervention. The certainty of evidence regarding these outcomes was downgraded due to risk of bias and imprecision.
- Adverse events: none of the studies included reported this outcome.

The authors of this review[21] concluded that music may be effective for improving subjective sleep quality among adults with insomnia. They also highlighted that this intervention is easy to administer. It is imperative that further RCTs should be conducted to increase the certainty of the evidence and to investigate more aspects of this intervention. For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD010459.pub2/full.

Pharmacotherapies for sleep disturbances in dementia

This review[22] evaluated the effects of any drug treatment versus placebo for sleep disorders among people with dementia. Six RCTs were eligible for inclusion, but only two included participants with insomnia as a sleep disturbance, and these assessed melatonin (15 participants; 1 RCT) and trazodone (30 participants; 1 RCT). All the participants had dementia due to Alzheimer's disease (AD), in association with their insomnia. The main results are presented below.

Melatonin (2 mg of slow-released melatonin, once daily, before bedtime) versus placebo

- Caregivers' sleep quality (change from baseline): no difference between groups (MD = 0.74 minutes; 95% CI = - 0.40 to 1.89; 2 RCTs; 15 participants).
- Activities of daily living (change from baseline): no difference between groups (MD = - 0.66; 95% CI = -1.9 to 0.58; 15 participants; 1 RCT).

No serious adverse effects from melatonin were reported in the studies included.

Trazodone (50 mg) versus placebo (for two weeks)

- Total nocturnal duration of sleep: benefit of trazodone (MD = 42.46 minutes; 95% CI = 0.9 to 84.0; 30 participants; 1 RCT).

- Sleep efficiency: benefit from trazodone (MD = 8.53%; 95% CI = 1.9 to 15.1; 30 participants; 1 RCT).
- Time spent awake after sleep onset: no difference between groups (MD = -20.41; 95% CI = -60.4 to 19.6; 30 participants; 1 RCT).
- Number of nocturnal awakenings: no difference between groups (MD = -3.71; 95% CI = -8.2 to 0.8; 30 participants; 1 RCT).

No effects on daytime sleep, cognition or activities of daily living were found. No serious adverse effects from trazodone were reported.

For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD009178.pub3/full.

Physical exercise for treating sleep problems among adults aged 60+

This review[23] assessed physical exercise among older adults (60 years and over). Only one RCT was included (43 participants). The patients received 16 weeks of moderate-intensity community-based exercise training or no treatment (these patients were on a waiting list).

Compared with the control group, the patients in the exercise training group showed significant improvement in the Pittsburgh Sleep Quality Index (scale from 0 to 21, on which lower scores mean better sleep quality) at 16 weeks (MD = -3.4 points; 95% CI = -1.9 to -5.4; one RCT; 43 participants).

The reports on the other sleep-related outcomes were not presented numerically. Moreover, this review was published in 2002 and therefore needs to be updated. It also did not assess any safety outcomes and this should be considered in further studies. For further details, refer to the original abstract, available at: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD003404/full.

DISCUSSION

The present review included seven Cochrane systematic reviews (SRs) that assessed the effects of different interventions for insomnia in the general population or among groups with specific disorders (people with dementia and the elderly). Overall, the current evidence from Cochrane SRs shows that acupuncture, music, physical exercise, paroxetine, doxepin, trimipramine and trazodone seem to present some benefit for patients with insomnia. However, the certainty of the evidence provided by these SRs ranged from very low to moderate, which means that it is likely or very likely that further studies may change the current evidence.

Three of the SRs included are out of date, since they were published in 2002 and 2003.[19,20,23] Updates for these reviews are urgently needed, to aid in searching for new studies and to revise the analyses in line with newer recommendations.

There are seven ongoing Cochrane SRs (protocols) that will be published in the future. Their aims are to assess new non-benzodiazepine hypnotics (eszopiclone, zaleplon, zolpidem and zopiclone),[24-27] ramelteon (melatonin receptor agonist)[28] and pharmacological and non-pharmacological interventions for treating insomnia during pregnancy.[29] Additionally, a network meta-analysis is being conducting to compare the efficacy and acceptability of all pharmacological treatments for insomnia among adults.[30] These SRs will be useful both for healthcare professionals and for patients, to aid in decision-making.

CONCLUSION

This review identified seven Cochrane systematic reviews that assessed pharmacological or non-pharmacological interventions for treating insomnia. Based on their findings, acupuncture, music, physical exercise, paroxetine, doxepin, trimipramine and trazodone seem to present some benefit for patients with insomnia. However, the uncertainty relating to these results means that no robust and definitive recommendations for clinical practice can be made until the benefits and harm from of each intervention for patients with insomnia have been confirmed through further studies.

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Address for correspondence:
Rafael Leite Pacheco
Pós-graduação de Medicina Baseada em Evidências, Universidade Federal de São Paulo (UNIFESP).
Rua Botucatu, 740 – 3º andar
Vila Clementino – São Paulo (SP) – Brasil
CEP 04023-900
Tel. (+55 11) 5576-4203
E-mail: rleitepacheco@hotmail.com

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Malignant transformation of abdominal wall endometriosis to clear cell carcinoma: case report

João Kleber de Almeida Gentile[I], Renato Migliore[I], Fábio Jorge Neubaner Kistenmacker[I], Marcio Menezes de Oliveira[II] Rodrigo Biscuola Garcia[III], Fang Chia Bin[IV], Pedro Marcos Santinho Bueno de Souza[III], José César Assef[IV]

Hospital do Servidor Público Municipal (HSPM-SP), São Paulo (SP), Brazil

[I]MD. Resident Physician, Department of Digestive Surgery, Hospital do Servidor Público Municipal (HSPM-SP), São Paulo (SP), Brazil. orcid.org/0000-0001-8650-2703

[II]MD. Resident of General Surgery, Department of General Surgery, Hospital do Servidor Público Municipal (HSPM), São Paulo (SP), Brazil.

[III]MD. Attending Physician, Department of Digestive Surgery, Hospital do Servidor Público Municipal (HSPM-SP), São Paulo (SP), Brazil.

[IV]MD. Department of Digestive System Surgery, Hospital do Servidor Público Municipal (HSPM-SP), São Paulo (SP), Brazil.

KEY WORDS:
Endometriosis.
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Cell transformation, neoplastic.
Adenocarcinoma, clear cell.

ABSTRACT

BACKGROUND: Malignant transformation of endometriosis in the abdominal wall is a rare and still poorly understood event. Less than 30 cases have been reported in the worldwide literature. Most cases of solid tumors are report in a previous abdominal scar with malignant transformation of a focus of endometriosis. Presence of lymph node metastases in nearby chains is frequent and is associated with poor prognosis.

CASE REPORT: We report a case of a 42-year-old woman with a history of abdominal surgery (Pfannenstiel) to resect abdominal wall endometriosis. Physical examination revealed a solid mass of approximately 10 cm x 6 cm in the anterior wall of the abdomen. Computed tomography (CT) of the abdomen and pelvis showed a heterogeneous, predominantly hypoattenuating expansive formation measuring 10.6 cm x 4.7 cm x 8.3 cm. The patient underwent exploratory incisional laparotomy, block resection of the abdominal mass and lymphadenectomy of the external and inguinal iliac chains. The abdominal wall was reconstructed using a semi-absorbable tissue-separating screen to reconstitute the defect caused by resection of the tumor. Histological evaluation revealed infiltration by malignant epithelioid neoplasia, thus confirming the immunohistochemical profile of adenocarcinoma with clear cell components. Lymphadenectomy showed metastatic involvement of an external iliac chain lymph node.

CONCLUSION: Resection of the mass along with the abdominal wall, with wall margins, is the most effective treatment. Reconstruction is a challenge for surgeons. The patient has been followed up postoperatively for eight months, without any evidence of disease to date.

INTRODUCTION

Endometriosis is defined as the presence of stroma and endometrial glands outside the uterine cavity. It affects approximately 15-40% of women of childbearing age. The most common site is the abdominal cavity, specifically in the pelvis and occasionally at extra-pelvic sites.[1,2] Abdominal wall endometriosis accounts for 0.4-2% of the cases, and is mostly found in the umbilical scar and in the scar of previous abdominal incisions, especially in cesarean scars, laparoscopies and appendectomies.[2]

In patients with abdominal wall endometrioma, the mean time taken to reach the diagnosis is 6 to 20 years after the initial surgery, and 14.3-26% of the cases show an association with pelvic endometriosis.[2] The endometrioma is diagnosed preoperatively only in 20-50% of the cases, and the typical complaint is most frequently cyclical menstrual pain. The differential diagnoses for an abdominal mass associated with a previous surgical incision in the abdominal wall include abscess, hematoma, hernia, desmoid tumors, sarcomas and metastatic disease.[1]

Malignant transformation of an abdominal wall endometrioma is an extremely rare event. Extensive local excision with surgical margins seems to be the only effective treatment, and it is almost always necessary to correct the defect of the abdominal wall with prosthetic surgical or cutaneous flaps for the closure of the abdominal wall.

Here we report a case of abdominal wall endometrioma that evolved into clear cell carcinoma of the abdominal wall with metastases to the lymphatic system.

CASE REPORT

The patient was a 42-year-old female, with one previous pregnancy, with a history of cesarean section seven years previously and resection of endometriosis of the cephalic scar (Pfannenstiel) two years previously at another service, for which a histopathological diagnosis of abdominal wall endometriosis was made.

Her condition evolved with progressive expansion in the region previously resected, for eight months, leading to presence of a bulging mass in the right side of the anterior abdominal wall, with cyclical local pain. During the investigation period, the patient said that she did not have any genitourinary or gastrointestinal symptoms, or any presence of lymph nodes or systemic symptoms.

Physical examination revealed a solid mass of approximately 10 cm x 6 cm in the anterior wall of the abdomen bordering the pubis. It extended inferiorly to the umbilical scar and laterally to the upper border of the iliac crest. At the time of the physical examination, there was no lymph node swelling in the inguinal region.

Laboratory tests and tumor marker investigations (CA 125, CA 19-9, CEA and alpha-fetoprotein) were requested and these were found to be within normal limits. Computed tomography (CT) of the abdomen and pelvis revealed a heterogeneous expansive formation that was predominantly hypoattenuating, with images suggestive of internal septation. It measured around 10.6 cm x 4.7 cm x 8.3 cm along the major transverse, anteroposterior and longitudinal axes, respectively, and was located in the anterior pelvic wall, with the largest axis to the right of the midline, involving the rectus abdominis muscle (**Figure 1**).

The patient underwent exploratory laparotomy by means of a Pfannenstiel incision, followed by block resection of the abdominal mass (**Figure 2**) with margins to the peritoneum, along with lymphadenectomy of the external and inguinal iliac chains. The abdominal wall was reconstructed to reconstitute the defect caused by resection of the tumor, using a semi-absorbable tissue-separating screen composed of a polypropylene parietal face and a visceral face coated with carboxymethyl cellulose. This rectangular sodium hyaluronate mesh measured 20.3 cm x 30.5 cm (Sempramesh IP Composite Bard Davol Inc.).

Histological analysis on the abdominal mass revealed infiltration by malignant epithelioid neoplasia into soft tissues, thus confirming the immunohistochemical profile of adenocarcinoma with clear cell components (**Figure 3**). The antigens investigated in the immunohistochemical evaluation are listed in **Table 1**. Lymphadenectomy showed metastatic involvement of an external iliac chain lymph node (1/8), and that other

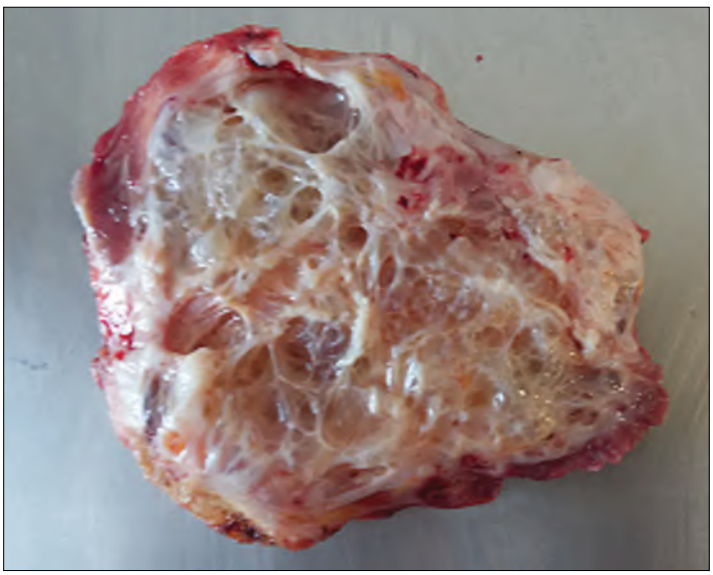

Figure 2. Macroscopic appearance demonstrating areas of cystic and trabecular components.

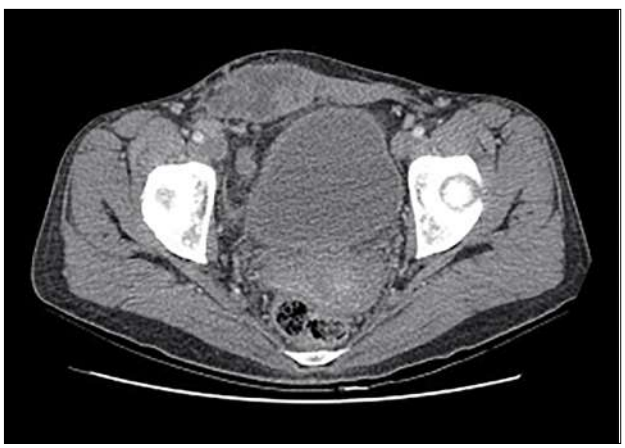

Figure 1. Computed tomography scan of the abdomen and pelvis (portal phase) showing an expansive process in the anterior abdominal wall and pelvis and lymph node enlargement in the external and inguinal iliac chains.

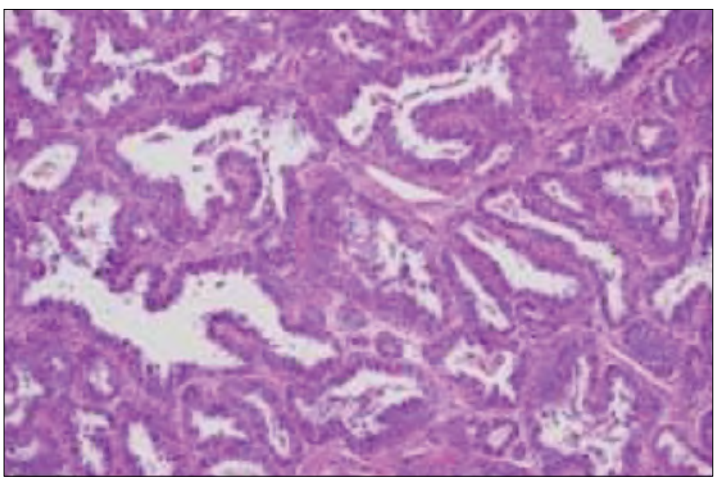

Figure 3. Histological section of clear cell endometrioid adenocarcinoma demonstrating loss of glandular architecture and stroma (hematoxylin and eosin; x 400).

lymph nodes of the iliac and inguinal chains presented lymphoid hyperplasia (0/11).

Our patient is in her second postoperative month, without having presented any clinical or surgical intercurrence to date. She is being followed up by the oncology sector and an adjuvant chemotherapy scheme has been indicated.

DISCUSSION

Malignant transformation of endometriosis is quite rare and affects less than 1% of the female population suffering from this condition. In the literature, the most common site of malignant transformation of endometriosis foci is the ovaries, while 20% of the cases occur at extragonadal sites, including the rectovaginal septum, colon and distal organs such as the abdominal wall. Less than 5% of these cases are carcinomas of clear cell origin like the case reported here.[3]

This malignant transformation in the abdominal wall is an extremely rare event, with less than 30 case reports in the worldwide literature. These cases consisted of endometrioid carcinoma (70%), sarcoma (25%) and clear cell carcinoma (5%).[4]

Sampson was one of the first authors to report a case of malignancy of an endometriosis outbreak. He proposed three criteria for diagnosing malignant transformation of endometriosis:

1. Demonstration of neoplastic and benign endometrial tissue in the tumor;
2. Histological type compatible with endometrial origin;
3. No other primary site identified.[5]

In 1953, Scott added a fourth criterion: histological presence of benign endometriosis and carcinoma with glandular transformation with atypias. Few reported cases have met all four proposed criteria, and the etiogenesis of such cases remains unknown.[6]

Malignancy of a focus of endometriosis on a previous scar on the abdominal wall is very rare, with a reported prevalence of 0.03%. It can affect all layers of the abdominal wall and the growth of such masses is exponential, reaching diameters greater than 10 cm.[7]

In our case, the mass appeared in the anterior wall of the abdomen without evidence of abdominal cavity involvement from abdominal CT. The abdominal wall itself was limited by the peritoneum. Our patient underwent preoperative screening for a primary focus of neoplasia, by means of upper gastrointestinal endoscopy, colonoscopy and thyroid ultrasonography. All of these were negative for neoplasms.

We reviewed the literature through MEDLINE, PubMed, Embase and LILACS using the English keywords "endometriosis", "cell transformation", "adenocarcinoma" and "abdominal wall". We found only 17 reports, as shown in **Table 2**, and 15 reports had clinical presentation similar to the reported case. **Table 3**[7-21] lists the reports in the literature describing the different types of treatment for clear cell carcinomas of the abdominal wall that were derived from an endometrioid focus on a previous abdominal scar. Local invasion is an important biological feature for transformation of endometriosis into invasive carcinoma. On the other hand, although lymphatic dissemination may be present, it has only been reported in three cases.[7-9]

At the time of the pre-surgical evaluation, it was difficult to make a diagnosis of lymph node involvement. However, the presence of lymph node enlargement in the inguinal region and in the external iliac chain was observed on CT scans. This was investigated using computerized tomography with 18-fluorodeoxyglucose positron emission tomography (FDG-PET).

Presence of a compromised lymph node in the 2-cm external iliac chain was demonstrated, with standardized uptake values (SUV) for the abdominal mass of 4.16 and 2.51 in the iliac lymph node. There were no other signs of FDG uptake.

In our case, lymphadenectomy of the external and inguinal iliac chain was performed, and the metastatic involvement of the lymph node caused by carcinoma was confirmed through histological analysis.

Table 1. Immunohistochemical profile and antigens investigated

Antigen	Result
D AE1/AE3	Positive
CD 34	Negative
CK 7	Positive
CK 20	Negative
Estrogen receptor	Negative
WT-1	Negative
Vimentin	Negative

Table 2. Search of the literature in medical databases for cases of degeneration of abdominal wall endometriosis for clear cell carcinoma. (Search was conducted on April 14, 2017)

Database	Search strategies	Papers found	Reports of cases with lymphatic dissemination
MEDLINE (via PubMed)	endometriosis and cell transformation and adenocarcinoma and abdominal wall "case reports" [publication type]	17	2
Embase (via Elsevier)	endometriosis and cell transformation and adenocarcinoma and abdominal wall "case reports" [publication type]	0	0
LILACS (via Bireme)	endometriosis and cell transformation and adenocarcinoma and abdominal wall	16	1

Table 3. Reported cases of clear cell carcinoma of the abdominal wall derived from focus of endometriosis

Author	Treatment	Follow-up (months)	Outcome
Schineber and Wagner-Kolb[8] (a)	HTA + SOB, R-Ad, Progesterone	18	Death
Hitti et al.[9] (a)	Resection, HTA + SOB	30	Alive without evidence of disease
Miller et al.[10] (a)	Resection, HTA + SOB, R-Ad, Q-Ad	60	Alive without evidence of disease
Park et al.[11] (a)	Resection, R-Ad	NA	Not reported
Ishida et al.[12] (a)	Resection, R-Ad	48	Death
Sergent et al.[13] (a)	HTA + SOB, Q-Ad	9	Death
Alberto et al.[14] (a)	Resection, Q-Ad, R-Ad	NA	Not reported
Rust et al.[15]	Resection	NA	Not reported
Bats et al.[7] (a)	Q-Neo, Resection, HTA + SOB	NA	Not reported
Razzouk et al.[16] (b)	Resection, Q-Ad	6	Death
Williams et al.[17] (a)	Resection, HTA + SOB, Q-Ad	11	Death
Yan et al.[18]	Resection, Q-Ad	24	Alive without evidence of disease
Mert et al.[19] (a)	Resection, HTA + SOB, R-Ad	31	Alive without evidence of disease
Markopoulos et al.[20] (b)	Resection, HTA + SOB	24	Alive without evidence of disease
Gücer et al.[21] (b)	Resection, HTA + SOB, Q-Ad, R-Ad, Progesterone	20	Death
Present case	Resection	8	Alive without evidence of disease

TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy; R = radiotherapy; Q = chemotherapy; Ad = adjuvant; Neo = neoadjuvant; a: clear cell serous carcinoma; b: clear cell and endometrioid carcinoma

Radical resection is considered to be the primary treatment for endometrioid carcinoma of the wall. Carboplatin-based chemotherapy and radiation therapy schemes have been proposed without any evidence of improved prognosis or survival.[7]

Due to the rarity of this tumor, the long-term survival following treatment is unknown. However, some recent reports have shown that aggressive radical surgery with total tumor excision with free margins, together with lymphadenectomy of the inguinal and iliac chains may be beneficial for these patients' disease-free survival.

CONCLUSION

Malignant transformation to clear cell carcinoma from a focus of endometriosis on the abdominal wall is a rare and poorly understood complication. Most recent studies have shown that aggressive surgical resection with safety margins associated with lymphadenectomy is still the most effective treatment with the highest survival rates. The role of adjuvant therapy remains unclear and therefore further studies to assess the long-term benefits are required.

In our case, lymphadenectomy of the external and inguinal iliac chain was performed, and the metastatic involvement of the lymph node caused by carcinoma was confirmed through histological analysis.

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Address for correspondence:
João Kleber de Almeida Gentile
Seção de Técnica de Cirurgia Digestiva, Hospital do Servidor Público Municipal (HSPM-SP)
Rua Castro Alves, 60
São Paulo (SP) — Brasil
CEP 01532-000
Tel. (+55 11) 3726-8591
E-mail: joaokleberg@gmail.com

Anticholinergic toxicity in a one-year-old male following ingestion of *Lupinus mutabilis* seeds: case report

Adrian Ernesto Flores-Pamo[I], Elinor Pisano[II], Nilton Yhuri Carreazo[III]

Pediatric Emergency Hospital, Lima, Perú

[I]MD. Resident Physician, School of Medicine, Universidad Nacional San Agustín de Arequipa (UNSA), Arequipa, Peru.
[II]MD. Resident Physician, Medstar Georgetown University Hospital, Washington, DC, United States.
[III]MD. Professor, School of Medicine, Universidad Peruana de Ciencias Aplicadas (UPC), Lima, Peru and Attending Physician, Pediatric Intensive Care Service, Hospital de Emergencias Pediátricas, Lima, Peru. orcid.org/0000-0002-5269-4855

KEY WORDS:
Lupinus.
Foodborne diseases.
Anticholinergic syndrome.
Cholinergic antagonists.
Alkaloids.

ABSTRACT

CONTEXT: The seeds from *Lupinus mutabilis* Sweet, also called "chocho", are an important part of the diet in several countries in South America. Prior to consumption, processing is required to remove toxic alkaloids. These alkaloids are known to have pharmacological properties as antiarrhythmics, antimuscarinics and hypoglycemics.

CASE REPORT: We report a case in which a one-year-old male initially presented with altered mental status and respiratory distress and subsequently developed symptoms of anticholinergic toxicity, after ingesting a large amount of chocho seeds.

CONCLUSION: In spite of going through a difficult clinical condition, the subject evolved favorably through receiving supportive treatment. The seeds from *Lupinus mutabilis* provide nutritional benefits when consumed, but people need to know their risks when these seeds are consumed without proper preparation.

INTRODUCTION

Ingestion of toxic substances is very common among young children. While household items such as medications are commonly ingested due to their similarity in appearance to candy, young patients can present with intoxication due to ingestion of plant matter as well. The majority of these occurrences are among patients less than six years of age.[1] The true prevalence of intoxication due to ingestion of plant matter is difficult to determine, since many presentations are mild and may not be brought to medical attention.

Some of the most commonly known toxic plants are the deadly nightshade (*Atropa belladonna*) and jimson weed (*Datura stramonium*). These plants produce the anticholinergic alkaloids atropine, scopolamine and hyoscyamine. Yet these are not the only anticholinergic alkaloids present in plant matter: even the unripe buds or flowers of common garden plants, such as eggplants and tomatoes, can also cause an anticholinergic toxidrome if ingested.[2]

Supportive care is generally sufficient for treatment of anticholinergic toxicity. However, treatment with physostigmine may be indicated for severely affected patients.

CASE REPORT

A one-year-old male child presented to the emergency room with acute onset of respiratory distress while sleeping, perioral cyanosis and severe cough. The patient's mother said that she worked in a farmer's market, on a stall where beans and vegetables were sold, and that she took her son to work with her every day. Among the harvested goods sold at the farmer's center are unprocessed seeds from *Lupinus mutabilis* Sweet, known as "chocho". The child had been seen placing a chocho seed in his mouth three hours before arrival at the hospital.

The physical examination upon presentation to the hospital was notable for stridor, which prompted bronchoscopic evaluation for a foreign body. However, no foreign body was found in the airway. Endoscopy revealed the presence of grains of chocho in the gastric cardia and stomach.

Six hours after initial presentation, the patient became irritable. He developed altered consciousness and shallow breathing. On examination, he demonstrated dilated, sluggishly reactive

pupils and dry mucus membranes. His abdomen was distended and tympanic to percussion. His heart rate was 100/minute, respiratory rate 26/minute and oxygen saturation 98% on 30 FiO_2 (fraction of inspired oxygen) through a nasal cannula. The arterial blood gas demonstrated pH 7.5, pCO_2 23, pO_2 135 and HCO_3 18.4. The blood parameters were: sodium 144 mEq/l, potassium 3.4 mEq/l, white blood cells (WBC) 8,800/mm^3, hemoglobin 9.9 g%, platelets 316,000/mm^3, glucose 107 mg/dl, creatinine 0.8 mg/dl and C-reactive protein (CRP) negative.

His altered mental status prompted admission to the intensive care unit (ICU) for monitoring and further evaluation. He was empirically started on intravenous fluids, ceftriaxone and mannitol. Computed tomography of the head was normal and lumbar puncture revealed normal cerebrospinal fluid; for this reason, all medications were discontinued and he was kept in the ICU for monitoring and supportive care.

After 24 hours, his mental status had improved and irritability diminished; his pupils returned to normal size and were reactive. On the following day, he developed liquid stools with elimination of the chocho seeds, and examinations demonstrated no remaining neurological signs. On the third day, he was discharged home.

DISCUSSION

The Andean lupin, commonly known as "tarwi" or "chocho" (*Lupinus mutabilis* Sweet), is a legume native to the Andean region of South America, and is the only domesticated and cultivated species of the genus *Lupinus* (**Figure 1**). It is distributed from Colombia to the north of Argentina; with important presence in the agriculture of Ecuador, Peru and Bolivia.

Figure 1. Typical appearance of processed chocho.

Chocho is an important part of the native diet, with high protein and fat content.[3] However, for consumption, the seed requires pre-treatment. This eliminates the toxic substances that it contains and which the plant uses for its natural defenses. This process is known as debittering ("desamargamiento") and includes cleaning the harvested seeds of impurities (plant residues, soil and small stones), then soaking for a day, cooking in water for an hour, placing in an appropriate container (burlap sack or basket) and exposing to running water for 4-5 days (N).[4]

The alkaloids present in chocho are responsible for the bitter flavor and toxicity of this legume and can reach a content of 3.3% in unprepared seeds. The principal alkaloids present are lupinine, sparteine and hydroxylupinine. These compounds have been reported to have effects as central nervous system depressants, antiarrhythmics, hypoglycemics and antimuscarinics. The debittering process can reduce the concentration of these toxic substances to 0.003%.[5] Given these innate toxic properties, it is common for farmers to use the seed without chemical pesticides.

The diagnosis of intoxication by *Lupinus mutabilis* is based on the clinical picture and antecedents of ingestion of chocho seeds. There have been previous case reports of symptoms after ingestion of the water that was used to process chocho, ingestion of the flour made of this legume and ingestion of the unprocessed seeds.[6-10] The symptoms described are consistent with those found in cases of typical anticholinergic toxicity: dry mucus membranes, mydriasis, tachycardia, ileus, urinary retention and altered mental status (commonly remembered via the mnemonic "Dry as a bone, Blind as a bat, Mad as a hatter").

This case is the first description of intoxication due to ingestion of the unprocessed beans by a pediatric patient. Even though the infant presented with the symptoms that have been described in this type of intoxication, the predominant clinical picture in this case was one of neurological symptoms, which may manifest as a range from mild disorientation to delirium and coma. In our patient, the alteration of consciousness directed the differential diagnosis towards possible meningitis or encephalitis. The treatment is supportive, with strict monitoring of vital signs; and the prognosis is favorable.[9,10]

We reviewed the literature in MEDLINE, Cochrane Library, LILACS and EMBASE using the keywords "Lupinus mutabilis" (**Table 1**). We found two related papers. A 1995 study (LILACS)

Table 1. Search of the literature in medical databases for case reports on *Lupinus mutabilis*. The search was conducted on February 28, 2017

Database	Search strategies	Papers found	Related papers
PubMed	"Lupinus mutabilis"[All Fields] AND Case Reports[ptyp]	1	1
Cochrane Library	Lupinus mutabilis	1	0
LILACS	"lupinus mutabilis" AND (instance:"regional") AND (db:("LILACS"))	12	1
EMBASE	"Lupinus mutabilis"	30	1

reported that three adult patients in the city of Trujillo (Peru) became intoxicated due to consumption of chocho water ("*agua de chocho*").[4] The search in Medline and EMBASE located a second paper, published in 2012 (present in both databases): a 48-year-old woman who became intoxicated due to consumption of *agua de chocho*.[5] We did not find any reports on pediatric cases.

Considering that the nutritional properties of chocho are continuing to be investigated (for example, its role as a hypoglycemic for treating diabetes mellitus), it can be expected that consumption of this product will increase.[11,12] For this reason, it is important to inform consumers about the hazards of ingesting products containing *Lupinus* that have not been processed.

CONCLUSIONS

In spite of going through a difficult clinical condition, the subject evolved favorably through receiving supportive treatment. The seeds from *Lupinus mutabilis* Sweet provide nutritional benefits when consumed, but people need to know their risks when these seeds are consumed without proper preparation.

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Address for correspondence:
Nilton Yhuri Carreazo
Escuela de Medicina, Universidad Peruana de Ciencias Aplicadas
Alameda San Marcos, cuadra 2, Lima 09
Lima — Peru
E-mail: yhuroc@gmail.com

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Genital myiasis associated with genital piercing. Case report

Daniel Melecchi Freitas[I], Flavio Aranovich[II], José Nicolau Olijnyk[I], Renan Lemos[II]

Department of Urology, Hospital Conceição, Porto Alegre (RS), Brazil

[I]Professor, Department of Urology, Hospital Conceição, Porto Alegre (RS), Brazil.
[II]Resident, Department of Urology, Hospital Conceição, Porto Alegre (RS), Brazil.

KEY WORDS:
Myiasis.
Penile diseases.
Body piercing.

ABSTRACT

CONTEXT: Myiasis is caused by larval infestation that usually occurs in exposed wounds. *Dermatobia hominis* is the most common fly species responsible for this parasitic infection. Genital piercing is an ornamental practice used in certain social circles. At placement, it transverses the skin surface and, as such, may be related to complications.

CASE REPORT: We report a case of a 31-year-old man with a history of wound infection secondary to genital piercing who was exposed to an environment with flies, leading to myiasis. Mechanical removal and systemic antiparasitic drugs are possible treatments for myiasis. However, prevention that includes wound cleaning and dressing is the best way to avoid this disease.

CONCLUSIONS: Genital piercing can lead to potential complications and myiasis may occur when skin lesions are not properly treated.

INTRODUCTION

Although adornment with genital piercings has been increasing over recent years, the clinical implication of this practice is still under debate.[1] Minor and major problems, such as secondary infections, have been reported on an increasing scale.[2] Moreover, subjects who have undergone this procedure and develop any complication seek medical advice late.[3]

Myiasis is a parasitic infestation in body tissues caused by flies' larvae. It occurs in healthy tissues (primary myiasis) or wounded tissues (secondary myiasis). *Dermatobia hominis* is the most common species of fly that parasitizes humans. The larvae are more frequently found in infected areas that present necrotic tissue.[4]

The treatment for myiasis may range from local extraction of larvae to systemic or topical use of antiparasitic drugs, depending on the severity of the infestation. We sought to report a case of secondary myiasis in a patient who developed wound infection after genital piercing. To the best of our knowledge, this is the first case reporting this medical condition (**Table 1**).

CASE REPORT

A 31-year-old man came to the emergency room (ER) complaining about genital ulcers and diffuse erythema and edema in the pubic and genital area. The patient had a history of piercing placement at the base of the penis one month previously. He reported that a wound infection at the piercing site developed seven days after the procedure. At that time, the patient extracted the piercing at home and did not take any medication to treat the infection. He started then to go to sleep without clothes because of pain at the site of infection. He also reported that he kept the windows of his bedroom opened to be able to tolerate the bad smell. Two weeks later, the patient noticed an erythematous area at the pubis and genitalia that itched. He also saw some black spots and small ulcers at the previous piercing site. He described episodes of acute stabbing pain, in these areas.

During physical examination, his vital signs (blood pressure, pulse, respiratory rate and temperature) were within normal limits. Examination of his genitals showed that there was a large area of

Table 1. Search of the literature in medical databases for case reports on genital myiasis associated with genital piercing. The search was conducted on February 12, 2017

Database	Search strategies	Papers found	Related papers
MEDLINE (via PubMed)	"genital myiasis" AND "genital piercing" AND case reports (publication type)	0	0
MEDLINE (via PubMed)	"genital myiasis" OR "genital piercing" AND case reports (publication type)	36	0

erythematous plaque in the pubic region with desquamation and pustules. Four necrotic ulcers were found; the largest was at the base of the penis with a diameter of 4 cm, where the urogenital piercing was previously inserted (**Figures 1A** and **B**). Inside the ulcers, several larvae were found (**Figure 2**). Laboratory tests demonstrated that blood parameters were normal and sexual transmitted disease tests were all negative.

The patient was taken to the operating room for surgical debridement and extraction of the myiasis under regional anesthesia. Given the large number of larvae, systemic ivermectin was prescribed after the procedure, along with antibiotics and local dressings. The patient was discharged on day seven with use of topical antibiotics and corticoids to treat and avoid new infection and decrease inflammatory reaction (**Figure 3**).

DISCUSSION

Genital myiasis is a rare medical condition caused by infestation with fly larvae. It is related to poor hygiene conditions and is more prevalent in rural zones. Although uncommon, its association with genital wounds, such as those caused by genital piercing, needs to be taken into account nowadays.

In addition to genital myiasis, genital piercing can also lead to other types of complications such as bleeding, skin irritation, urinary and sexual problems.[3,5,6] However, the lack of reporting probably stems from the fact that people with genital piercings usually seek non-medical care after complications occur.

Some studies have demonstrated that genital myiasis can mimic inflammatory or ulcerous lesions.[4,7,8] In fact, this parasitic infestation has been associated with certain urogenital diseases. For example, Tavares et al. reported a case of a man who underwent emergency penectomy due to extensive larval infestation.[7] In that case, myiasis was also associated with penile squamous cell carcinoma. In another case, an infectious furunculoid scrotal lesion that was thought to be neoplastic was actually caused by myiasis.[8] In our case, the patient underwent genital piercing with subsequent wound infection. The patient presented with a wound infested by larvae inside penile ulcers.

Although cases of penile myiasis had previously been published, the correlation with genital skin lesions caused by piercing had never been reported before. Genital piercing is a form of cultural expression that can lead to several complications. Furthermore, some cases of sexually transmitted disease, like human immunodeficiency virus infection and hepatitis, have been associated with this practice.[9] Thus, given that genital piercing causes a skin lesion at

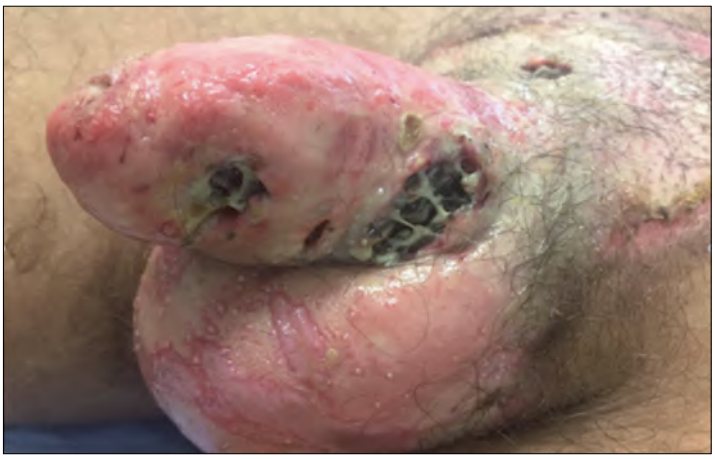

Figure 2. Genital ulcers following infection for genital piercing. Several larvae found inside genital ulcers.

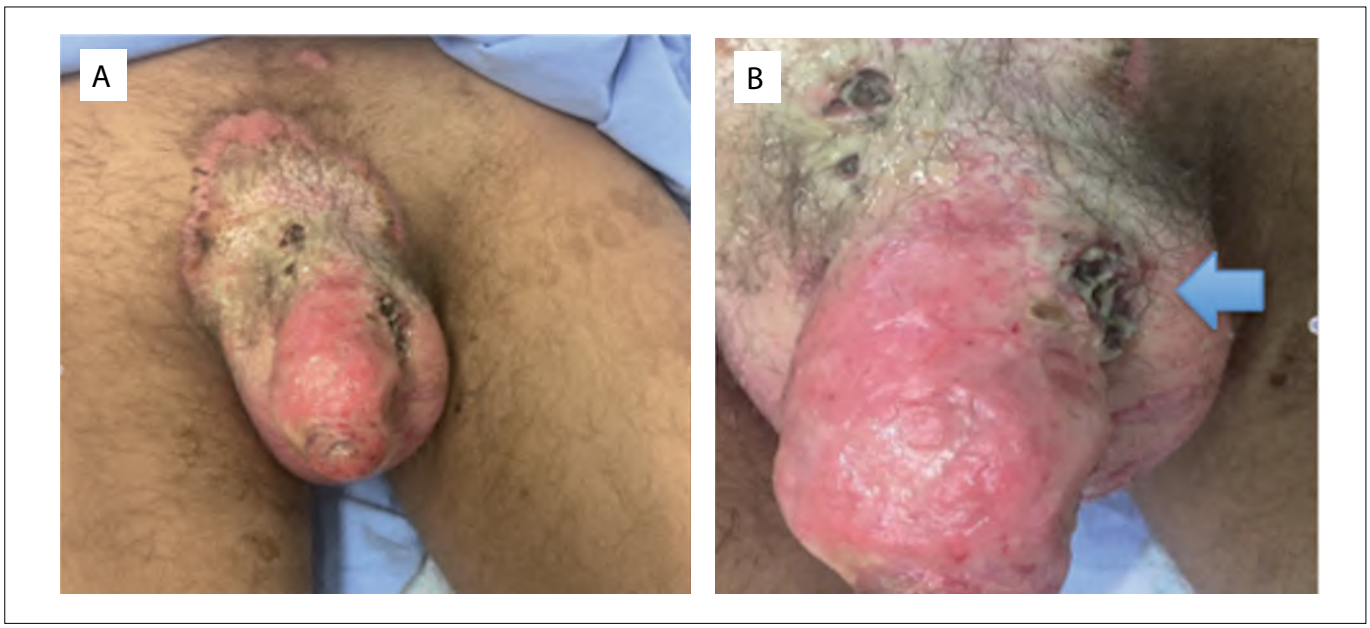

Figure 1. Aspect of the genitals at presentation to the emergency room. A. Ulcers. B. Arrow shows site where genital piercing had previously been placed.

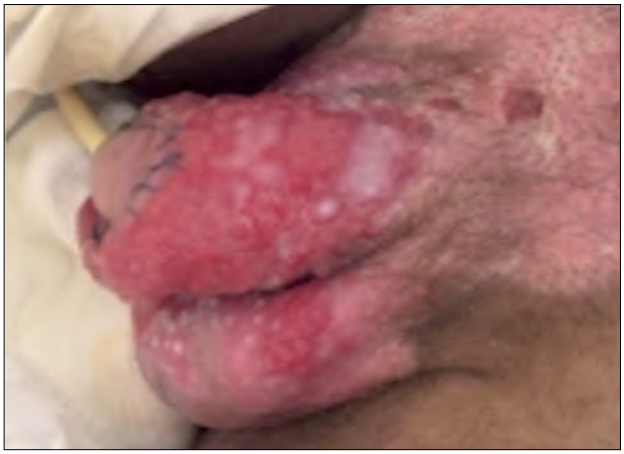

Figure 3. Postoperative appearance after debridment and extraction of myiasis in genitals.

the placement site, certain hygiene conditions need to be achieved in order to avoid wound infection and exposure to flies.

There are several ways to treat myiasis. Regarding genital myiasis, mechanical removal of larvae is the most frequent method.[10] In general, wound closure that makes it impossible for the larvae to breath, thus causing larval death, is a common treatment. Recently, use of systemic or topical antiparasitic drugs has been described, for example ivermectin. This is a broad-spectrum antiparasitic medication that is used in selected cases that present unusual and widespread infestation and in immunocompromised patients.[11] Nevertheless, wound closure and mechanical removal are much more cost-effective in uncomplicated cases.

The purpose of this case report was to draw attention to possible medical issues relating to genital piercing, which is a form of cultural expression. Firstly, genital piercing can lead to potential complications. A recent case series reported that almost 20% of subjects who underwent piercing in the genitalia experienced complications.[12] Secondly, penile myiasis should be suspected in patients with genital ulcers who have low socioeconomic status and poor hygiene habits. Thirdly, although antiparasitic drugs such as ivermectin are well tolerated and have a broad spectrum, their use should only be considered in selected cases. Fourthly, prevention of infestation through cleaning and dressing of wounds is fundamental.

CONCLUSIONS

In conclusion, among subjects who undergo genital piercing, myiasis infestation may potentially be associated with wound infection secondary to the procedure. Doctors need to be aware of this complication so as to be able to provide the best treatment for this condition and give medical counseling about the potential problems of genital piercing.

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Address for correspondence:
Daniel Melecchi Freitas
Department of Urology, Grupo Hospitalar Conceição (GHC)
Av. Francisco Trein Suite, 3B2
Porto Alegre (RS) — Brasil
CEP 91350-200
Tel. (51) 3357-2000
E-mail: danielmelecchi@hotmail.com

Intramural duodenal hematoma secondary to pancreatitis: case report and review of the literature

João Henrique Botto de Oliveira[I], Raiza Samenica Esper[I], Rodrigo Campos Ocariz[I], Flora Specian Sartori[I], Lucas Marcelo Dias Freire[II], Elinton Adami Chaim[III], Francisco Callejas-Neto[IV], Everton Cazzo[V]

Department of Surgery, Centro Médico de Campinas (CMC), Campinas (SP), Brazil

[I]MD. Resident Physician, Department of Surgery, Centro Médico de Campinas (CMC), Campinas (SP), Brazil

[II]MD. Attending Physician, Endovascular Surgery Unit, Centro Médico de Campinas (CMC), Campinas (SP), Brazil

[III]MD, PhD. Full Professor, Department of Surgery, Faculdade de Ciências Médicas da Universidade Estadual de Campinas (FCM/UNICAMP), Campinas (SP), Brazil.

[IV]MD, MSc. Assistant Professor, Department of Surgery, Faculdade de Ciências Médicas da Universidade Estadual de Campinas (FCM/UNICAMP), Campinas (SP), Brazil.

[V]MD, PhD. Adjunct Professor, Department of Surgery, Faculdade de Ciências Médicas da Universidade Estadual de Campinas (FCM/UNICAMP), Campinas (SP), Brazil.

KEY WORDS:
Pancreatitis.
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Duodenal diseases.

ABSTRACT

CONTEXT: Spontaneous intramural duodenal hematoma is uncommon and is usually associated with coagulopathy, anticoagulant therapy and endoscopic procedures. The aim here was to describe a case of intramural duodenal hematoma caused by chronic exacerbation of pancreatitis.

CASE REPORT: A 46-year-old male with chronic alcoholic pancreatitis was admitted to hospital due to abdominal pain, melena and low hemoglobin. An intramural duodenal hematoma with active bleeding was detected and selective angioembolization was warranted. The patient evolved with a perforated duodenum and underwent laparotomy with exclusion of the pylorus and Roux-en-Y gastrojejunostomy. He was discharged nine days later.

CONCLUSION: Intramural duodenal hematoma is a rare complication of pancreatitis. Selective embolization is the preferred treatment for hemorrhagic complications of pancreatitis. However, the risk of visceral ischemia and perforation should be considered.

INTRODUCTION

The first description of an intramural duodenal hematoma was made by McLauchlan in 1838. This condition is usually associated with blunt abdominal trauma. Spontaneous intramural duodenal hematoma is uncommon and has been linked to coagulopathy, anticoagulant therapy and endoscopic procedures.[1-3] Other causes include several pancreatic diseases, collagenosis, peptic ulcers and pancreaticoduodenal aneurysm.[4-7] To date, the exact mechanism leading to intramural hematoma in cases of pancreatitis has not yet been fully elucidated and the prognosis has not yet been completely defined, mainly due to its scarcity.[1,3,5-9]

This study sought to describe a case of an intramural duodenal hematoma caused by chronic exacerbation of pancreatitis.

CASE REPORT

The patient (JCAP) was a 43-year-old white male who had been a chronic abuser of alcohol for 25 years (two liters of distilled liquor/day), with an antecedent of acute pancreatitis five years before the present case report. He had been complaining of strong typical pain for three days, along with melena.

At admission to hospital, the following test results were noted: leukogram = 15,000 u/l, hemoglobin = 16 g/dl and amylase = 99 IU/l. A computed tomography (CT) scan showed signs of chronic pancreatitis and a bulky submucosal duodenal hematoma from the bulb to the third portion of the duodenum with intramural active bleeding in the region of the gastroduodenal artery (**Figure 1A**). Upper digestive tract endoscopy revealed a large submucosal hematoma in the duodenum, without any active bleeding into the lumen (**Figure 2**).

On the following day, the hemoglobin level decreased to 12 g/dl and selective angioembolization was indicated. During angiography, active bleeding was detected (**Figure 3A**). After embolization, no more signs of active bleeding were observed (**Figure 3B**).

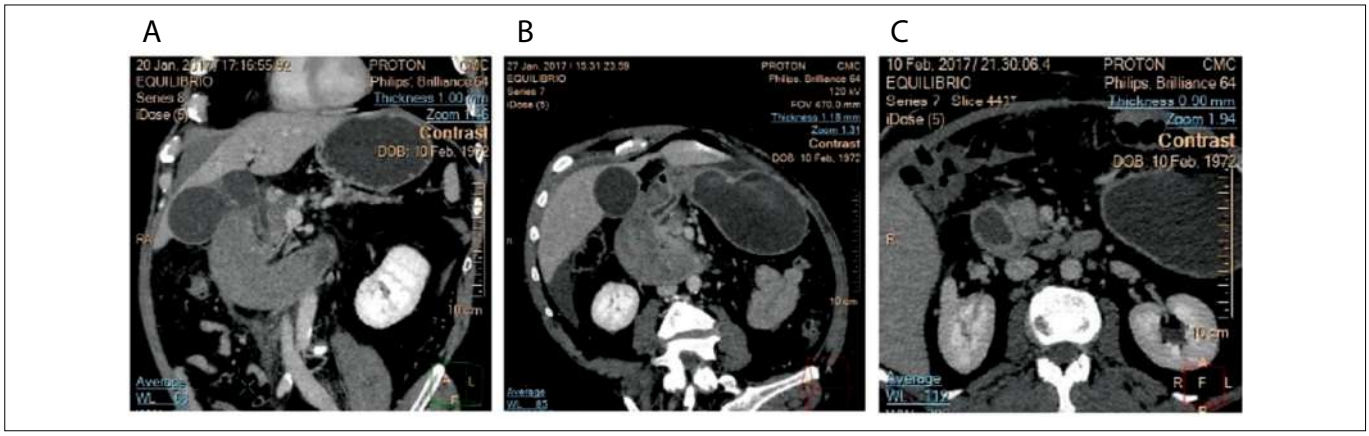

Figure 1. Computed tomography scans: A) at admission; B) post-embolization; C) post-surgical control.

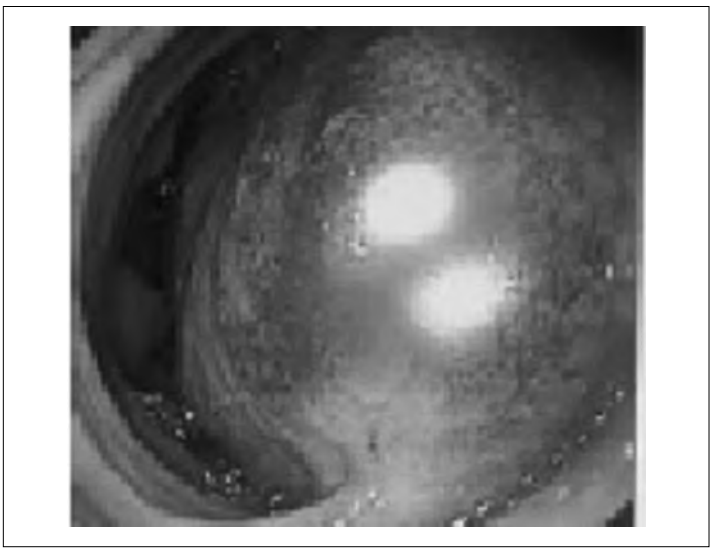

Figure 2. Upper gastrointestinal endoscopy.

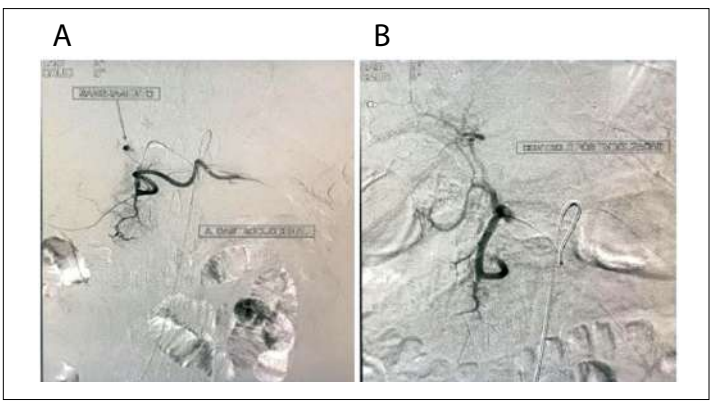

Figure 3. Arterial embolization: A) active bleeding in the region of the gastroduodenal artery; B) post-embolization control.

One day after this procedure, there was worsening of the pain. A CT scan showed signs of visceral perforation (**Figure 1B**). Emergency laparotomy was warranted and revealed the following: an ischemic duodenum with a bulky wall hematoma and a 6-cm duodenal rupture from the bulb to the second portion of the duodenum, along with signs of acute pancreatitis. The duodenal rupture was closed, along with exclusion of the pylorus, and Roux-en-Y gastroenteroanastomosis was carried out.

The patient then progressed with a high-output duodenal fistula. Treatment consisting of parenteral nutrition, octreotide and antibiotic therapy was started, and this led to regression over a nine-day period. A control CT scan demonstrated regression of the hematoma, while the signs of chronic pancreatitis continued to be present, but without evidence of exacerbation (**Figure 1C**). A contrasted upper gastrointestinal radiographic series showed exclusion of the pylorus and patent gastroenteroanastomosis. The patient was then followed up for six months, with uneventful evolution.

DISCUSSION

Duodenal hematomas have been described as complications of both acute and chronic pancreatitis. Acute pancreatitis is a common disease, with an incidence of 20 to 40 cases/100,000 person-years of life and a mortality rate close to 5%, and the vast majority of the cases are of biliary etiology.[10] On the other hand, chronic pancreatitis is a progressive inflammatory disorder characterized by irreversible destruction of the pancreatic parenchyma and may

Table 1. Database search results for duodenal hematomas caused by pancreatitis

Electronic databases	Search strategies	Results
MEDLINE (PubMed)	(Duodenum) AND (Hematoma) AND (Pancreatitis)	13 case reports
LILACS (BVS)	(((Duodenum) OR (Duodeno)) AND (Hematoma) AND ((Pancreatitis) OR (Pancreatite) OR (Pancreatítis)))	1 case report

Table 2. Previously reported cases of intramural duodenal hematoma secondary to pancreatitis[1-9,12-16]

Author	Age (years)	Gender	Etiology of pancreatitis	Anticoagulant/ antiplatelet therapy or coagulopathy	Treatment	Outcome
Bodnár et al.[1]	33	Male	Hypertriglyceridemia	No	Conservative	Evolved with a pancreatic abscess that required CT-guided aspiration; no specific therapy for the duodenal hematoma; discharged after six weeks
Leundji et al.[2]	45	Male	Alcoholic	Yes (thrombopenic due to portal hypertension)	Conservative	Asymptomatic one year afterwards
Eurboonyanun et al.[3]	27	Male	Alcoholic	No	Conservative	Uneventful; discharged after 17 days
Dugernier et al.[4]	32	Male	Biliary	No	Conservative	Evolved with infected necrosis that required repeated surgical debridement and drainage; no specific therapy for the duodenal hematoma was carried out due to the poor clinical condition; discharged after six months
Fukunaga et al.[5]	49	Male	Alcoholic	No	Surgical drainage and biopsy	Uneventful; discharged after 18 days
Neuzillet et al.[6]	62	Male	Alcoholic	No	Pancreaticoduodenectomy	Uneventful
Lee et al.[7]	47	Male	Dengue fever	Yes (dengue hemorrhagic fever)	Conservative	Evolved with a peripancreatic abscess that required drainage 51 days after admission; uneventful evolution after drainage
Lee et al.[8]	55	Male	Alcoholic	No	Endoscopic drainage	Uneventful after drainage; follow-up CT demonstrated a smaller intramural mass in the duodenum and upper endoscopy showed a small duodenal ulcer
Veloso et al.[9]	64	Male	Unknown	Yes (aspirin and clopidogrel due to a previous myocardial infarction)	Conservative	Uneventful; discharged after 14 days
Ma et al.[12]	32	Male	Alcoholic	No	Pancreaticoduodenectomy after failure of conservative therapy	Discharged two weeks after surgery; uneventful late postoperative evolution
Dubois et al.[13]	55	Male	Alcoholic	No	Conservative	Uneventful
Khurana et al.[14]	73	Female	Pancreatic malignancy	Yes (warfarin for deep venous thrombosis)	Conservative	Uneventful; after resolution of duodenal hematoma, the patient underwent distal pancreatic resection
Farhoud et al.[15]	71	Female	Obstructive	Yes (warfarin for deep venous thrombosis)	Conservative	Uneventful; discharged after 10 days
Fesenmeyer et al.[16]	71	Male	Unknown	No	Conservative	Uneventful

CT = computed tomography.

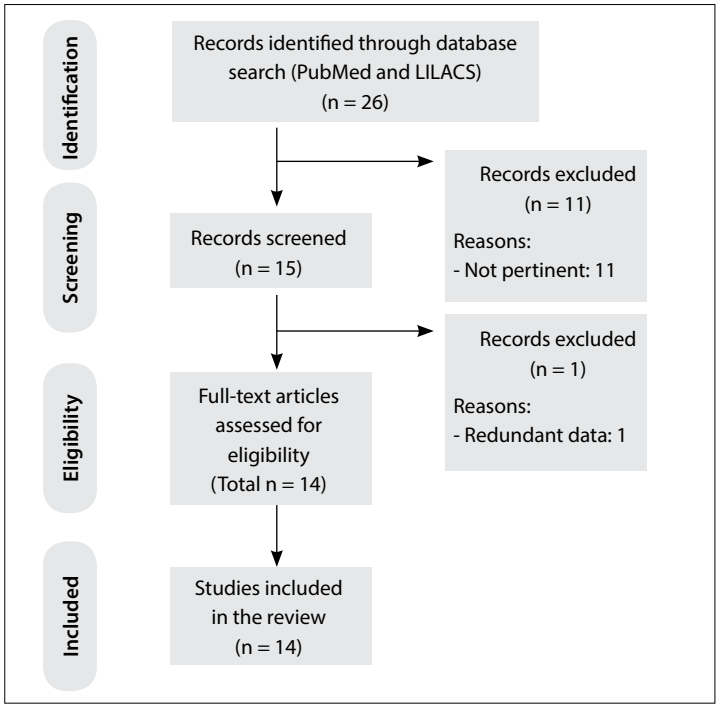

Figure 4. Flow diagram of the narrative review of the literature.

be associated with disabling chronic pain and permanent loss of exocrine and endocrine function. The majority of such cases are alcohol-related. Their prevalence is 3-4 cases/100,000 people.[11]

One of the rarest and most fatal complications of pancreatitis is spontaneous bleeding of intestinal vessels. A review of the literature was conducted through an online search for the MeSH terms "duodenum", "hematoma" and "pancreatitis" in MEDLINE (via PubMed) and LILACS (via BVS) in papers published over the last 20 years (**Table 1**). We included original studies that reported single cases or case series of this disease. All the papers were checked according to their titles and abstracts (screening). Full papers were obtained from journals available through the website of the Commission for Improvement of Higher Education Personnel (Comissão de Aperfeiçoamento de Pessoal de Nível Superior, CAPES), of the Brazilian Ministry of Education. Articles that were unavailable were requested from their authors. Articles presenting potentially relevant studies were read and analyzed to assess the inclusion criteria. We excluded articles that consisted of *in vitro* or animal studies, articles in which the participants' characteristics did not match those mentioned above, poster session abstracts, review articles and other types of publications. Articles that described traumatic or iatrogenic duodenal hematomas were also excluded. Other papers were used for contextualization and discussion.

After extensive online research, we identified 14 studies, which were all single case reports. **Table 2**[1-9,12-16] summarizes the main articles found and their reported outcomes. **Figure 4** presents a flow diagram of the articles selected.

Vascular erosion occurs due to extravasation of proteolytic enzymes. Potentially fatal bleeding, characterized by a decrease of two hemoglobin points, are rare complications occurring in about 1% to 5% of patients with acute or chronic pancreatitis. The vessels most affected are the gastroduodenal, pancreatoduodenal, splenic, gastroepiploic and left gastric arteries, because of their proximity to the pancreas, along with small branches of the inferior mesenteric arteries. The symptoms include pain, melena, hematemesis and retroperitoneal bleeding. Upper endoscopy, CT scans and angiography are involved in making the diagnosis.[17]

The majority of the previously reported cases showed that conservative therapy was possible, but this method is associated with lengthier hospital stay and a need for transfusion of blood components. Interventional therapies may include surgical repair using endoscopic and endovascular techniques. The benefits of endoscopy include its minimally invasive nature, compared with other treatment options, and the absence of radiation exposure. However, it has limitations, given that it cannot be used for areas of bleeding that are inaccessible to the intestinal lumen.[8] Arterial embolization seems to be effective for management of bleeding. Embolization is considered successful when both radiologically and clinically there is evidence of bleeding control characterized by hemoglobin stabilization and absence of signs and symptoms of shock.[18,19] Laparotomy for bleeding therapy should only be considered for hemodynamically unstable patients. Advances in endovascular radiology have led to this method becoming the preferred treatment option.[18]

The present case demonstrates the need for a high degree of suspicion that the diagnosis could comprise bleeding caused by pancreatitis, as well as the need to remain open to the possibility of a severe and rare complication after embolization (visceral perforation). This complication requires rapid intervention, because the risk of mortality that it presents is up to 10 times greater than that commonly observed in cases of acute pancreatitis.[20,21] The currently available evidence consists solely of single case reports, which thus precludes final conclusions regarding the optimal therapy. Hence, further research is necessary.

CONCLUSION

Intramural duodenal hematoma is a rare complication of pancreatitis. Selective embolization is the preferred treatment, but the risk of visceral ischemia and perforation should be considered.

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Address for correspondence:
Everton Cazzo
Departamento de Cirurgia da Facudade de Ciências Médicas da Universidade Estadual de Campinas (FCM/UNICAMP)
Rua Alexander Fleming, s/nº
Cidade Universitária Zeferino Vaz — Campinas (SP) — Brasil
CEP 13085-000
Tel. (+55 19) 3521-9450
E-mail: notrevezzo@yahoo.com.br

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Chlorella-induced thrombocytopenia

Irfan Yavasoglu[I], Atakan Turgutkaya[II], Zahit Bolaman[III]

Division of Hematology, Adnan Menderes University Medical Faculty, Aydın, Turkey

[I]MD. Professor, Division of Hematology, Adnan Menderes University Medical Faculty, Aydın, Turkey.
orcid.org/0000-0003-1703-2175
[II]MD. Fellow, Division of Hematology, Adnan Menderes University Medical Faculty, Aydın, Turkey.
orcid.org/ 0000-0001-8428-4730
[III]MD. Professor, Division of Hematology, Adnan Menderes University Medical Faculty, Aydın, Turkey.
orcid.org/ 0000-0003-0651-5462

Dear Editor,

Many improvements to alternative medicine have emerged recently. In speaking about the effects of these drugs, it is sometimes impossible to consider their adverse effects adequately.

Chlorella vulgaris is a unique single-celled species of freshwater microalga with grass-like odor. It has been used as a form of nutritional support. Clinical trials have suggested that supplementation with *Chlorella vulgaris* can ameliorate hyperlipidemia and hyperglycemia, and protect against oxidative stress, cancer and chronic obstructive pulmonary disease.[1] Here, we present a case in which thrombocytopenia was developed after use of this product as an anti-aging treatment.

A 49-year-old healthy female patient who was a medical doctor was admitted to our hospital because of thrombocytopenia. She reported that she not had any previous chronic drug use, but that she was taking *Chlorella* in tablet form, at a dose of 1,080 mg/day. Twenty days after starting to take the *Chlorella*, her platelet count was found to be 27,000/mm^3, while her leukocyte and hemoglobin levels were normal. We learned that a hemogram that had been produced one month earlier (i.e. before she started to use this medicine) was completely normal. The findings from a blood smear were also consistent with thrombocytopenia. Therefore, use of this drug was immediately stopped. Her platelet count then began to improve and became normal after two weeks.

So far, 39 cases of adverse effects from *Chlorella* have been notified to the United States Food and Drug Administration (FDA), and three of them (7.69%) have involved thrombocytopenia. Among these cases, three were males aged over 60 years and all of them had histories of using ornithine and imatinib.[2] Our case was female and did not have any medical illness.

The adverse effects of alternative products that are made available without their having gone through the various phases of drug trials always need to be considered. Use of these drugs should always be questioned, without exception, if a low platelet count is found.

INFORMED CONSENT

Informed consent was obtained from the patient who is reported in this study.

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Address for correspondence:
Irfan Yavasoglu
Adnan Menderes University Medical Faculty, Division of Hematology
Zafer Street, Adnan Menderes University Road, Adnan Menderes University
09100 Aydın — Turkey
Tel. +90-256-212 00 20
E-mail: dryavas@hotmail.com

AIM AND EDITORIAL POLICY

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THE MANUSCRIPT AND TYPES OF ARTICLES

General guidelines: for all types of articles

All manuscripts must be submitted in English with a covering letter signed by the corresponding author. The letter must contain the following five essential items relating to the manuscript:

1. a declaration that the manuscript is original and that the text has not been nor will be submitted for publication in any other journal.
2. a statement that the manuscript has been approved by all authors, who agree to cede the copyrights to the Journal, disclose all sources of funding and declare all potential conflicts of interest.
3. a statement that implementation of the study was endorsed by an Internal Review Board (Ethics Committee), including the date and number of the approval (in the case of original articles).
4. a brief description of contributorship.
5. a list of a minimum of five potential referees outside of the authors' institutions.

The Journal recommends that all articles submitted must comply with the editorial quality standards established in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (available at www.icmje.org).[1] This means that each type of study must be described in accordance with the specific quality guidelines for papers reporting on clinical trials (CONSORT),[2] systematic reviews and meta-analyses (PRISMA),[3,4] observational studies (STROBE),[5,6] case reports (CARE)[7] and accuracy studies on diagnostic tests (STARD).[8,9]

Abbreviations must not be used, even those in everyday use. Drugs or medications must be referred to using their generic names, avoiding casual mention of commercial or brand names. All drugs should be followed by the dosage and posology used. Any product cited in the Methods section, such as diagnostic or therapeutic equipment, tests, reagents, instruments, utensils, prostheses, orthoses and intraoperative devices must be described together with the manufacturer's name and place (city and country) of manufacture in parentheses.

Grants, bursaries and any other financial support for studies must be mentioned separately, after the references, in a section named "Acknowledgements." This section should also be used to acknowledge any other contributions from individuals or professionals who have helped in producing the study. The Journal supports the position taken by the International Committee of Medical Journal Editors (http://www.icmje.org) regarding authorship. This body's recommendations should be read to obtain clarifications regarding the criteria for authorship.

For any manuscript, all statements in the text that do not result from the study presented for publication in the São Paulo Medical Journal but from other studies must be accompanied by a quotation of the source of the data. All statements regarding health statistics and epidemiological data should generally be followed by references to the sources that generated this information, even if the data is only available electronically.

Articles must also include an abstract and three to five keywords in English. The keywords must be selected from the MeSH list only, available from: https://www.ncbi.nlm.nih.gov/mesh (no other keywords will be accepted).

Texts must be submitted exclusively through the Internet, using the electronic submission system, which is available at http://mc04.manuscriptcentral.com/spmj-scielo. Submissions sent by e-mail or through the post will not be accepted.

Authorship

Authors of articles published in São Paulo Medical Journal should all have contributed actively to the discussion of the study results and should review and approve the final version to be released. The corresponding author is the primary guarantor of all ethical issues relating to the manuscript, before, during and after its publication. However, São Paulo Medical Journal considers that all authors are held fully responsible for the study, regarding the accuracy or integrity of data and data interpretation in the text.

All authors must create an ORCID ID record (in www.orcid.org) before submitting their article and link the submission to their existing ORCID ID in the electronic submission system. ORCID identifications help to distinguish researchers with similar names.

During submission, the authors will be asked to indicate the names of three to five referees. All of them should be from outside the institution where they work and at least two should preferably be from outside Brazil.

FORMAT

Title page (cover page)

The title page must contain:

1. Type of paper (original article, review or updating article, short communication or letter to the editor).
2. Title of the paper in English, which must be brief but informative, and must contain the study design (clinical trial, cohort, cross-sectional or case control study, systematic review and case report are the most common).
3. Full name of each author (the editorial policy of the São Paulo Medical Journal is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full or omitted, without using abbreviations); his/her background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or undergraduate student); and his/her position currently held (for example, Master or Doctoral Student, Assistant Professor, Associate Professor or Professor, but not Head of Department, Dean, Provost or Rector), in the department and institution where he/she works, and the city and country (affiliations).
4. Each author should indicate the way his/her name should be used in indexing. For example: for a "João Costa Andrade", indexing should be "Costa-Andrade J." or "Andrade JC"?
5. Each author should present his/her ORCID identification number (as obtained in www.orcid.org).
6. Place or institution where the work was developed, city and country.
7. Date and venue of the event at which the paper was presented, if applicable, such as congresses or dissertation or thesis presentations.
8. Sources of support in the forms of finance for the project, study bursaries or funding for purchasing equipment or drugs. The protocol number for the funding must be presented.
9. For Brazilian authors, all grants that can be considered to be related to production of the manuscript must be declared, such as fellowships for undergraduate, master and doctoral students; along with possible support for postgraduate programs (such as CAPES) and for the authors, such as awards for established investigators (*Produtividade* - CNPq), accompanied by the respective grant numbers.
10. Description of any conflicts of interest held by the authors. We recommend that the item "Conflicts of interest" at http://www.icmje.org should be read to obtain clarifications regarding what may or may not be considered to be a conflict of interest.
11. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). The author should also indicate a postal address, e-mail address and telephone number that can be published together with the article. São Paulo Medical Journal recommends that office (nor residential) addresses are informed for publication.

Main document

Second page: abstract and keywords

The second page must include the title and a 250-word abstract in English (case reports with 100 words). Do not cite references in the abstract.

Use the following headings:

1. Background: Describe the rationale for the study including the research question or the scientific hypothesis.
2. Design and setting: Declare study design correctly,[11] and the setting.
3. Methods: Describe methods briefly.
4. Results: Describe primary results with quantitative results describing the sampling strategy.
5. Conclusions: Make a succinct statement of data interpretation answering the research question presented previously.
6. Clinical Trial Registration. Mandatory for clinical trials and systematic reviews, optional for observational studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.

Insert 3 to 5 key words after the abstract, with terms differing from the title. The words must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh.

Text

- Typical main headings include Introduction, Methods, Results, Discussion and Conclusion. The authors can use short subheadings too.
- Number the pages.
- Abbreviations must be avoided.
- A maximum of 3000 words in the main text, from the Introduction to the Conclusions; 1000 words for short communications.
- Maximum number of figures and/or tables is 5
- Maximum number of references is 35 (except for systematic reviews).

References

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

The reference list should be inserted after the conclusions and before the tables and figures. In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the journal's readers will be taken to the exact document cited, and not to a general website.

In the end of each reference, please insert the "PMID" number (for papers indexed in PubMed) and the "doi" number if available.

Authors are responsible for providing a complete and accurate list of references, so that all references cited in the text must appear in the reference list, and every item in the reference list must be cited in text. Also, citations must be in the correct sequence.

The reference list should be inserted after the conclusions and before the tables and figures.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent a resolution of 300 DPI and/or minimum size of 2500 pixels (width) and be recorded in ".jpg" or ".tif" format. Do not attach images inside Microsoft PowerPoint or Microsoft Word documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Graphs prepared in Microsoft Excel (do not send them in image formats) spreadsheets must be accompanied by the tables of data from which they have been generated.

All the figures and tables should be cited in the text.

All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded to indicate the magnification used. The staining agent should be specified in the figure legend.

Original articles

Clinical trials; cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies; case series (i.e. case reports on more than three patients analyzed together); and systematic reviews with or without meta-analysis, are considered to be full-text original articles, with a maximum of 3000 words.

Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured in the same way as original articles.

Short communications and case reports must be limited to 1000 words (from the introduction to the end of the conclusion). The abstracts in short communications should not be structured and have a maximum of 100 words.

Authors will be required to comply with the guidelines for writing each type of original article, as follows:

1. Observational articles: STROBE Statement[5,6]
2. Clinical trials: CONSORT Statement[2]
3. Accuracy studies on diagnostic tests: STARD Statement[8,9]
4. Systematic reviews of the literature and meta-analyses: PRISMA[4]
5. Case reports: CARE[7]

São Paulo Medical Journal supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, since 2008, manuscripts on clinical trials have only been accepted for publication if they have received an identification number from one of the clinical trial registers (the options are stated at http://www.icmje.org).

The identification number should be declared at the end of the abstract. Articles describing systematic reviews must provide the protocol registration number in the PROSPERO database. Authors of randomized clinical trials and systematic reviews must thus register their studies before submitting them for publication in the São Paulo Medical Journal.

Results from cases with DNA sequences must be deposited in appropriate public databases. The protocol number or URL can be requested at any time during the editorial review. Publication of other research data in public repositories is also recommended, since it contributes towards replicability of research, increases article visibility and possibly improves access to health information.

Short communications, case reports, case series and narrative reviews

Short communications and case reports must be limited to 1000 words (from the introduction to the end of the conclusion), a maximum of five references and one figure or table. They should be structured in the same way as original articles. Individual case reports should contain the following sections: Introduction, Case Report, Discussion and Conclusion. Reports on case series constitute observational studies and these should be structured in accordance with the norms of the STROBE Statement.[5]

Both short communications and case reports must be submitted with abstracts and keywords. The abstracts in short communications should not be structured and have a maximum of 100 words.

The São Paulo Medical Journal is interested in publishing rare or instructive case reports, accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[11] The search strategy for each database and the number of articles obtained from each database must be shown in a table. The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms are appropriate to be utilized for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT) the search dates should be indicated in the text or in the table.

Narrative reviews may be accepted by the São Paulo Medical Journal provided that a systematic search is made, and they should be structured as Original Articles. The search strategy and results should be presented as described above for case reports. By invitation from the Editor-in-Chief, narrative reviews addressing historical personal or collective experiences relating to clinical health sciences, epidemiology and public health may be accepted, but with no more than two authors.

Individual case reports should contain Introduction, Case Report, Discussion and Conclusion. Case reports should be structured in accordance with the norms of the CARE Statements.[7] Case reports published in São Paulo Medical Journal must be submitted with abstracts and keywords.

Letters to the editor

Letters to the editor may address articles published in the São Paulo Medical Journal publication or may deal with health issues of interest. Case reports must not be submitted as letters. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

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