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New findings about atherosclerosis in Brazil from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

Novas descobertas sobre aterosclerose no Brasil: Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil)

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.

Do not panic if you confuse "atherosclerosis" and "arteriosclerosis". Few people, including teachers and researchers in the medical world, can describe in few words the difference between these pathological conditions, which have distinct impacts on clinical practice and epidemiological studies. The confusion has become very well established. The American Heart Association publishes a journal called "Arteriosclerosis, Thrombosis, and Vascular Biology" in which most of the articles address the experimental, clinical and epidemiological aspects of atherosclerosis. In contrast, the same association publishes "Hypertension", which has a permanent section on "arteriosclerosis". The differentiation between arteriosclerosis and atherosclerosis comes from one of the fathers of modern cardiology, George Pickering.[1] I prefer to summarize this discussion with two clear definitions:

1. Atherosclerosis is a complex inflammatory process associated with the presence of oxidized low-density lipoprotein (LDL)-cholesterol in the intima and media of the arterial wall.
2. Arteriosclerosis consists of functional depletion of large-artery elasticity.

My assessment is supported by a recent meta-analysis on 76 studies that revealed that only blood pressure and age correlate with the pulse wave velocity (i.e. arteriosclerosis), thereby explaining more than half of the variability of pulse wave velocity.[2] On the other hand, it is well known that high cholesterol, hypertension, diabetes and smoking are risk factors for atherosclerosis.

Fifty years ago, Professor Mario Montenegro led a seminal study addressing atherosclerosis in Brazil and the Americas using autopsy cases.[3,4] However, subsequently, the approach towards atherosclerosis remained not very well developed in Brazil or elsewhere until the emergence of non-invasive imaging tools. For epidemiological and clinical studies such as the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), atherosclerosis was assessed through measuring the intima-media thickness (IMT) in the common carotid arteries, either by means of ultrasound or from determination of calcium scores in the coronary artery tree through computed tomography. On the other hand, arteriosclerosis was measured by determining the carotid-femoral pulse wave velocity.[5-13]

ELSA-Brasil was the first observational study to report in our population both the IMT and coronary artery calcium (CAC), thereby enabling comparison of these measurements in clinical practice.[6,7] Moreover, ELSA-Brasil was able to verify associations with migraine,[8] anxiety/depression,[9] cognition,[10] insulin resistance,[11] inflammatory biomarkers[12] and neck circumference.[13]

One fascinating finding was the association found in ELSA-Brasil between ideal cardiovascular health as proposed by the American Heart Association and the presence of coronary calcium.[14] The relationship is not exactly direct and linear (i.e. the higher the frequency of risk factors, the greater the quantity of calcium in the coronary tree also would be). ELSA-Brasil enrolled 4,077 participants with no prior history of cardiovascular disease aged 35 to 74 years who underwent CAC measurement. The ideal risk factors (IRFs) were defined using data on

smoking, physical activity, diet, blood pressure, glucose/cholesterol levels and body mass index. Zero IRF score was the worst situation and seven was the best. The distribution of the participants according to the IRFs was: 0 to 1 (n = 1,025; 25.1%), 2 (n = 1,200; 29.4%), 3 to 4 (n = 1,551; 38.1%) and 5 to 7 (n = 301; 7.4%). By comparing the presence or absence of CAC, it could be seen that people with IRF = 2 had a 25% lower frequency of calcium in the coronary arteries. Individuals with IRF = 4 had a 40% lower CAC rate and participants with IRF = 5 to 7, a 50% lower CAC rate. The authors concluded that even among individuals with better cardiovascular health (IRF = 5 to 7), it is possible to find CAC greater than zero. This reflects the notion that IRF measurements do not fully account for all factors that result in coronary artery disease.

These findings will need to be confirmed during the ELSA-Brasil follow-up. However, they indicate that subclinical atherosclerosis markers can play a major role in prevention of cardiovascular disease.

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Human herpesvirus 8 (HHV-8) detected by nested polymerase chain reaction (PCR) in HIV patients with or without Kaposi's sarcoma. An analytic cross-sectional study

Herpesvírus humano 8 (HHV-8) detectado por reação da polimerase em cadeia do tipo *nested* PCR em pacientes HIV-positivos com ou sem sarcoma de Kaposi. Um estudo transversal analítico

Paula Renata Lima Machado[I], Kleber Juvenal Silva Farias[I], Maira Gabriela Martins Pereira[II], Patrícia Pereira da Silva de Freitas[III], Benedito Antônio Lopes da Fonseca[IV]

Virology Research Center, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), São Paulo, Brazil

[I]PhD. Biomedical Scientist, Virology Research Center, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brazil.

[II]MSc. Biologist, Virology Research Center, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brazil.

[III]PhD. Veterinarian, Virology Research Center, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brazil.

[IV]MD, PhD. Associate Professor, Virology Research Center, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brazil.

KEY WORDS:
Sarcoma, Kaposi.
Herpesvirus 8, human.
HIV.
Polymerase chain reaction.
Serology.

PALAVRAS-CHAVE:
Sarcoma de Kaposi.
Herpesvirus humano 8.
HIV.
Reação em cadeia da polimerase.
Sorologia.

ABSTRACT

CONTEXT AND OBJECTIVE: Kaposi's sarcoma (KS) is a common neoplastic disease in AIDS patients. The aim of this study was to evaluate the frequency of human herpesvirus 8 (HHV-8) infection in human immunodeficiency virus (HIV)-infected patients, with or without KS manifestations and correlate HHV-8 detection with KS staging.

DESIGN AND SETTING: Analytic cross-sectional study conducted in a public tertiary-level university hospital in Ribeirão Preto, São Paulo, Brazil.

METHODS: Antibodies against HHV-8 lytic-phase antigens were detected by means of the immunofluorescence assay. HHV-8 DNA was detected in the patient samples through a nested polymerase chain reaction (nested PCR) that amplified a region of open reading frame (ORF)-26 of HHV-8.

RESULTS: Anti-HHV-8 antibodies were detected in 30% of non-KS patients and 100% of patients with KS. Furthermore, the HHV-8 DNA detection rates observed in HIV-positive patients with KS were 42.8% in serum, 95.4% in blood samples and 100% in skin biopsies; and in patients without KS, the detection rate was 4% in serum. Out of the 16 serum samples from patients with KS-AIDS who were classified as stage II, two were positive (12.5%); and out of the 33 samples from patients in stage IV, 19 (57.6%) were positive.

CONCLUSION: We observed an association between HHV-8 detection and disease staging, which was higher in the serum of patients in stage IV. This suggests that detection of HHV-8 DNA in serum could be very useful for clinical assessment of patients with KS and for monitoring disease progression.

RESUMO

CONTEXTO E OBJETIVO: Sarcoma de Kaposi (SK) é uma doença neoplásica comum em pacientes com aids. O objetivo deste estudo foi avaliar a frequência da infecção por herpesvírus humano 8 (HHV-8) em pacientes infectados por HIV, com ou sem SK e associar a detecção do HHV-8 com o estadiamento do SK.

TIPO DE ESTUDO E LOCAL: Estudo transversal analítico realizado em hospital universitário público terciário de Ribeirão Preto, São Paulo, Brasil.

MÉTODOS: Anticorpos contra antígenos de fase lítica do HHV-8 foram detectados por imunofluorescência. O DNA viral de HHV-8 foi detectado em amostras de pacientes pela reação em cadeia da polimerase do tipo *nested* (*nested* PCR), que amplificou uma região do fragmento de leitura aberta (ORF)-26 do HHV-8.

RESULTADOS: Anticorpos anti-HHV-8 foram detectados em 30% dos pacientes sem SK e 100% dos com SK. Além disso, a detecção de HHV-8 DNA observada em pacientes HIV-positivos com SK foi de 42,8% no soro, 95,4% em amostras de sangue e 100% em biópsias de pele, e em pacientes sem SK foi de 4% no soro. Das 16 amostras de soro de pacientes com SK-AIDS classificados como estádio II, duas foram positivas (12,5%) e, das 33 amostras de pacientes no estádio IV, 19 (57,6%) foram positivas.

CONCLUSÃO: Observamos associação entre a detecção do HHV-8 e o estadiamento da doença, que foi maior no soro de pacientes no estágio IV. Isso sugere que a detecção do HHV-8 no soro poderia ser muito útil para a avaliação clínica de pacientes com SK e para o monitoramento da progressão da doença.

INTRODUCTION

Human herpesvirus 8 (HHV-8) is associated with three neoplastic disorders: Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman's disease.[1-3] KS is an angioproliferative disease that is particularly frequent and aggressive in patients with AIDS. It commonly presents as multifocal disease, frequently in the upper body, head and neck, with a rapid course regarding both local progression of lesions to tumors and visceral dissemination, leading to organ dysfunction and high mortality.[4] The most common sites for visceral involvement by KS are the lungs (37%), gastrointestinal tract (50%) and lymph nodes (50%).[5]

Since HHV-8 has not been isolated in cell cultures, HHV-8 infection is identified by means of either serological methods or molecular biology assays. Several qualitative and quantitative amplification techniques for HHV-8 detection in different biological samples have been developed.[6-8] HHV-8 viral sequences have been successfully detected by means of the polymerase chain reaction (PCR) in various specimens, such as in KS lesions. HHV-8 sequences can also be detected in plasma and in peripheral blood mononuclear cells with very high specificity and sensitivity. The HHV-8 viral load in peripheral blood mononuclear cells of KS patients has been shown to correlate with tumor burden, but this approach has only in frequently been used to monitor KS patients in clinical practice.[9]

Although high rates of HHV-8 antibodies (19.6-57.4%) have been found in Brazilian Amerindians,[10,11] only low rates of HHV-8 antibodies have been found in blood donors from other parts of Brazil (2.8-7.4%).[12,13] The HHV-8 antibody prevalence among healthy children and young adults in different cities in the state of São Paulo ranges from 1.0 to 4.1% in different age groups. Among AIDS patients, the prevalence has been found to be 39.2% (51/130).[14]

OBJECTIVES

The aims of the present study were to evaluate the frequency of HHV-8 infection in human immunodeficiency virus (HIV)-infected patients, with or without KS manifestations, and to assess the association between HHV-8 detection and KS staging.

METHODS

This analytic, cross-sectional study was conducted in the Infectious Disease Unit of the University Hospital of Ribeirão Preto, São Paulo, Brazil. The subjects examined in this study comprised HIV-1 positive patients with KS, and HIV-1 positive patients without KS. The number of HIV patients was calculated as 50 (considering 5% alpha, test power of 80% and prevalence of HHV-8 infection of at least 5%). The sample size was calculated using the PS Power and Sample Size Calculations 2.1.30 software. The study was approved by the Ethics Committee of this institution (no. 12999).

The diagnosis of KS was clinically suspected and histologically confirmed. The histopathological criteria for diagnosing KS included spindle cell proliferation, erythrocyte-filled vascular slits and proliferation of small vessels, with vessels showing evidence of extracellular hemorrhage and hemosiderin deposition. KS staging was performed in accordance with the Mitsuyasu and Groopman system.[15]

BCBL-1 cells were cultured in RPMI medium with 20 ng/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma) for 96 hours. The cells were then washed twice with phosphate-buffered saline (PBS), placed on slides (10 µl/well), fixed in cold acetone for 10 minutes and stored at -20 °C. The slides were incubated with human serum (1:40 dilution in PBS with 3% fetal bovine serum) at 37 °C for 30 minutes. They were then washed, incubated with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G (IgG) (Dako) at 1:256 dilution in Evans blue at 37 °C for 30 minutes, washed again and air dried. Coverslips were mounted with buffered glycerol. Whole-cell fluorescence in about 20% of the TPA-treated cells was considered positive for antibodies against lytic-phase antigens.

Nested PCR was performed on DNA extracted from serum, peripheral blood cells and skin tissue. DNA was extracted from 200 µl of serum and peripheral blood cells using the QIAamp DNA blood kit (QIAGEN) and from 25 mg of skin tissue using the QIAamp DNA tissue kit (QIAGEN), in accordance with the manufacturer's instructions, and DNA was eluted with 50 µl of adding the elution buffer AE. For all PCR reactions, 5 µl of deoxyribonucleic acid (DNA) obtained from extraction of serum, peripheral blood cells or tissue were used.

In a total volume of 50 µl, the PCR mixture contained 0.2 mM of each dNTP, 25 pmol of each sense and anti-sense primer (5'-AGCCGAAAGGATTCCACCAT-3' and 5'-TCCGTGTTGTCTACGTCCAG-3')[1], 1.5 mM of $MgCl_2$, 2.5 U of Taq DNA polymerase (Invitrogen) and 5 µl DNA extracted from each sample. PCR amplification of HHV-8 was done at 94 °C for 1 minute, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 58 °C and 1 minute at 72 °C, with a final extension step (10 minutes at 72 °C) to allow complete extension of the amplicons. The nested PCR amplification mixture contained 1 µl of the first PCR mixture and the same PCR reagents described above, except that 25 pmol of each sense and anti-sense internal primers (5'-TTCCACCATTGTGCTCGAAT-3' and 5'-TACGTC CAGACGATATGTGC-3')[1] were used. Again, the amplification was done at 94 °C for 1 minute, followed by 40 cycles of 1 minute

at 94 °C, 1 minute at 60 °C, 1 minute at 72 °C and a final extension of 10 minute at 72 °C. Ten microliters of each reaction mixture were analyzed by means of electrophoresis on 2% agarose gel. Positive reactions yielded an amplicon of 211 bp, which was easily viewed through ethidium bromide staining.

As a positive control, b-globin was amplified using PCR from each specimen (blood samples and skin tissue) using the primers GL1 and GR2.[16] A set of negative controls (sterile water and a negative clinical sample) was included during all steps of the DNA isolation and amplification.

Recombinant plasmids containing amplicons of the HHV-8 ORF-26 were prepared in order to determine the nested-PCR sensitivity. The amplicon of 233 bp was amplified from a KS patient, purified using the Wizard SV Gel and PCR Clean-Up System kits (Promega), ligated to the pDrive cloning vector (QIAGEN) and used to transform *Escherichia coli* DH5α competent cells. The recombinant plasmids were then recovered using the Perfectprep Plasmid mini-kit (Eppendorf). Plasmids were sequenced using the ABI Prism Big Dye Terminator Cycle sequencing-ready kit (Applied Biosystems) with M13 forward or reverse primers, and the nucleotide sequence contained in this plasmid was compared with HHV-8 sequences retrieved from the GenBank database. These recombinant plasmids were used to analyze the absolute quantity of HHV-8 DNA detected by the nested PCR. Serial tenfold dilutions of a mixture containing a known copy of HHV-8 plasmids were amplified using nested PCR, and the analytical sensitivity of this technique was considered to be the last dilution presenting an amplicon band.

The variations of positivity for HHV-8 were determined using Fisher's exact test, with a significance level of 0.05. Calculations were performed using the BioEstat 5.0 software.[17]

RESULTS

Out of the 49 HIV-positive patients with a clinical or histopathological diagnosis of KS, 43 (87.8%) were males and 6 (12.2%) females, with ages ranging from 20 to 79 years (mean: 40 years). Out of the 50 HIV-positive patients without manifestations of KS who agreed to participate in this study, 36 (72%) were males and 14 (28%) females, ranging in age from 29 to 47 years (mean: 37.8 years).

Regarding the topography of AIDS-KS lesions, 32.7% of the patients presented cutaneous lesions alone; 30.6% cutaneous and digestive tract lesions; 16.3% cutaneous and respiratory tract lesions; 10.2% cutaneous, digestive tract and respiratory lesions; and 10.2% disseminated disease with lymph node involvement.

Out of the 49 patients, 16 were classified as stage II (more than one anatomical area and more than 10 cutaneous lesions) and 33 as stage IV (skin and visceral involvement). None of them were classified as stage I (one anatomical area with less than 10 lesions) or stage III (visceral involvement alone).

All of the AIDS-KS patients were positive for HHV-8 antibodies in the immunofluorescence assay (IFA), whereas only 15/50 of the patients without KS were positive (100% versus 30%).

The analytical sensitivity of nested PCR, analyzed in terms of the ability to detect plasmids containing the HHV-8 DNA sequence, was 17 copies of HHV-8 DNA. Forty-nine serum samples, 21 peripheral blood samples and 13 skin biopsies from patients with AIDS-KS, and 50 serum samples from HIV-positive/KS-negative patients were subjected to molecular detection of HHV-8. The HHV-8 DNA detection rates for HIV-positive patients with KS were 42.8% (21/49) in serum, 95.4% (21/22) in peripheral blood samples and 100% (13/13) in skin biopsies. In HIV-positive patients without KS, the detection rate for HHV-8 DNA was 4% (2/50) in serum **(Table 1)**. The efficiency of DNA extraction from blood samples and biopsies was confirmed by means of PCR amplification of the human β-globin gene.

When broken down according to disease stage, out of the 16 serum samples from patients with AIDS-KS classified as stage II, two (12.5%) were positive for HHV-8 through PCR. Out of the 33 samples from patients in stage IV, 19 (57.6%) were positive (P = 0.0048) **(Table 2)**. Thus, it was seen that HHV-8 DNA was more readily detected in patients with disseminated disease.

Table 1. Prevalence of HHV-8 DNA in different samples from HIV-positive patients with and without Kaposi's sarcoma (KS) as evaluated by nested polymerase chain reaction (nested PCR) test

Patients	n	Positive
HIV-positive/KS-positive		
Serum	49	21 (42.8%)
Blood samples	22	21 (95.4%)
Biopsies	13	13 (100%)
HIV-positive/KS-negative		
Serum	50	2 (4%)

HHV-8 = human herpesvirus 8; DNA = deoxyribonucleic acid; HIV = human immunodeficiency virus.

Table 2. Prevalence of HHV-8 DNA in serum samples from HIV-positive patients with Kaposi's sarcoma according to stage as evaluated by nested polymerase chain reaction (nested PCR) test

Stage	n	Positive in nested PCR (serum)	P-value
II	16	2 (12.5%)	0.0048
IV	33	19 (57.6%)	
Total	49	21 (42.8%)	

HHV-8 = human herpesvirus 8; DNA = deoxyribonucleic acid; HIV = human immunodeficiency virus.

In the group of HIV-positive/KS-positive patients, it was possible to obtain 22 peripheral blood samples (19 from stage IV and 3 from stage II patients). Presence of HHV-8 DNA was detected in all stage IV patients and in two of the stage II patients. Analysis on these 22 peripheral blood samples according to their corresponding serum samples showed that nested PCR was positive in 12/19 and 1/3 of the stage IV and II patient samples (63.1% versus 33.3%), respectively. No significant difference was detected between these groups (P = 0.1364).

DISCUSSION

Epidemiological studies have determined that HHV-8 seropositivity in various populations is strongly correlated with the population's risk of developing KS[4,14,18,19] and several longitudinal studies have shown that HHV-8 infection precedes the onset of KS.[4,13,20] Among men who are infected with both HIV and HHV-8, the hazard ratio for KS is estimated to be 5.04%, and the 10-year probability of developing KS is up to 49.6%. The incidence of AIDS-KS has declined considerably since the use of highly active antiretroviral therapy (HAART) became widespread. The effect of the treatment may result from direct action of antiretroviral drugs on HIV, which is known to trigger KS, or may constitute direct antiviral action against HHV-8. Furthermore, immune reconstitution following HAART may lead to better recognition and clearance of HHV-8 through specific immune responses.[4]

Our study found HHV-8 antibody percentages of 30% and 100% in HIV-positive/KS-negative and HIV-positive/KS-positive patients, respectively. Other studies conducted in Brazil have found rates ranging from 13.9% to 39.2% in KS-free HIV-infected patients,[11,14,18,19,21,22] from 80.0 to 98.7% in HIV-positive/KS-positive patients[14,19,21] and from 2.5 to 25.1% in the general population.[14,19,23-25] Several groups have reported variable seroprevalence rates for HHV-8 in the general populations of several countries. Depending on the assay used and the countries examined, the seroprevalence of HHV-8 antibodies ranges from 0 to 53% in the general population.[26] These results should take into consideration the fact that the current serological tests present some accuracy problems with regard to detecting antibodies against HHV-8, especially in patients with asymptomatic infection.[27] It should be pointed out that these results also vary with the type of immunofluorescence assay used, since the lytic IFA is more sensitive than IFA-latency-associated nuclear antigen (LANA) even though IFA-LANA is more specific than lytic IFA.[28]

HHV-8 DNA can be amplified from KS tissue at different clinical stages of the disease. Furthermore, semi-quantitative analysis has established that the HHV-8 DNA load is higher in patients with multicentric and visceral involvement than in those with localized disease, and also that the nodular stage is associated with a higher viral load than the patch and plaque stages, thereby showing a correlation between viral load and disease severity.[29]

There are conflicting reports regarding the prevalence of HHV-8 DNA in human tissues and body fluids, because most reports are based on data obtained through PCR of varying sensitivity and specificity. Nested PCR has been used to detect HHV-8 in paraffin-embedded tissues from biopsies on KS and multicentric Castleman's disease cases, lymphoid tissues from PEL patients, semen, plasma, peripheral blood and saliva.[30]

In the present study, HHV-8 DNA was detected in 95.4% (21/22) of blood samples, thus indicating that HHV-8 can be found in peripheral blood mononuclear cells (PBMC) from individuals carrying HHV-8. In areas of low prevalence, such as the United Kingdom, France, United States and Canada, nested PCR has shown that there is no positive test in PBMC among healthy individuals. HHV-8 has been detected in PBMC in 9% of HIV-1 patients in parts of Italy. In Uganda, a country with high incidence of KS, HHV-8 was detected in PBMC in 14% of the total population.[30] High frequency (82%) of detectable HHV-8 DNA in PBMC from 36 AIDS-KS cases from the Central African Republic was detected using a real-time PCR quantitative assay.[31] The high prevalence of HHV-8 DNA found in the presence study might represent selection bias, since all of our patients presented very severe disease.

In a study on the molecular epidemiology of HHV-8 among Cuban and German patients with KS and asymptomatic sexual contacts, HHV-8 DNA was isolated from PBMC and amplified by means of nested PCR for ORF-K1 in 41.7% (10/24) of the cases of asymptomatic sexual contact.[32]

Out of the 49 serum samples from patients with AIDS-KS, 21 (42.8%) were HHV-8 positive in our study, thus indicating that these patients presented viremia, an important event in the pathogenesis of KS. Viral DNA was detected in 12.5% (2/16) of the serum samples from patients in KS stage II and 57.6% (19/33) of the patients in stage IV, thereby demonstrating that detection of HHV-8 in serum appears to be associated with disease stage. In HIV-positive patients without AIDS-KS, 4% (2/50) were positive in nested PCR, in agreement with the seropositivity obtained by means of HHV-8 IFA on the serum samples from these two patients. These patients certainly present higher risk of developing AIDS-KS.

Thus, nested PCR applied to serum samples can be used to assess the effects of HAART on HHV-8 viremia and of the specific drugs used for treating KS. In addition, detection of herpes virus DNA in lymphocytes could possibly represent latent infection, while detectable DNA in serum or plasma is usually associated with disease.[33]

The nested PCR applied in the present study was very sensitive: it was able to detect approximately 17 copies of HHV-8 and

provided a sensitive and quick diagnosis. Thus, this test can be used for confirmation of histopathological examinations, especially in cases of early vascular lesions, in which histopathological diagnosis is difficult.

Detection of HHV-8 by means of PCR is important for making the differential diagnosis of KS and for therapy. Virus detection in serum may be very useful for clinical assessment of patients with KS and for monitoring disease progression, which is important for clinical practice, thereby avoiding unnecessary treatments. Finally, this study provides the basis for developing further studies on Kaposi's sarcoma and HHV-8 in our hospital.

CONCLUSION

The percentage of anti-HHV-8 antibodies, detected by lytic IFA, was 30% for non-KS patients and 100% for patients with KS, and we observed an association between HHV-8 detection and disease staging, which was higher in serum from patients in stage IV.

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Address for correspondence:
Paula Renata Lima Machado
Av. Bandeirantes, 3.900
Ribeirão Preto (SP) — Brasil
CEP 14049-900
Tel. (+55 16) 3602-3251
E-mail: paulamachado@usp.br

Post-analysis methods for lactate threshold depend on training intensity and aerobic capacity in runners. An experimental laboratory study

Métodos de pós-análise do limiar do lactato dependem da intensidade de treinamento e da capacidade aeróbica dos corredores. Um estudo laboratorial experimental

Tiago Lazzaretti Fernandes[I], Rômulo dos Santos Sobreira Nunes[II], Cesar Cavinato Cal Abad[III], Andrea Clemente Baptista Silva[IV], Larissa Silva Souza[IV], Paulo Roberto Santos Silva[V], Cyro Albuquerque[VI], Maria Cláudia Irigoyen[VII], Arnaldo José Hernandez[VIII]

Laboratório do Estudo do Movimento (LEM), Instituto de Ortopedia e Traumatologia (IOT), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Sports Medicine Group of FMUSP, FIFA Medical Centre of Excellence, IOT HC-FMSUP, São Paulo, Brazil

[I]MD, MSc. Doctoral Student and Attending Physician, Sports Medicine Group, FIFA Medical Centre of Excellence, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto de Ortopedia e Traumatologia (IOT), Hospital das Clínicas (HC), São Paulo, Brazil.

[II]Undergraduate Student, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[III]MSc, PhD. Heart Institute, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto do Coração (InCor), São Paulo, Brazil.

[IV]MD. Sports Medicine Group, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto de Ortopedia e Traumatologia (IOT), Hospital das Clínicas (HC), São Paulo, Brazil.

[V]PhD. Sports Medicine Group, FIFA Medical Centre of Excellence, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto de Ortopedia e Traumatologia (IOT), Hospital das Clínicas (HC), São Paulo, Brazil.

[VI]MSC, PhD. Assistant Professor, Department of Mechanical Engineering, Centro Universitário da FEI, São Bernando do Campo, Brazil.

[VII]MD, PhD. Professor, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto do Coração (InCor), São Paulo, Brazil.

[VIII]PhD. Assistant Professor, Director of Sports Medicine Group, FIFA Medical Centre of Excellence, Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Instituto de Ortopedia e Traumatologia (IOT), Hospital das Clínicas (HC), São Paulo, Brazil.

KEY WORDS:
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Sports medicine.

PALAVRAS-CHAVE:
Ácido láctico.
Resistência física.
Limiar anaeróbio.
Consumo de oxigênio.
Teste de esforço.
Medicina esportiva.

ABSTRACT

CONTEXT AND OBJECTIVE: This study aimed to evaluate different mathematical post-analysis methods of determining lactate threshold in highly and lowly trained endurance runners.

DESIGN AND SETTING: Experimental laboratory study, in a tertiary-level public university hospital.

METHOD: Twenty-seven male endurance runners were divided into two training load groups: lowly trained (frequency < 4 times per week, < 6 consecutive months, training velocity ≥ 5.0 min/km) and highly trained (frequency ≥ 4 times per week, ≥ 6 consecutive months, training velocity < 5.0 min/km). The subjects performed an incremental treadmill protocol, with 1 km/h increases at each subsequent 4-minute stage. Fingerprint blood-lactate analysis was performed at the end of each stage. The lactate threshold (i.e. the running velocity at which blood lactate levels began to exponentially increase) was measured using three different methods: increase in blood lactate of 1 mmol/l at stages (DT1), absolute 4 mmol/l blood lactate concentration (4 mmol), and the semi-log method (semi-log). ANOVA was used to compare different lactate threshold methods and training groups.

RESULTS: Highly trained athletes showed significantly greater lactate thresholds than lowly trained runners, regardless of the calculation method used. When all the subject data were combined, DT1 and semi-log were not different, while 4 mmol was significantly lower than the other two methods. These same trends were observed when comparing lactate threshold methods in the lowly trained group. However, 4 mmol was only significantly lower than DT1 in the highly trained group.

CONCLUSION: The 4 mmol protocol did not show lactate threshold measurements comparable with DT1 and semi-log protocols among lowly trained athletes.

RESUMO

CONTEXTO E OBJETIVO: O objetivo do presente estudo é avaliar modelos matemáticos de pós-análise do limiar de lactato em grupos de corredores de longa distância muito ou pouco treinados.

TIPO DE ESTUDO E LOCAL: Estudo laboratorial experimental. Hospital Público Universitário Terciário.

MÉTODO: Vinte e sete corredores homens foram divididos em: pouco treinados (frequência < 4 vezes por semana, < 6 meses, velocidade ≥ 5,0 minutos/km) e muito treinados (frequência ≥ 4 vezes por semana, ≥ 6 meses, velocidade < 5,0 minutos/km). Os participantes foram submetidos a protocolo de esteira escalonado (1% inclinação) = 1 km/h por fase (4 minutos). Ao fim de cada estágio, análise da "impressão digital" metabolômica foi realizada. O limiar do lactato (i.e. velocidade em que o lactato sanguíneo aumenta exponencialmente) foi medido utilizando-se três métodos: aumento de 1 mmol/l da concentração, concentração absoluta de 4 mmol e método semi-log. ANOVA foi utilizada para comparar os diferentes limiares de lactato e grupos.

RESULTADO: Atletas muito treinados apresentaram limiares de lactato maiores que os corredores pouco treinados, independentemente do método de cálculo utilizado. Comparando todos os corredores juntos, as análises de aumento de 1 mmol/l e semi-log não foram diferentes, enquanto a concentração absoluta de 4 mmol/l foi significativamente mais baixa que as dos dois outros métodos. Essas mesmas tendências foram observadas ao se compararem os métodos de limiar de lactato no grupo menos treinado. Entretanto, a análise absoluta de 4 mmol/l foi menor do que a do aumento de 1 mmol/l no grupo muito treinado.

CONCLUSÃO: O método concentração absoluta de 4 mmol não mostrou mensurações comparáveis de limiar do lactato quando comparado com os protocolos aumento de 1 mmol/l e semi-log nos atletas pouco treinados.

INTRODUCTION

Blood lactate evaluation commonly complements endurance training regimens.[1,2] It has been recommended as an efficient method for evaluating training intensity and recovery, and for improving the performance of endurance athletes.[3-6] During incremental exercise, the lactate threshold (LT) is defined as the abrupt transition from slow increases to rapid exponential increases in blood lactate levels.[7]

The evaluation of lactate threshold in athletes has evolved, from the 4 mmol universal lactate threshold, to the more individualized Onset of Blood Lactate Accumulation, and to the current Maximal Lactate Steady State standard. This progression has been due to better understanding of the physiological processes of lactate production and clearance, and the role of lactate during prolonged and submaximal exercise.[5,8-12]

However, most published studies on lactate threshold have compared homogeneous groups of athletes with similar aerobic capacity, or have made regression analyses on these data.[13-16] Comparisons between different methods on lactate threshold acquisition also remain controversial in the literature.[17-19] To our knowledge, there is no comparative study evaluating lactate threshold methods in both lowly and highly trained endurance athletes.

OBJECTIVE

The purpose of this study was to evaluate different lactate threshold methods, and determine which methods are most reliable for athletes with different physical conditioning and training programs.

METHODS

This was an experimental laboratory study performed within the Sports Medicine Group of Faculdade de Medicina da Universidade de São Paulo. Twenty-seven male endurance runners were recruited for this study from university campus running clubs. For the primary outcome (post-analysis method for the lactate threshold in the same group), the sample size was calculated after a five-athlete pilot study, taking $P < 0.05$ and power = 80%. The sample size was estimated as 10 individuals per group. We added a minimum of 20% more subjects to account for potential data loss.

The subjects were divided into two distinct groups based on the responses to a questionnaire: 15 highly trained runners (minimum of 4 training runs per week for 6 consecutive months, and a long-distance training pace less than or equal to 5.0 min/km) and 12 lowly trained runners (long-distance training pace greater than 5.0 min/km, with a maximum of 3 runs per week and a maximum of 6 consecutive training months). The exclusion criteria were previous cardiorespiratory disease and musculoskeletal running-related injuries. No athlete was currently taking any medications.

Oxygen consumption (VO_2) was measured continuously and monitored by means of a breath-by-breath gas analyzer on a treadmill (h/p/cosmos, Pulsar, Germany) using a metabolic analyzer (CPX/D Med Graphics, St. Paul, USA)

The mean physiological characteristics of the highly trained group were: age 33.7 ± 10.3 years; training velocity: 4.0 ± 0.6 min/km; resting heart rate 68.7 ± 14.7 bpm; and VO_{2max}: 52.4 ± 5.3 ml/kg/min. Characteristics of the lowly trained group were: age 37.2 ± 9.3 years; training velocity: 5.3 ± 0.9 min/km; rest heart rate 79.3 ± 15.2 bpm; and VO_{2max}: 43.4 ± 5.7ml/kg/min.

The Institutional Review Board approved this research and informed consent was obtained from each subject prior to participation. This research followed the Helsinki Declaration principles.[20]

Lactate protocol

A washout period of 24 hours with no physical activity was requested for all participants prior to the experiment. The subjects then performed an incremental treadmill test to directly measure their lactate threshold. All subjects did the test at the same location, with the same equipment, and under similar thermal conditions (temperature 21-26°C, humidity 33-66%, barometric pressure 688 mmHg). Throughout the protocol, treadmill elevation was kept constant at a 1% grade to duplicate the energy cost of over-ground running.[21]

The subjects first performed a 3-minute warm-up run at 30% of their long-distance training velocity. At the beginning of the incremental test, the treadmill velocity was set at 70% of the estimated long distance training velocity, depending on the running ability of each participant (it is known that performance in competition is an appropriate criterion for valid laboratory tests).[3,22]

Heart rate and Borg scale were recorded each minute. Stage length was set at 4 minutes,[21] with running velocity increases of 1 km/h per stage until volitional exhaustion was reached (as measured from the Borg scale). Fingerprint whole-blood samples were taken between the points of 3.5 and 4 minutes in each stage and were immediately analyzed in an automated blood-lactate analyzer (Accutrend Lactate, Typ3012522) without treadmill protocol interruption. Blood samples were collected for two additional stages following exponential inflection of the lactate point.

Calculating lactate threshold

The basis for determining the lactate threshold is that there is an inflection point at a given workload (i.e. running velocity) where blood lactate exponentially increases with a corresponding increase in workload.[17,18,21,23,24] It is used to define the highest work rate or O_2 uptake (oxygen consumption) at which athletes can maintain their efforts over a specified time frame.[25] An individual blood-lactate

profile was created for each subject by plotting running velocity (km/h) at each stage of the test (x-axis) versus blood-lactate concentration attained at each stage (y-axis).[10,21,24,26,27]

Three methods commonly cited in the literature were used to define the inflection point (**Figure 1**):

1. Increase of 1 mmol/l blood lactate (DT1): the work rate that just precedes a rise in blood lactate concentration of > 1 mmol/l between two stages estimates the lactate threshold.[10,21,26,27]
2. Absolute value of 4 mmol/l blood lactate (4 mmol): workload when the concentration of lactate in the blood reaches 4 mmol/l.[10,21,26,27]
3. Semi-log method (semi-log): based on a logarithmic scale (blood lactate) in which the exponential blood lactate curve is divided into two linear segments that cross each other; the point of intersection is the lactate threshold.[17,18]

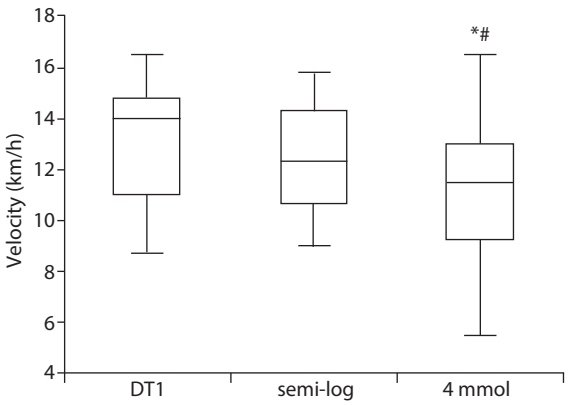
Statistical analysis

The normality curve was addressed by means of histograms and it was decided to use parametric tests. Baseline characteristics were analyzed first to demonstrate homogeneity. The threshold values of each method were compared with repeated-measurement analyses of variance (ANOVA). When a significant difference was attained, Tukey's post-hoc test was performed. Statistical significance was denoted as $P < 0.05$ (STATA-9 for Windows).

RESULTS

Before analyzing the relationship between lactate threshold and velocity, the associations between lactate threshold and baseline characteristics such as age, heart rate, VO_{2max} and training regularity were assessed. This analysis showed that neither demographic nor baseline characteristics could explain associations with lactate threshold, except, logically, for the dependent variables of training regularity between groups and VO_{2max}.

As expected, the lactate thresholds of the highly trained group were obtained at higher velocity stages than those of the lowly trained group in all tested methods. When considering all subjects (both the highly trained and the lowly trained groups), comparison of lactate threshold methods showed significant differences between the DT1 and 4 mmol methods, and between the semi-log and 4 mmol methods. There was no statistical difference between DT1 and semi-log (**Figure 2**).



*Significant difference between DT1 and 4 mmol methods ($P < 0.05$), and #significant difference between semi-log and 4 mmol ($P < 0.05$), ANOVA (analysis of variance), Bonferroni post-hoc test.

Figure 2. Box plot of the velocity of the lactate threshold of all subjects obtained using the DT1, semi-log and 4 mmol post-analysis methods.

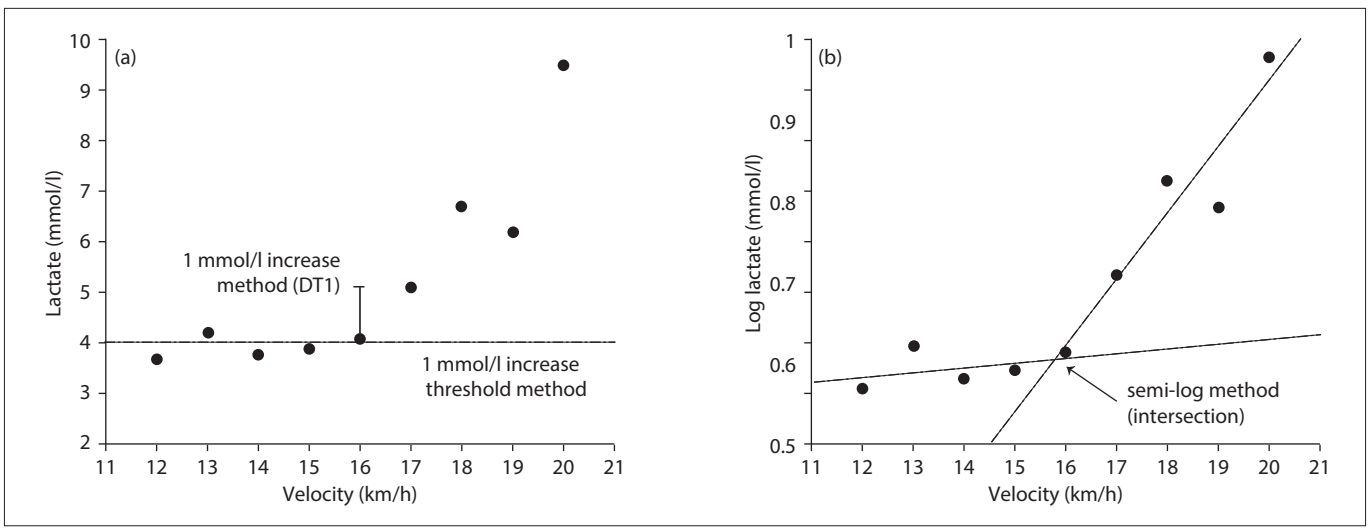


Figure 1. Example of the three post-analysis methods for lactate threshold applied to one subject: (a) the velocity before the increase of 1 mmol/l blood lactate (DT1); the velocity at which the blood lactate exceeds the value of 4 mmol/l (4 mmol); and (b) the velocity at the intersection of two interpolated lines on the semi-logarithmic scale (semi-log).

When the groups were compared separately (highly trained and lowly trained), the 4 mmol measurement was found to be significantly lower than the DT1 and semi-log measurements in the lowly trained group. The DT1 and semi-log measurements were not statistically different in this group (**Figure 3**). In the highly trained group, a significant difference was only found between the DT1 and the 4 mmol methods.

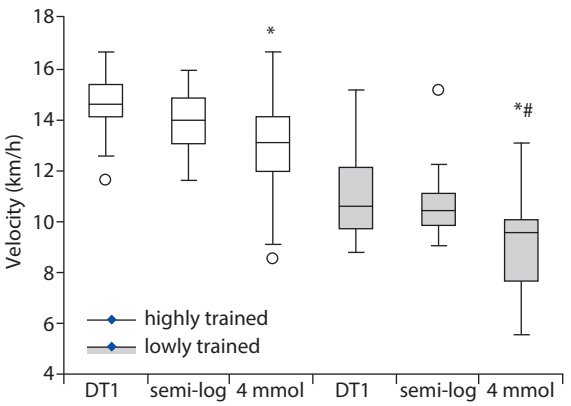
DISCUSSION

The most important finding of this study was the differences in lactate threshold measurement methods between highly and lowly trained endurance runners. The method with fixed blood lactate of 4 mmol/l underestimated the lactate threshold in the lowly trained group.

Sargent et al.[28] identified differences in lactate threshold between different groups of subjects, such as men versus women. On the other hand, Smekal et al.[29] reported that blood lactate concentration at the maximal lactate steady state was independent of both endurance capacity and sex. Other authors have showed comparisons between trained and untrained individuals through using cardiorespiratory tests.[30,31] One notable characteristic of our study is that we only used male subjects and made comparisons between controlled training levels (high and low) instead of between trained and sedentary subjects.

One explanation for the different values of measurement methods is that error is introduced when the curves do not follow the mathematical physiological functions.[19]

Subjects with different performance levels often have different mechanical running responses and consequently different metabolic demands.[32] Our study also agreed with the literature regarding higher lactate threshold values in trained individuals. Kumagai et al.[33] showed that aerobic training increased the lactate threshold, with a concomitant improvement in both endurance and middle-distance performance.



*Significant difference between DT1 and 4 mmol methods ($P < 0.05$), and #significant difference between semi-log and 4 mmol ($P < 0.05$), using ANOVA (analysis of variance) and the Bonferroni post-hoc test.

Figure 3. Box plot of the velocities at the lactate threshold obtained using the DT1, semi-log and 4 mmol post-analysis methods in each group (highly trained and lowly trained).

Individuals with greater endurance capacity have faster oxygen kinetics.[34] The higher values of lactate thresholds in the highly trained subjects may reflect more efficient peripheral and central exchange during exercise.[34] During low-intensity exercise, blood lactate formation and removal depends on the intracellular/tissue balance among the glycolytic (cytosol) and oxidative (mitochondria) processes.[35] It seems that in trained individuals, these variables are more predictable and have controlled behavior.

Joyner et al.[36] suggested that running performance could be explained by VO_2max, running economy and fractional utilization of VO_2max. Moreover, they suggested that the lactate threshold integrates all three of these variables and is the best physiological predictor of distance running performance[1], given that it is detectable in both trained and untrained individuals.[10]

It is known that the average value for the lactate threshold in normal subjects is 3.7 mmol and that serum blood lactate at the lactate threshold is not equal for all individuals (range: 1.5 to 7.5 mmol) and also changes in a single individual.[37] Although the 4 mmol lactate protocol is an easy method for estimating lactate threshold, the fixed value of 4.0 mmol does not take these physiological conditions into consideration and may underestimate lactate threshold, as shown in this study.[38]

The clinical relevance of this study relates to the populations tested. Most people are not competitive endurance athletes, yet still need predictions of aerobic threshold and exercise prescriptions for health issues. Our results suggest that lowly trained subjects would benefit from semi-log or DT1 lactate threshold methods in clinical practice.

The main limitation of this study relates to the treadmill protocol, such as the stage duration and initial running velocity. Due to the large variation of treadmill protocols in the lactate threshold literature, direct comparisons of our results with previous studies may not be appropriate. Despite training group characteristics that were very specific (frequency, intensity and duration of training), we believe that they represent objective inclusion criteria and, because of that, the results may be reproducible. Future studies should examine lactate threshold methods and cardiorespiratory performance in both highly trained and lowly trained groups. We suggest that these groups should be stratified according to training frequency, intensity and duration.

CONCLUSION

The 4 mmol protocol did not show lactate threshold measurements comparable with with DT1 and semi-log protocols among lowly trained athletes.

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Address for correspondence: Tiago Lazzaretti Fernandes
Laboratório do Estudo do Movimento
Instituto de Ortopedia e Traumatologia, Hospital das Clínicas
Faculdade de Medicina da Universidade de São Paulo
Dr. Ovídio Pires de Campos, 333 — 2º andar
São Paulo (SP) — Brasil
CEP 05403-010
Tel. (+55 11) 2661-6486
E-mail: tiago.lazzaretti@usp.br

Gene mutations of platelet glycoproteins and response to tirofiban in acute coronary syndrome

Mutações gênicas das glicoproteínas plaquetárias e resposta ao tirofiban na síndrome coronariana aguda

Antonio de Padua Mansur[I], Alessandra Roggerio[II], Júlio Yoshio Takada[III], Pérola Michelle Vasconcelos Caribé[IV], Solange Desirée Avakian[III], Célia Maria Cassaro Strunz[V]

Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil

[I]MD, PhD. Associate Professor, Department of Cardiopulmonology, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.
[II]BSc, PhD. Biochemist, Department of Cardiopulmonology, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.
[III]MD, PhD. Attending Physician, Department of Cardiopulmonology, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.
[IV]MD, MSc. Doctoral Student, Department of Cardiopulmonology, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.
[V]BSc. Central Laboratory Director, Department of Cardiopulmonology, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil.

KEY WORDS:
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Myocardial infarction.

PALAVRAS-CHAVE:
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ABSTRACT

CONTEXT AND OBJECTIVES: Glycoprotein inhibitors (abciximab, eptifibatide and tirofiban) are used in patients with unstable angina and non-ST-segment elevation myocardial infarction before percutaneous coronary intervention. Of these, tirofiban is the least effective. We hypothesized that the response to tirofiban might be associated with glycoprotein gene mutations.

DESIGN AND SETTING: Prospective study at Emergency Unit, Heart Institute (InCor), University of São Paulo.

METHOD: Intrahospital evolution and platelet aggregation in response to tirofiban were analyzed in relation to four glycoprotein mutations in 50 patients indicated for percutaneous coronary intervention: 17 (34%) with unstable angina and 33 (66%) with non-ST-segment elevation myocardial infarction. Platelet aggregation was analyzed using the Born method. Blood samples were obtained before and one hour after tirofiban infusion. Glycoproteins Ia (*807C/T*), Ib *(Thr/Met)*, IIb (*Ile/Ser*) and IIIa (*PIA*) were the mutations selected.

RESULTS: Hypertension, dyslipidemia, diabetes, smoking, previous coronary artery disease and stroke were similar between the groups. Mutant glycoprotein IIIa genotypes had lower platelet aggregation before tirofiban administration than that of the wild genotype (41.0% ± 22.1% versus 55.9% ± 20.8%; P = 0.035). Mutant glycoprotein IIIa genotypes correlated moderately with lower platelet inhibition (r = -0.31; P = 0.030). After tirofiban administration, platelet glycoprotein Ia, Ib, IIb and IIIa mutations did not influence the degree of inhibition of platelet aggregation or intrahospital mortality.

CONCLUSIONS: Mutations of glycoproteins Ia, Ib, IIb and IIIa did not influence platelet aggregation in response to tirofiban in patients with unstable angina and non-ST-segment elevation myocardial infarction.

RESUMO

CONTEXTO E OBJETIVOS: Inibidores da glicoproteína (abciximab, eptifibatide, tirofiban) são utilizados em pacientes com angina instável e infarto do miocárdio sem elevação do segmento ST (IAMSSST) antes da intervenção coronária percutânea. Dentre eles, o tirofiban é o menos eficaz. Nossa hipótese é que a resposta ao tirofiban possa estar associada a mutações no gene da glicoproteína.

DESENHO E LOCAL: Estudo prospectivo na Unidade de Emergência do Instituto do Coração (InCor), Universidade de São Paulo (USP).

MÉTODOS: Foram analisadas a evolução intra-hospitalar e agregabilidade plaquetária em resposta ao tirofiban de 4 mutações da glicoproteína em 50 pacientes com indicação para intervenção coronária percutânea, 17 (34%) com angina instável e 33 (66%) com IAMSSST. A agregação plaquetária foi analisada pelo método de Born. Amostras de sangue foram obtidas antes e uma hora após infusão do tirofiban. As glicoproteínas Ia (*807C/T*), Ib (*Thr/Met*), IIb (*Ile/Ser*) e IIIa (*PIA*) foram as mutações selecionadas.

RESULTADOS: Hipertensão, dislipidemia, diabetes, tabagismo, doença coronariana e acidente vascular cerebral prévios foram semelhantes entre os grupos. Observou-se menor agregabilidade plaquetária dos genótipos mutantes da glicoproteína IIIa antes da administração de tirofiban do genótipo selvagem (41% ± 22% *versus* 56% ± 21%; P = 0,035). Genótipos mutantes da glicoproteína IIIa correlacionaram-se moderadamente com menor inibição plaquetária (r = -0,31; P = 0,030). Após a administração tirofiban, as mutações das glicoproteínas Ia, Ib, IIb, e IIIa não influenciaram o grau de inibição da agregação plaquetária e mortalidade intra-hospitalar.

CONCLUSÕES: Mutações das glicoproteínas Ia, Ib, IIb e IIIa não influenciaram a agregação plaquetária em resposta ao tirofiban nos pacientes com angina instável e IAMSSST.

INTRODUCTION

Tirofiban, a platelet surface receptor glycoprotein IIbIIIa (GPIIbIIIa) inhibitor, is recommended for patients with unstable angina and non-ST-segment elevation myocardial infarction (NSTEMI) before a planned percutaneous coronary intervention, in order to reduce periprocedural coronary events. GPIIbIIIa plays a central role in thrombus formation through binding fibrinogen, von Willebrand factor and fibronectin.[1] Tirofiban, like fibrinogen, has a similar affinity to the active GPIIbIIIa receptor. Thus, tirofiban binds to GPIIbIIIa and prevents formation of fibrinogen bridges, thereby reducing the thrombogenesis process.[2] Clinical studies have demonstrated the efficacy of tirofiban in reducing coronary events in patients with unstable angina and NSTEMI who undergo percutaneous coronary intervention.[3,4] However, studies have shown unfavorable results from use of tirofiban for reducing ischemic events in patients undergoing percutaneous coronary intervention and also less inhibition of platelet aggregation with tirofiban, compared with two other commercially available inhibitors of platelet glycoprotein, abciximab and eptifibatide.[5,6] The possibility of a paradoxical effect of platelet inhibitors on platelet aggregation has also been discussed.[7] The possible hypotheses for these findings relate to lower intensity of platelet inhibition, antagonist-induced platelet activation and interaction between platelets and inflammation.[8] Another hypothesis to explain the interindividual variability in the antiplatelet effect of tirofiban may involve platelet glycoprotein gene polymorphisms.[9] Mutations of GPIa (*807C/T*), Ib (*Thr/Met*), IIb (*Ile/Ser*) and IIIa (*PIA*) have been inconsistently associated with increased risk of major acute coronary events and resistance to antiplatelet drugs.[10,11] We hypothesized that the smaller effect of tirofiban might be associated with platelet GP gene mutations.

METHODS

Baseline clinical and admission laboratory characteristics, coronary artery disease risk factors, in-hospital outcomes, angiography and treatments were analyzed in relation to 50 consecutive patients admitted to the emergency room with unstable angina or NSTEMI between May 2008 and November 2010. They were indicated to receive tirofiban before undergoing coronary angiography for possible percutaneous coronary intervention, as advocated in the ACC/AHA 2002 guidelines for management of patients with unstable angina and non-ST-segment elevation myocardial infarction.[12]

All the patients received 200 mg of aspirin or were put on aspirin at least 3 hours before tirofiban was started, along with heparin. Initially, a bolus dosage regimen of 0.4 μg/kg/min was given for 30 minutes, followed by a maintenance dose of 0.1 μg/kg/min for at least 24 hours.

High-risk unstable angina was defined as chest pain at rest lasting for more than 20 minutes, with dynamic electrocardiographic changes on the electrocardiogram and normal levels of MB isoenzyme of creatine kinase (CKMB) mass or troponin I. NSTEMI was diagnosed when these findings were associated with increased blood levels of CKMB and troponin I. The clinical outcome was defined as in-hospital death from all causes. The study was approved by the local institutional ethics committee. Informed consent was obtained from all patients.

Laboratory analysis

Troponin I and CKMB mass levels were analyzed in serum samples using specific kits, in the automated ADVIA Centaur equipment (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The kit used for troponin determination was TnI-Ultra, and all assays were conducted in accordance with the manufacturer's instructions. Platelet aggregation tests in vitro were analyzed before and one hour after tirofiban administration. Venous blood was collected in citrated tubes before and one hour after the introduction of tirofiban. Plasma samples were obtained after centrifugation and were analyzed as soon as possible. Platelet-rich plasma (PRP) was obtained by means of centrifugation at 250 g for 4 minutes from citrated blood. The time between sample collection and the tests did not exceed two hours. The platelet aggregation was performed by means of the optical method and consisted of treating 400 ul of PRP with 10 uM ADP, in the Chrono–Log 440 equipment (Havertown, PA, USA) in accordance with the Born method.[13] The mixture was stirred at 37 °C using Teflon-coated magnetic rods, and aggregation curves were recorded using an aggregometer. The analysis was performed in duplicate, and the aggregation rate was measured from the maximum variation of light transmittance from the system.

Platelet glycoprotein genotype determination

Samples for DNA extraction were collected in EDTA tubes and maintained at 4 °C until use. Genomic DNA was isolated from peripheral blood lymphocytes in accordance with the method of Miller et al.[14] Platelet membrane glycoprotein Ia, Ibα, IIb and IIIa genotypes were analyzed by means of amplification of DNA using the polymerase chain reaction (PCR), as described in previously published methods,[15-17] and using the oligonucleotide sequencing primers described in **Table 1**. The mutations analyzed were selected because these mutations present high frequency in our population.[11]

Genotyping of glycoprotein Ia (*807C/T*) polymorphism was performed by means of enzymatic digestion of a 115 bp fragment using Taq I at 37 °C, overnight, followed by identification of the fragments by means of electrophoresis on 2.5% agarose gel.[15] Presence of the 92 bp fragment indicates homozygosis of the wild genotype (C/C), while presence of the 115 bp fragment indicates homozygosis of the mutant allele.

Presence of the platelet glycoprotein Ibα (*Thr/Met*) genotype characterizes substitution of a cytosine by a thymine at position 1018 and results in amino acid dimorphism (*Thr/Met*) in the 145 position of GP Ibα. A 353 bp sequence of nucleotides[16] resulting from PCR was subjected to digestion using BsaHI at 37 °C overnight, followed by identification of fragments through electrophoresis.

The normal allele has a restriction site for BsaHI, thus resulting in two fragments of 242 and 111 bp. Presence of the mutant allele leads to loss of the restriction site (353 bp fragment).

Presence of the platelet glycoprotein IIb (*Ile/Ser*) genotype was reflected in Ile/Ser dimorphism,[16] which occurred at position 843 of GPIIb, thus resulting in substitution of thymine for guanine at nucleotide 2622 in exon 26. A 253 bp sequence of nucleotides was amplified using PCR and was digested with Fok I at 37 °C overnight; the fragments were identified by means of electrophoresis.

The normal allele had a restriction site for Fok I, thus resulting in two fragments of 127 and 111 bp. Presence of the mutant allele leads to loss of the restriction site and a fragment of 253 bp.

Presence of the platelet glycoprotein IIIa (*PIA*) genotype resulted from the C/T transition at position 1565 in exon 2 of GP IIIa.[17] The PCR reaction product, comprising a sequence of 266 bp of nucleotides was digested using MspI, at 37 °C overnight, followed by identification of fragments by means of electrophoresis. The normal allele had a restriction site for MspI, thus resulting in two fragments of 221 and 45 bp. The presence of polymorphism adds another restriction site to the 266 bp fragment, which then leads to presentation of three degradation products: 171, 50 and 45 bp.

Statistical analysis

The chi-square and Student t tests were used for baseline comparisons. Each GP mutation was divided into two genotype groups for bivariate analysis (homozygous wild versus heterozygous and homozygous mutants). The Spearman test was used for correlations between GP mutations and platelet inhibition data. Using in-hospital death as a dependent variable, we performed logistic regression that included independent variables with $P < 0.25$. The significance level used for the statistical tests was 5% ($P < 0.05$). Statistical analyses were performed using the SAS for Windows software (Statistical Analysis System), version 9.2 (SAS Institute Inc., 1989-1996; Cary, NC, USA).

RESULTS

The patients' mean age was 61.8 ± 11.7 years and 30 (60%) were men. Hypertension, dyslipidemia, previous coronary artery disease and diabetes were highly prevalent among the patients and occurred, respectively, in 47 (94%), 39 (78%), 27 (54%) and 25 (50%) of the patients. The prevalence of diabetes was 25%, and 24% were active smokers. Previous stroke was reported by 10% of the patients. In-hospital death occurred in the cases of seven patients (14%): six (12%) due to cardiogenic shock, and one (2%) due to septicemia. The distributions of these data according to the genotype group (homozygous wild versus heterozygous and homozygous mutant) of each GP mutation are shown in **Table 2**. The prevalences of hypertension, dyslipidemia, diabetes, smoking, previous coronary artery disease, previous stroke and all causes of death were similar between the groups. Platelet aggregations before tirofiban administration were also similar between the GPIa, Ib and IIb mutation groups, but not for the GPIIIa group. The GPIIIa mutation had lower baseline aggregation than the wild genotype (41.0% ± 22.1% versus 55.9% ± 20.8%; $P = 0.035$). A moderate correlation was observed between GPIIIa mutation and baseline platelet aggregation ($r = -0.31$; $P = 0.032$). After tirofiban administration, platelet GPIa, Ib, IIb and IIIa mutations did not alter inhibition of platelet aggregation or all causes of death (**Table 2**). Multivariate logistic regression did not reveal any GP mutation that was an independent variable for in-hospital death (**Table 3**).

DISCUSSION

The four polymorphisms of platelet glycoprotein analyzed in this study did not have any influence on the level of platelet inhibition in response to a standard dose of tirofiban, a specific inhibitor of GPIIbIIIa, in patients with acute coronary syndrome. The mutations analyzed were selected because these mutations present high frequency in our population.[11] The patients were treated in accordance with the guidelines of the American Heart Association for patients with unstable angina or NSTEMI.[5] Aspirin was administered at least three hours before introduction of tirofiban. Blood samples were obtained before and one hour after tirofiban administration, for analysis on platelet aggregation in response to tirofiban.

Table 1. Primer sequences and expected sizes of the products in base pairs (BP)

Oligonucleotides	Primer sequences	Size of product
Glycoprotein Ia[15]	sense: 5' GTGTTTAACTTGAACACATAT 3' antisense: 5' ACCTTGCATATTGAATTGCTT 3'	115 bp
Glycoprotein Ibα[16]	sense: 5' CCT TCA ACC GGC TGA CCT CGC TGC C 3' antisense: 5' TTC AGC ATT GTC CTG CAG CCA GC 3'	353 bp
Glycoprotein IIb[16]	sense: 5' CTC AAG GTA AGA GCT GGG TGG AAG AAA GAC 3' antisense: 5' CTC ACT ACG AGA ACG GGA TCC TGA AGC CTC 3'	253 bp
Glycoprotein IIIa[17]	sense: 5' TTC TGA TTG CTG GAC TTC TCT T 3' antisense: 5' TCT CTC CCC ATG GCA AAG AGT 3'	266 bp

Prior tirofiban administration

There was a lower degree of inhibition of platelet aggregation with aspirin among the patients with the glycoprotein IIIa PLA2 mutation, before administration of tirofiban. This mutation was most often associated with increased resistance to inhibition among the platelet polymorphisms analyzed in this study.[18] Szczeklik et al. assessed the association between genotypes with PlA2 mutation and platelet aggregation in response to aspirin, in 80 healthy subjects. Individuals carrying the mutation PlA2 were associated with greater resistance of platelet aggregation to aspirin.[19] Among 82 patients on aspirin and clopidogrel, Angiolillo et al. showed that there was higher platelet aggregation in response to the agonists collagen, epinephrine and ADP, in patients with the GPIa C807T mutation.[20] Furthermore, Gonzalez-Conejero et al. did not observe any association between the mutations GPIIbIIIa PlA2 and GPIaIIa C807T and the efficacy of aspirin for platelet inhibition.[21] The effectiveness of platelet inhibition was higher among subjects treated with higher doses of aspirin. These authors concluded that aspirin resistance was unlikely, but when it occurred, it would probably be dose-dependent and not influenced by mutations of GPIIbIIIa and GPIa/IIa.

The association between mutations of GPIIbIIIa and GPIa/IIa and higher resistance of platelet inhibition to aspirin and clopidogrel is very questionable. Recent systematic reviews and meta-analyses by Floyd et al. showed conflicting associations between the GPIIIa PlA2 allele and resistance to antiplatelets and to cardiovascular diseases.[22] In these studies, the authors found an association between PlA2 mutation and ischemic stroke; however, no association was observed between the mutation and resistance to platelet inhibition with aspirin and clopidogrel, either in healthy subjects or in patients with cardiovascular diseases.[23]

After tirofiban administration

Our study showed that mutations of the glycoproteins analyzed did not influence platelet aggregation one hour after administration of tirofiban. However, it can be argued that inhibition of platelet aggregation using tirofiban is less effective than that of other GPIIbIIIa inhibitors.[5,6,24] This lower efficacy could be related to the genetic variability of the glycoproteins involved in thrombus formation.

Nevertheless, the results from studies on the influence of genetic polymorphisms of platelet glycoproteins towards lower

Table 2. Demographic variables and laboratory data relating to 50 patients with unstable angina and non-ST-segment elevation myocardial infarction

Variables	All patients n = 50(%)	GPIa 1 n = 31 (62)	GPIa (2+3) n = 19 (38)	GPIb 1 n = 35 (70)	GPIb (2+3) n = 15 (30)	GPIIb 1 n = 15 (31)	GPIIb (2+3) n = 34 (69)	GPIIIa 1 n = 35 (70)	GPIIIa (2+3) n = 15 (30)
Age (years)	61.8 ± 11.7	60.5 ± 12.0	63.8 ± 11.2	61.7 ± 11.7	62.1 ± 11.9	59.2 ± 11.4	63.0 ± 11.9	61.9 ± 11.9	61.7 ± 11.6
Sex (M/F)	30 (60)/20 (40)	20 (64)/11 (36)	10 (53)/9 (47)	24 (69)/11 (31)	6 (40)/9 (60)	10 (67)/5 (33)	19 (56)/15 (44)	19 (54)/16 (46)	11 (73)/4 (27)
UA/AMI	17 (34)/33 (66)	10 (32)/21 (68)	7 (37)/12 (63)	13 (37)/22 (63)	4 (27)/11 (73)	6 (40)/9 (60)	10 (29)/24 (71)	13 (37)/22 (63)	4 (27)/11 (73)
BMI (kg/m^2)	28.6 ± 4.7	27.8 ± 5.2	29.7 ± 3.8	27.6 ± 4.7	30.5 ± 4.3	30.2 ± 5.6	28.2 ± 4.1	27.5 ± 4.6	31.8 ± 3.7
Previous CAD	27 (54)	15 (48)	12 (63)	21 (60)	6 (40)	10 (67)	16 (47)	19 (54)	8 (53)
Hypertension	47 (94)	29 (94)	18 (95)	32 (91)	15 (100)	14 (93)	32 (94)	35 (100)	12 (80)
Dyslipidemia	39 (78)	24 (77)	15 (79)	26 (74)	13 (87)	14 (93)	25 (73)	27 (77)	12 (80)
Diabetes	25 (50)	14 (45)	11 (58)	17 (49)	8 (53)	15 (67)	10 (44)	16 (46)	9 (60)
Smoking	12 (24)	9 (29)	3 (16)	8 (23)	4 (27)	3 (20)	8 (23)	7 (20)	5 (33)
Previous stroke	5 (10)	3 (10)	2 (10)	5 (14)	0 (0)	0 (0)	5 (15)	3 (9)	4 (13)
CK mass (ng/ml)	28.7 ± 70.8	35.4 ± 88.8	17.7 ± 16.9	32.3 ± 83.5	20.1 ± 21.9	44.2 ± 126.3	22.6 ± 22.3	17.9 ± 18.0	53.9 ± 125.6
Troponin (pg/ml)	12.6 ± 22.3	12.5 ± 23.5	12.6 ± 20.8	13.1 ± 23.8	11.4 ± 19.0	13.1 ± 26.3	12.7 ± 21.0	8.9 ± 14.4	21.1 ± 33.6

GP = glycoprotein; 1 = homozygous wild; 2 = heterozygous; 3 = homozygous mutant; UA = unstable angina; AMI = acute myocardial infarction; M = male; F = female; CAD = coronary artery disease; CK = creatine kinase. Value in parentheses are percentages.

Table 3. Aggregation data and incidence of death among 50 patients with unstable angina and non-ST-segment elevation myocardial infarction

Variables	All patients n =50 (%)	GPIa 1 n =31 (62)	GPIa (2+3) n = 19 (38)	GPIb 1 n = 35 (70)	GPIb (2+3) n = 15 (30)	GPIIb 1 n = 15 (31)	GPIIb (2+3) n = 34 (69)	GPIIIa 1 n = 35 (70)	GPIIIa (2+3) n = 15 (30)
Baseline aggregation (%)	51.9 ± 22.0	49.6 ± 23.8	55.7 ± 18.5	54.7 ± 22.0	45.1 ± 21.2	47.8 ± 23.5	52.7 ± 21.4	55.9 ± 20.8	41.0 ± 22.1*
One-hour aggregation (%)	15.3 ± 13.6	16.6 ± 15.7	13.1 ± 8.7	17.2 ± 14.7	10.3 ± 8.6	15.8 ± 10.6	14.3 ± 14.1	14.7 ± 10.1	16.8 ± 20.5
Baseline to one hour (%)	32.5 ± 23.6	33.1 ± 27.4	43.6 ± 18.8	37.6 ± 26.8	35.5 ± 19.9	32.1 ± 24.0	38.9 ± 25.6	41.7 ± 18.2	24.4 ± 35.1
In-hospital death	7 (14)	4 (13)	3 (16)	4 (11)	3 (20)	2 (13)	5 (15)	5 (14)	2 (13)

*P = 0.035; GP = glycoprotein; 1 = homozygous wild; 2 = heterozygous; 3 = homozygous mutant; one-hour aggregation and inhibition (%) refer to the percentage aggregation or inhibition one hour after tirofiban administration. Value in parentheses are percentages.

platelet response to GPIIbIIIa inhibitors have been contradictory. O'Connor et al. showed that there was higher incidence of major coronary events among patients with acute coronary syndrome with GPIIIa PlA2 mutation who were treated with orbofiban, an oral GPIIbIIIa antagonist, thus signaling that there might be an association between this mutation and coronary thrombosis.[25] Wheeler et al. showed that there was less platelet inhibition through using abciximab in patients with the PlA2 mutation who underwent percutaneous coronary intervention.[26] Weber et al. conducted an *in vitro* study on the antiplatelet effects of three GPIIbIIIa inhibitors (abciximab, tirofiban and eptifibatide) among healthy individuals and patients with stable coronary artery disease.[27] They confirmed that there was great variability of the inhibitory response to GPIIbIIIa inhibitors, but that this variability was not associated with GPIIbIIIa PlA2 mutation. Verdoia et al. analyzed the platelet response to GPIIbIIIa inhibitors among 80 patients undergoing percutaneous coronary revascularization (40 patients with abciximab; 40 patients with eptifibatide or tirofiban).[28] Aggregation tests were performed at baseline and after 10 min, 1 h and 4 h after GPIIbIIIa inhibitor administration. PlA2 mutation was present in 26 patients (32.5%). The clinical and angiographic features were similar between groups of carriers and noncarriers of the PLA2 mutation, except with regard to in-stent restenosis, which was more frequent among patients with PlA2. The PlA2 mutation did not affect the platelet response to GPIIbIIIa inhibitors.

Although the initial studies signaled that genetic polymorphisms of platelet glycoproteins had an influence on platelet response, this interaction was not observed in our study or proven by other more recent studies.[25-28] However, variability of platelet response to GPIIbIIIa inhibitors exists and can be correlated with other factors, such as drug dose-dependence, the method used to assess platelet aggregation, pharmacodynamics, concomitant use of dual antiplatelet aggregation, rheological characteristics of blood coagulation and other such things. Khaspekova et al. analyzed the platelet response among patients with acute coronary syndrome and found that the expression of platelet GPIb and GPIIbIIIa correlated with the average volume of platelets and not the genetic polymorphism of the GPIIIa Leu33Pro and GPIbα Thr145Met mutations.[29] Schneider et al. showed that the lesser degree of initial inhibition of platelet aggregation using tirofiban was dose-dependent compared to abciximab."[30] A recent meta-analysis showed that there was a reduction in major adverse coronary events through addition of tirofiban to aspirin and clopidogrel, among patients with ST-elevation myocardial infarction prior to percutaneous coronary intervention.[31] Blood rheological changes, such as blood viscosity, increased serum levels of fibrinogen, inflammatory cytokines and others, could explain the variability of platelet response. It is well known that these factors favor thrombotic process by acting at various levels of the coagulation system.[32]

CONCLUSION

In conclusion, this study showed that the GPIa, Ib, IIb and IIIa mutations analyzed did not influence platelet aggregation in response to tirofiban in patients with acute coronary syndrome. This result suggests that, despite the possibility that interindividual differences in platelet response to GPIIbIIIa inhibitors might have a genetic component, this probably depends on deeper interaction between genes and not just the interaction of a few single glycoprotein mutations. The small number of patients, homozygous and heterozygous mutant genotype groupings and influence of prior use of aspirin may be important limitations of this study. Similarly, it may not be possible to generalize our findings to other antiplatelet drugs.

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Address for correspondence:
Antonio de Padua Mansur
Av. Dr. Enéas de Carvalho Aguiar, 44
São Paulo (SP) — Brasil
CEP 05403-000
Tel. (+55 11) 2661-5387
Email: apmansur@yahoo.com

Association between variation in the genes DDAH1 and DDAH2 and hypertension among Uygur, Kazakh and Han ethnic groups in China

Associação entre variação de genes DDAH1 e DDAH2 com hipertensão nos grupos étnicos uygur, kazakh e han na China

Zhong Wang[I], Shaoze Chen[II], Lina Zhang[III], Guilin Lu[I], Chengming Zhou[III], Dao Wen Wang[III], Li Wang[I], Bayinbate Badengmu[IV], Zhihong Zhai[I], Lian Qin[I]

Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University, Shihezi, China

[I]MD. Head of Department, Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University, Shihezi, China.

[II]MD. Head of Department, Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University, Shihezi, China. Doctoral Student, Department of Internal Medicine and Genetic Diagnosis Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

[III]MD. Doctoral Student, Department of Internal Medicine and Genetic Diagnosis Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

[IV]MD. Head of Department, Department of Cardiology, The People 's Hospital of Boertala, Bole, China.

KEY WORDS:
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PALAVRAS-CHAVE:
Sistema cardiovascular.
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ABSTRACT

CONTEXT AND OBJECTIVE: Dimethylarginine dimethylaminohydrolase enzymes (DDAH), which are encoded by the genes DDAH1 and DDAH2, play a fundamental role in maintaining endothelial function. We conducted a case-control study on a Chinese population that included three ethnic groups (Han, Kazakh and Uygur), to systemically investigate associations between variations in the genes DDAH1 and DDAH2 and hypertension.

DESIGN AND SETTING: Experimental study at the Department of Internal Medicine and Genetic Diagnosis, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

METHODS: This case-control study included 1,224 patients with hypertension and 967 healthy unrelated individuals as controls. DDAH1 -396 4N (GCGT) del>ins, rs3087894, rs805304 and rs9267551 were genotyped using the TaqMan 5′ nuclease assay.

RESULTS: The G/C genotype of rs3087894 in DDAH1 was a risk factor for hypertension in the Kazakh group in the co-dominant model (G/C versus G/G) (OR 1.39; 95% CI: 1.02-1.88; P < 0.05), with the same result in the dominant model (G/C + C/C versus G/G) (OR 1.38; 95% CI: 1.03-1.84; P < 0.05). In contrast, the C/C genotype of rs3087894 seemed to be a protective factor against hypertension in the Uygur group in the recessive model (C/C versus G/G + G/C) (OR 0.62; 95% CI: 0.39- 0.97; P < 0.05). Similar findings for rs3087894 were also observed after adjusting the variable for the age covariate.

CONCLUSION: Our results indicated that the C-allele of rs3087894 in DDAH1 was a risk factor for hypertension in the Kazakh group but a protective factor in the Uygur group.

RESUMO

CONTEXTO E OBJETIVO: Enzimas dimetilarginina dimetilaminohidrolase (DDAH), codificadas por genes DDAH1 e DDAH2, desempenham papel fundamental na manutenção da função endotelial. Realizamos estudo tipo caso-controle na população chinesa, com três grupos étnicos (han, kazakh e uygur) para investigar sistematicamente a associação entre a variação de genes DDAH1 e DDAH2 e a hipertensão.

DESENHO E LOCAL: Estudo tipo caso-controle no Departamento de Medicina Interna e Diagnóstico Genético, Hospital de Tongji, Tongji Medical College, Universidade de Ciência e Tecnologia de Huazhong.

MÉTODOS: Este estudo incluiu 1.224 pacientes com hipertensão e 967 indivíduos saudáveis, sem parentesco, como controles. DDAH1 -396 4 N (GCGT) del > ins, rs3087894, rs805304 and rs9267551 foram genotipados usando o ensaio nuclease TaqMan 5′.

RESULTADOS: O genótipo G/C de rs3087894 no DDAH1 foi um fator de risco para a hipertensão arterial no grupo kazakh em modelo codominante (G/C *versus* G/G; OR 1,39; IC 95%: 1,02-1,88; P < 0,05), com o mesmo resultado no modelo dominante (G/C + C/C *versus* G/G; OR 1,38; IC 95%: 1,03-1,84; P < 0,05). Em contraste, o genótipo C/C de rs3087894 parecia ser um fator de proteção para a hipertensão no grupo uygur no modelo recessivo (C/C *versus* G/G + G/C; OR 0,62; IC 95%: 0,39-0,97; P < 0,05). Achado semelhante para rs3087894 também foi observado depois de se ajustar a variante à covariante idade.

CONCLUSÃO: Os nossos resultados indicaram que o C-alelo de rs3087894 no DDAH1 foi fator de risco para a hipertensão no grupo de kazakh, mas fator de proteção no grupo de uygur.

INTRODUCTION

Raised blood pressure (BP) or hypertension is a multifactorial disorder and a major risk factor for stroke and ischemic heart disease. The World Health Organization has estimated that hypertension affects over one billion people and that will rise to 1.5 billion by 2020, thus contributing 13.5 million deaths worldwide annually.[1-3] Hypertension is a complex trait influenced by genetic or environmental factors (including age, weight, ethnicity and diet), or their interactions.[4-8] Many studies have demonstrated that estimates of heritability of BP range from 31% to 68%,[9-11] and this has prompted extensive efforts to identify genes associated with hypertension. A legible genetic map of hypertension will highlight potential drug targets for its prevention or treatment and reduce the risk of cardiovascular events among people with hypertension.[12,13] A large number of candidate genes for hypertension have now been widely studied and, among these, the genes DDAH1 and DDAH2 seem to have an influence on hypertension, since they play a fundamental role in maintaining endothelial function.[14-16]

Dimethylarginine dimethylaminohydrolase enzymes (DDAH), which are encoded by the genes DDAH1 and DDAH2, clear asymmetric dimethylarginine (ADMA) from the kidneys and liver.[17] ADMA, as an endogenous inhibitor of nitric oxide (NO) synthase, reduces NO and thus regulates endothelial function. Based on detection of endothelial dysfunction at the origin of hypertension, ADMA has considerable clinical impact on hypertension. Studies have demonstrated that DDAH1 variants are associated with increased plasma ADMA and increased risk of thrombosis, stroke and coronary artery disease (CAD) in humans.[18,19] Similarly, Leiper et al. have shown that loss of DDAH1 activity leads to accumulation of ADMA and reduction in NO signaling, which in turn causes vascular pathophysiology, including endothelial dysfunction, increased systemic vascular resistance and elevated systemic and pulmonary blood pressure.[20]

OBJECTIVE

In view of the genetic heterogeneity among ethnic groups, in the present study we aimed to systemically evaluate associations between the DDAH1 and DDAH2 variants and hypertension in three ethnic groups within the Chinese population (Han, Kazakh and Uygur).

METHODS

Subjects

This case-control study included 1,224 patients with hypertension (660 males and 564 females; mean age 55.90 ± 11.23 years) who had histories of hypertension (systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg) or histories of use of anti-hypertension medication such as calcium channel blockers, beta-blockers, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors or diuretics. Patients with kidney disease (glomerular filtration rate, GFR, of less than 60 ml/minute) were excluded from the study. In addition, a group of 967 healthy unrelated individuals (504 males and 463 females; mean age 51.62 ± 13.50) without hypertension was studied as a control group. The control group was initially recruited in the same study and was matched to cases according to race, geographic location and date of data-gathering (within three months). Individuals from three ethnic groups (Han, Kazakh and Uygur) who came to Shihezi hospital or outpatient clinic, in Xinjiang province, China, between January 2013 and December 2013, were recruited. All participants signed an informed consent statement. This study was approved by the Medical Ethics Committee of the hospital and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

All participants underwent standard medical history-taking and physical evaluations. They answered a questionnaire that sought complete demographic information (name, age, sex, smoking, drinking, history of disease and so on). A complete general physical examination was carried out. BP was measured in a sitting position using a standard analogue sphygmomanometer. Plasma glucose, lipid and homocysteine levels were measured using standard methods, using an Olympus AU 2700 automatic biochemical analyzer (Olympus CO Ltd., Tokyo, Japan), after the patients had fasted for 12 h overnight.

DNA extraction and genotyping

Genomic DNA from whole blood containing EDTA was isolated using a DNA isolation kit (Tiangen Biotech, Beijing, China), in accordance with the protocol. DDAH1 -396 4N (GCGT) del>ins, c.17 G > C (rs3087894), DDAH2 c.531 A > C (rs805304) and c.662 G > C (rs9267551) were genotyped using the TaqMan 5' nuclease assay in the 7900HT fast real-time PCR system, using primers and probes synthesized by ABI (Applied Biosystems, Foster City, CA, USA), under standard conditions.[21] The sequences of probes and primers for DDAH1 -396 4N (GCGT) del>ins were as follows: forward primer, 5' CAGGTAAAGACCAGGAAGCCC 3'; reverse primer, 5' GGACCTCGGCGAAAAGC 3'; probe 1 del, 5' CGCAGGTGCACAC 3'; and probe 2 ins, 5' AGGTGCACGCACAC 3'. The product codes of the other TaqMan probes were, in turn, C_2518300_10, C_3233671_10 and C_27848488_10.

Statistical analysis

Continuous variables were shown as mean values, with their standard deviation and categorical variables expressed as absolute numbers with their prevalence. The Kolmogorov-Smirnov test was used to evaluate departure from normal distribution, and this was evaluated for all continuous variables. The independent-sample t test and Pearson's chi-square test were applied

in order to observe the differences between patient and control groups for continuous variables and categorical variables, respectively. The data relating to genotype frequencies of DDAH1 and DDAH2 variants in the patients and controls were obtained by means of direct counting, and significant differences among ethnic subgroups were assessed using Pearson's chi-square test or Fisher's exact test. The Hardy-Weinberg equilibrium was evaluated using Pearson's chi-square test in order to determine the variation in distribution of alleles and genotypes within the study population. Inheritance hypotheses were tested in accordance with three models: co-dominant, dominant and recessive. Binary logistic regression was built to determine associations between the DDAH1 and DDAH2 variants and hypertension.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), version 17.0. Double-sided P-values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

This case-control study recruited 1,224 patients with hypertension and 967 controls. Clinical information was collected at baseline from all subjects and was compared between patients and controls. The clinical characteristics and laboratory test data are shown in **Table 1**. No differences relating to sex, ethnicity, smoking or drinking were found between the patient and control groups (P > 0.05). The mean age of the patients was significantly higher than that of the controls (55.90 ± 11.23 versus 51.62 ± 13.50; P < 0.01). The histories of CAD, stroke and diabetes differed significantly between patients and controls (P < 0.05). In contrast with the controls, systolic BP, diastolic BP and body mass index were clearly higher among the patients. Moreover, the glucose, triglyceride, total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B and homocysteine levels were significantly higher among the patients (P < 0.05). There were no differences between the two groups regarding apolipoprotein A1 and high-density lipoprotein cholesterol.

Genotype distribution in the three ethnic groups

Before analyzing gene variation in relation to hypertension, it was noteworthy that the genetic background was markedly different between the ethnic groups. Based on the TaqMan results, we determined and recorded the genotype of each individual and calculated the genotype and allele frequencies of each variation (DDAH1 -396 4N de l > ins, c.17 G > C, DDAH2 c.531 A > C and c.662 G > C) manually for the Han, Kazakh and Uygur groups (**Table 2**). The genotype distribution of the four variations was in line with the Hardy-Weinberg equilibrium

Table 1. Baseline characteristics of patients and controls

Variables	Patients (n = 1224)	Controls (n = 967)	P-value
Female	46%	48%	0.355
Age	55.90 ± 11.23*	51.62 ± 13.50	0.000
Ethnic group			-
Han	31%	30%	-
Kazakh	35%	35%	-
Uygur	34%	35%	0.828
Smoking	18%	19%	0.528
Drinking	21%	23%	0.148
History of CAD	34%*	14%	0.000
History of stroke	4%†	2%	0.009
History of diabetes	15%*	5%	0.000
SBP (mmHg)	149.46 ± 18.97*	119.14 ±10.19	0.000
DBP (mmHg)	91.46 ± 12.41*	75.92 ± 6.82	0.000
BMI (kg/m^2)	26.62 ± 3.99*	25.55 ± 4.17	0.000
Glucose (mmol/l)	5.47 ± 1.95*	4.91 ± 1.30	0.000
Triglyceride (mmol/l)	1.50 ± 1.06*	1.30 ± 0.99	0.000
Total cholesterol (mmol/l)	4.92 ± 1.14*	4.60 ± 1.00	0.000
LDL-c (mmol/l)	2.84 ± 0.82*	2.70 ± 0.91	0.000
HDL-c (mmol/l)	1.46 ± 0.47	1.43 ± 0.46	0.154
Apolipoprotein A1 (g/l)	1.42 ± 0.29	1.39 ± 0.29	0.089
Apolipoprotein B (g/l)	0.96 ± 0.36*	0.89 ± 0.25	0.000
Homocysteine (μmol/l)	14.89 ± 7.34†	12.50 ± 7.37	0.037

*indicates P-value < 0.01; †indicates P-value < 0.05; CAD = coronary artery disease; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; LDL-c = low-density lipoprotein cholesterol; HDL-c = high-density lipoprotein cholesterol.

Table 2. Genotype distribution of DDAH1 and DDAH2 polymorphisms in Han, Kazakh and Uygur groups

Genotype	Han	Kazakh	Uygur	P-value
DDAH1 -396 4N (GCGT) del>ins				
del/del	512 (0.78)	491 (0.65)	511 (0.68)	
del/ins	135 (0.21)	245 (0.32)	209 (0.28)	
ins/ins	9 (0.01)	23 (0.03)	31(0.04)	0.000
MAF	0.12	0.19	0.18	
DDAH1 c.17 G>C (rs3087894)				
G/G	425 (0.65)	367 (0.49)	389 (0.52)	
G/C	195 (0.30)	309 (0.41)	281 (0.37)	
C/C	37 (0.06)	78 (0.10)	85 (0.11)	0.000
MAF	0.20	0.31	0.30	
DDAH2 c.531 A>C (rs805304)				
A/A	220 (0.33)	242 (0.32)	233 (0.31)	
A/C	334 (0.50)	360 (0.48)	400 (0.54)	
C/C	108 (0.16)	150 (0.20)	114 (0.15)	0.089
MAF	0.42	0.44	0.42	
DDAH2 c.662 G>C (rs9267551)				
G/G	629 (0.95)	678 (0.90)	701 (0.93)	
G/C	33 (0.05)	74 (0.10)	52 (0.07)	
C/C	0 (0.00)	3 (0.00)	0 (0.00)	0.001
MAF	0.03	0.05	0.03	

Data are shown as genotype number (proportion of genotype). P-values were obtained from comparisons between the three ethnic groups using Pearson's chi-square test. MAF = minor allele frequency.

(P > 0.05). The genotype distribution of all four variations except for DDAH2 c.531 A > C was significantly different in the three ethnic groups and the minor allele frequency (MAF) was lower in the Han group than in the Kazakh and Uygur groups.

Association between gene variation and hypertension

To evaluate associations between gene variation (DDAH1 -396 4N del > ins, c.17 G > C, DDAH2 c.531 A > C and c.662 G > C) and hypertension, we performed logistic regression analysis in accordance with three inheritance models: co-dominant, dominant and recessive. The results showed that there was no significant difference between hypertension patients and controls, irrespective of the ethnic factor.

Next, we assessed the effect of gene variation on hypertension in an ethnicity-specific case-control analysis. The G/C genotype of rs3087894 (c. 17 G > C) in DDAH1 was a risk factor for hypertension in the Kazakh group in the co-dominant model (G/C versus G/G) (OR = 1.39; 95% CI = 1.02-1.88; P < 0.05) and the same result was obtained in the dominant model (G/C + C/C versus G/G) (OR = 1.38; 95% CI = 1.03-1.84; P < 0.05). In contrast, the C/C genotype of rs3087894 seemed to be a protective factor against hypertension in the Uygur group in the recessive

Table 3. Odds ratios of DDAH variation in relation to hypertension in the Han, Kazakh and Uygur groups

Inheritance model	Han	Kazakh	Uygur	Total
Genotype	**n = 668**	**n = 760**	**n = 761**	**n = 2191**
DDAH1 -396 4N (GCGT) del>ins				
co-dominant				
del/del	1	1	1	1
del/ins	0.89 (0.61-1.30)	1.14 (0.83-1.55)	1.07 (0.77-1.47)	1.04 (0.86-1.26)
ins/ins	2.64 (0.54-12.82)	2.42 (0.94-6.23)	0.76 (0.37-1.56)	1.30 (0.78-2.19)
dominant				
del/del	1	1	1	1
del/ins + ins/ins	0.94 (0.65-1.37)	1.21 (0.89-1.63)	1.02 (0.75-1.39)	0.99 (0.83-1.19)
recessive				
del/del + del/ins	1	1	1	1
ins/ins	2.70 (0.56-13.12)	2.32 (0.90-5.94)	0.74 (0.36-1.52)	1.07 (0.86-1.35)
DDAH1 c.17 G>C (rs3087894)				
co-dominant				
G/G	1	1	1	1
G/C	0.96 (0.68-1.35)	**1.39 (1.02-1.88)**	1.09 (0.80-1.49)	1.14 (0.95-1.37)
C/C	1.01 (0.51-1.99)	1.35 (0.82-2.22)	0.64 (0.40-1.03)	0.92 (0.68-1.24)
dominant				
G/G	1	1	1	1
G/C + C/C	0.96 (0.70-1.33)	**1.38 (1.03-1.84)**	0.97 (0.73-1.29)	1.09 (0.92-1.29)
recessive				
G/G + G/C	1	1	1	1
C/C	1.03 (0.53-2.00)	1.17 (0.73-1.88)	**0.62 (0.39-0.97)**	0.87 (0.65-1.16)
DDAH2 c.531 A>C (rs805304)				
co-dominant				
A/A	1	1	1	1
A/C	1.13 (0.80-1.59)	0.79 (0.57-1.10)	1.04 (0.75-1.44)	0.97 (0.80-1.17)
C/C	1.17 (0.73-1.86)	0.91 (0.60-1.37)	1.13 (0.72-1.77)	1.05 (0.82-1.36)
dominant				
A/A	1	1	1	1
A/C + C/C	1.14 (0.82-1.58)	0.82 (0.60-1.12)	1.06 (0.77-1.44)	0.99 (0.83-1.19)
recessive				
A/A + A/C	1	1	1	1
C/C	1.09 (0.72-1.65)	1.04 (0.73-1.50)	1.10 (0.74-1.65)	1.07 (0.86-1.35)
DDAH2 c.662 G>C (rs9267551)				
dominant				
G/G	1	1	1	1
G/C + C/C	1.05 (0.52-2.14)	1.08 (0.67-1.73)	1.20 (0.68-2.13)	1.11 (0.80-1.53)

Data are shown as odds ratio (with 95% confidence intervals). Data in bold and italic are binary logistic results between case-control groups that showed statistically significant differences (P < 0.05).

model (C/C vs. G/G + G/C) (OR = 0.62; 95% CI = 0.39-0.97; P < 0.05), whereas there were no differences between patients and controls in the Han group (P > 0.05). No significant difference was observed in relating to the other three gene variation. The logistic regression and OR results, for possible associations between hypertension and gene variation are shown in **Table 3**. Similar findings for rs3087894 were also observed after adjusting the variable for the age covariate (**Table 4**).

DISCUSSION

To our knowledge, this was the first investigation of an association between variation in the genes DDAH1 and DDAH2 and hypertension in a multi-ethnic Chinese population. The main finding from this study was that it demonstrated that the C-allele of rs3087894 in DDAH1 is a risk factor for hypertension in the Kazakh group but a protective factor in the Uygur group. In addition, we did not find any genotype of DDAH1 and DDAH2 associated with hypertension in the Han group.

DDAH may play a crucial role in blood pressure regulation through clearance of ADMA, which inhibits NO synthase, thus having implications with regard to development of atherosclerosis.[22] Some studies, both on animals and on humans, have demonstrated that DDAH activity has a critical role in regulating NO synthesis *in vivo*. Hu et al. found that plasma and tissue ADMA levels in DDAH1 knockout mice were several times higher than in wild-type mice.[23] In human research, previous studies have shown that more than 70% of the clearance of ADMA was due to DDAH activity and that genetic variation in DDAH1 was related to ADMA levels.[24,25] These studies have shown that there could be functional variants in DDAH genes and that this is likely to affect the risk of vascular events.[18] Ding et al. found that DDAH1 polymorphism (-396 4N del>ins) in the promoter region was associated with increased risk of thrombosis, stroke and coronary heart disease (CHD) in Han groups in China.[19] Maas et al. found that the -1151 A/C and -449 G/C polymorphisms in the DDAH2 promoter region were associated with increased prevalence of hypertension.[26]

In the present study, we hypothesized that genetic variation in DDAH1 (-396 4N del > ins and c.17 G > C) and DDAH2 (c.531 A > C and c.662 G > C) would present an association with hypertension, and we tested this in three ethnic groups. Because of the different genetic backgrounds, the genotype distribution of all four variations except for DDAH2 c.531 A > C was significantly different between the three ethnic groups, and MAF was lower in the Han group than in the Kazakh and Uygur groups.

Our study highlighted conflicting results for rs3087894 in DDAH1 in the three ethnic groups. We found that the C-allele of rs3087894 was a risk factor for hypertension in the Kazakh group (G/C versus G/G: OR = 1.39; 95% CI = 1.02-1.88; G/C + C/C versus G/G: OR = 1.38; 95% CI = 1.03-1.84; P < 0.05) but a protective factor in the Uygur group (C/C versus G/G + G/C: OR = 0.62; 95% CI = 0.39 - 0.97; P < 0.05). This was not a significant factor in the Han group (P > 0.05).

The Uygur, Kazakh and Han groups are the three main ethnic groups living in the Xinjiang area, and they have total different genetic background, lifestyle and eating habits. Han individuals in this area have usually migrated from other provinces of China and have the typical way of life of the Han people. The Uygurs and Kazakhs are natives of Xinjiang. Uygurs are mainly engaged in farming and trade, but most Kazakhs still live a nomadic life. Moreover, Kazakhs have the highest incidence of hypertension among these three ethnic groups, while Uygurs are the contrary.

In our study, we found that the C-allele of rs3087894 in DDAH1 plays conflicting roles in these three ethnic groups. The mechanisms of this association need further investigation. The most possible explanation for the discrepancies lies within differences in linkage-disequilibrium structure between these ethnic backgrounds. Differences in gene-gene or gene-environment interactions in relation to the development of hypertension in these ethnic groups may also contribute towards the discrepancies. Lastly, the small sample size may also had had a certain degree of influence on the research results. Further research, specifically large-scale prospective cohort studies and further gene linkage-disequilibrium investigations, will be needed in order to confirm these findings.

Table 4. Association between rs3087894 in DDAH1 and hypertension in the Kazakh and Uygur groups after adjusting for the age covariate

Ethnic group	Inheritance model	Odds ratio	95% CI	P-value
Kazakh	G/C vs G/G	1.43	1.05-1.96	0.024
Kazakh	G/C + C/C vs G/G	1.39	1.04-1.87	0.027
Uygur	C/C vs G/G + G/C	0.61	0.38-0.95	0.031

CI = confidence interval

CONCLUSION

We identified that rs3087894 in DDAH1 was significantly associated with hypertension and showed conflicting results in different ethnic groups. This is therefore a candidate for further studies with the aim of helping to ascertain the mechanisms of hypertension in different populations.

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Address for correspondence:
Zhong Wang
Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University, Shihezi, Xinjiang 832008, China
Tel. +86 13899525766
E-mail: wangzshz@163.com

Visceral adiposity index and prognosis among patients with ischemic heart failure

Índice de adiposidade visceral e prognóstico em pacientes com insuficiência cardíaca isquêmica

Patrícia Vogel[I], Airton Stein[II], Aline Marcadenti[III]

Department of Cardiology, Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brazil

[I]BSc. Specialist in Clinical Nutrition, Institute of Education and Research, Hospital Moinhos de Vento (IEP/HMV), Porto Alegre, RS, Brazil.

[II]PhD. Professor, Department of Public Health, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil, Professor, Institute of Health Technology Assessment, Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brazil.

[III]PhD. Professor, Postgraduate Cardiology Program, Institute of Cardiology, Fundação Universitária de Cardiologia (IC/FUC), Porto Alegre, RS, Brazil, Professor, Department of Nutrition, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil.

KEY WORDS:
Anthropometry.
Body mass index.
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PALAVRAS-CHAVE:
Antropometria.
Índice de massa corporal.
Insuficiência cardíaca.
Mortalidade.
Obesidade abdominal.

ABSTRACT

CONTEXT AND OBJECTIVES: The obesity paradox has already been established in relation to heart failure, but it is not known which obesity indicator best reflects this phenomenon. The aim of this study was to evaluate the association between obesity indexes and mortality among patients with heart failure.

DESIGN AND SETTING: Cohort study conducted in the Department of Cardiology of Hospital Nossa Senhora da Conceição (Brazil).

METHODS: Clinical, demographic, socioeconomic, biochemical and anthropometric data on 116 patients aged 30 to 85 years with a diagnosis of heart failure were evaluated. Arm fat area, body mass index, body surface area, body adiposity index, lipid accumulation product (LAP) and visceral adiposity index (VAI) were calculated. Cox regression was used to perform survival analyses.

RESULTS: At baseline, the individuals with ischemic heart failure who remained alive showed higher VAI (3.60 ± 3.71 versus 1.48 ± 1.58; P = 0.04) and a trend towards higher LAP, in comparison with the individuals who died. After an average follow-up of 14.3 months, ischemic heart failure patients who had VAI > 1.21 showed 78% lower risk of death (HR 0.12; 95% CI: 0.02-0.67; P = 0.02) and the Kaplan-Meier survival curves showed better prognosis for these individuals (P = 0.005; log-rank test).

CONCLUSION: Our results suggest that VAI is a good predictor of better prognosis among ischemic heart failure patients.

RESUMO

CONTEXTO E OBJETIVOS: O paradoxo da obesidade já está consolidado na insuficiência cardíaca, mas não se sabe qual indicador de obesidade melhor reflete esse fenômeno. Este estudo teve como objetivo avaliar a associação entre índices de obesidade e a mortalidade entre pacientes com insuficiência cardíaca.

TIPO DE ESTUDO E LOCAL: Estudo de coorte realizado no Departamento de Cardiologia do Hospital Nossa Senhora da Conceição (Brasil).

MÉTODOS: Foram incluídos 116 pacientes com diagnóstico de insuficiência cardíaca de 30 a 85 anos. Dados clínicos, demográficos, socioeconômicos, bioquímicos e antropométricos foram avaliados. Área de gordura do braço, índice de massa corporal, área de superfície corporal, índice de adiposidade corporal, produto da acumulação lipídica e índice de adiposidade visceral foram calculados; regressão de Cox foi usada para realizar análises de sobrevida.

RESULTADOS: No início do estudo, indivíduos com insuficiência cardíaca isquêmica que se mantiveram vivos mostraram valores mais elevados de índice de adiposidade visceral (3,60 ± 3,71 contra 1,48 ± 1,58, P = 0,04) e uma tendência para produto da acumulação lipídica maior quando comparados com os indivíduos que morreram. Após acompanhamento médio de 14,3 meses, os pacientes com insuficiência cardíaca isquêmica que apresentaram índice de adiposidade visceral > 1,21 tiveram 78% menor risco de morrer (HR 0,12; IC 95% 0,02-0,67, P = 0,02) e as curvas de Kaplan-Meier para sobrevida mostraram melhor prognóstico nesses indivíduos (P = 0,005, teste *log-rank*).

CONCLUSÃO: Nossos resultados sugerem que o índice de adiposidade visceral é um bom preditor de melhor prognóstico em pacientes com insuficiência cardíaca isquêmica.

INTRODUCTION

Heart failure is a complex clinical conditional and it is considered to be an important public health problem. Despite significant therapeutic advances, heart failure is highly associated with morbidity and mortality in both developed and developing countries.[1]

Obesity is a well-known risk factor for cardiovascular diseases, including heart failure.[2-4] However, studies conducted among ischemic and non-ischemic heart failure patients have suggested that high adiposity indexes may be associated with better prognosis and longer survival than among patients with normal and lower weight.[5,6] This association is known as the "obesity paradox" or "reverse epidemiology".[7,8]

Most studies on the obesity paradox use body mass index to assess overweight and detect possible associations with the prognosis.[9,10] Although body mass index is the most common and practical method for classifying obesity, there has been some discussion about whether it is indeed a good marker for patients with chronic diseases.[11-13] Studies have been conducted with the aim of evaluating the prognostic value of other adiposity measurements such as waist circumference[14] and body composition assessed through cutaneous skinfolds,[15] dual-energy X-ray absorptiometry (DEXA) or bioelectrical impedance analysis[16,17] among subjects with heart failure.

The lipid accumulation product index[18] and the visceral adiposity index[11] have been proposed as indirect measurements of visceral adipose tissue and have been correlated with cardiometabolic risk, cardiovascular disease and mortality in the general population.[19,20] The body adiposity index is an alternative to the body mass index for determining the percentage of body fat,[21] and neck circumference has been characterized as a pathogenic fat depot correlated with visceral adipose tissue and worse metabolic profile.[22] However, none of these adiposity measurements has been tested among patients with ischemic or non-ischemic heart failure, within the context of the obesity paradox.

The purpose of this study was to evaluate associations shown by indexes of general and abdominal obesity in relation to mortality, among patients with ischemic and non-ischemic heart failure.

OBJECTIVE

To evaluate associations shown by indexes of general and abdominal obesity in relation to mortality, among patients with ischemic and non-ischemic heart failure.

METHODS

Setting and study design

A cohort study was conducted between July 2011 and January 2013 in the Department of Cardiology, Hospital Nossa Senhora da Conceição (Porto Alegre, Rio Grande do Sul, Brazil), which provides healthcare within the National Health System that is used mostly by patients belonging to lower social classes. The research protocol was approved by the Research Ethics Committee of the Conceição Hospital Group (CEP-GHC no. 10-118) and all the patients signed a consent statement in order to participate.

A sample of 116 patients aged 30 to 85 years who were admitted to the Cardiology Unit of Hospital Nossa Senhora da Conceição due to complications and symptoms relating to decompensated heart failure (for instance: dyspnea, fatigue or edema), of New York Heart Association classes I-IV, was consecutively selected. Patients with significant complications relating to heart failure (coronary artery disease, cerebrovascular disease or severe renal impairment) within the last 6 months, individuals with acute coronary syndrome within the last 90 days, patients with valvular heart disease, candidates for myocardial revascularization surgery, patients with a history of cancer over the last two years and patients who were not on a condition in which anthropometric data could be verified (those with amputation of lower limbs or sequelae of stroke) were excluded.

At baseline, demographic data (age, sex and self-reported skin color) and information regarding education (years at school) and lifestyle characteristics (smoking, abusive alcohol consumption [≥ 30 g for men and ≥ 15 g for women] and physical activity) were collected by trained personnel (physicians, medical students and nutritionists) using a standardized questionnaire. Clinical data were obtained directly from the medical records or after medical evaluation: angina (stable or unstable) was diagnosed by a trained cardiologist in accordance with current guidelines through reviewing the patient's electronic medical records;[23] atrial fibrillation was also defined by a trained cardiologist in accordance with the guidelines and after cardiac auscultation;[24] and blood pressure measurements were made using an aneroid sphygmomanometer with an adequately sized cuff around the arm circumference. Hypertension was defined as a diagnosis of systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mmHg, from a previous medical diagnosis or through use of antihypertensive pressure-lowering agents.[24] Fasting blood glucose ≥ 126 mg/dl or glycated hemoglobin ≥ 6.5% or a previous medical diagnosis were used to detect patients with type 2 diabetes mellitus.[25] Ejection fraction values, in percentages, were obtained from color Doppler-derived measurements on tissues, by means of transthoracic echocardiography, performed using the GE Vivid 3 system (General Electric, Norway).

Complete blood count, serum creatinine, HDL-cholesterol, serum triglycerides, glycated hemoglobin and fasting blood glucose were obtained from 10 ml venous blood samples from the patient, using standardized techniques at the hospital's certified laboratory. Hematological parameters were detected using a Sysmex XE-5000 analyzer (Sysmex, Kobe, Japan); serum creatinine values were obtained through the kinetic colorimetric Jaffé method; HDL-cholesterol and triglyceride levels were detected by means of the colorimetric enzymatic method; glycated

hemoglobin levels were examined through electrochemiluminescence; and fasting blood glucose values were obtained using the modified hexokinase enzymatic method.

After hospital discharge, the patients were contacted every six months by telephone to collect information about their vital status. All patients were contacted at least three times during the follow-up. In cases of death, this was confirmed from the medical records and/or the death certificate, which was brought to the researchers by a member of the family.

Anthropometric parameters

Weight, height, arm circumference, neck circumference, waist circumference, hip circumference and triceps skinfold thickness measurements were obtained. Weight (kg) was measured with the patients wearing light clothes and barefoot, standing on weighing scales with scale divisions of 100 g (Filizola model 31, São Paulo, Brazil), and height was obtained using a stadiometer with scale divisions of 0.1 cm (Tonelli model E120 A; IN Tonelli SA, Santa Catarina, Brazil). Circumferences were measured with a non-elastic measuring tape. Arm circumference was obtained at the midpoint between the acromion and the olecranon, with the arm extended down the side of the body and the palm of the hand facing the thigh. Waist circumference was measured at the largest part between the waist and the thigh, while the subjects were wearing thin clothes. the narrowest part between the hips and the ribs. The triceps skinfold thickness was measured using a plicometer at the midpoint between the acromion and the olecranon with the arm extended down the side of the body and the palm of the hand facing the thigh.[26] Neck circumference was obtained from the midpoint of the neck.[27]

Adiposity measurements were calculated as follows:

- Arm fat area (cm^2) = (arm circumference (cm)*[triceps skinfold thickness (mm)/10]/2) – (3.14*[triceps skinfold thickness (mm)/10]^2/4). The 90th percentile was taken to be the cutoff point for obesity.[28]
- Body mass index (kg/m^2) = weight (kg)/height^2 (m). The cut-off point for obesity was taken to be ≥ 30 kg/m^2.[29]
- Body surface area (m^2) = $(0.007184*(\text{height (cm)})^{0.725})*(\text{weight (kg)}^{0.425})$.[30]
- Body adiposity index (%) = (hip circumference (cm)/(height)$^{1.5}$) – 18.[21]
- Lipid accumulation product index (cm.mmol.l) = (waist circumference (cm) – 65)* triglycerides (mmol/l) for males; and lipid accumulation product index = (waist circumference (cm) – 58)* triglycerides (mmol/l) for females.[18]
- Visceral adiposity index = (waist circumference (cm)/(39.68 + (1.88*body mass index))*(triglycerides/1.03)*(1.31/HDL) for males; and visceral adiposity index = (waist circumference (cm)/(36.58 + (1.89*body mass index))* (triglycerides/0.81)*(1.52/HDL) for females.[11]

Statistical analysis

Analyses were performed in accordance with the etiology of heart failure (ischemic or non-ischemic), in order to explore the possible role of the etiology in relation to anthropometric indexes and mortality. Data were expressed as mean ± standard deviation or as frequencies (%). Student's t test (parametric variables), Wilcoxon and Mann-Whitney tests (nonparametric variables) and Fisher's exact test (categorical variables) were used for comparisons. We used the log-rank test, Kaplan-Meier curves and Cox regression model for survival analyses. All anthropometric data were categorized as percentiles, and the reference category was defined as ≥ 25th percentile. The lipid accumulation product index, visceral adiposity index and body adiposity index do not have specific cut-off points, but all the adiposity indexes were standardized at the 25th percentile in order to maintain comparability.

Analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.0 (SPSS, IL, USA). For each analysis, α-level = 0.05 was considered significant, and 95% confidence intervals were shown.

RESULTS

During the follow-up period (mean duration of 14.3 ± 10.2 months), the general mortality rate was 20.6%. It was 30% (six deaths) among the patients with ischemic heart failure and 18.8% (18 deaths) among those with non-ischemic heart failure. No statistically significant correlation was identified between the heart failure etiology and mortality rate (P = 0.3).

The patients' mean age was 61.8 ± 12.3 years; 62.1% were males; 71.6% were whites; the mean number of years of school attendance was 5.0 ± 3.4; 12.1% were current smokers; and 8.6% presented abusive consumption of alcohol. According to the body mass index, 41 (35.4%), 36 (31%) and 39 (33.6%) presented, respectively, normal weight, overweight and obesity. Regarding the New York Heart Association functional class, 88 (75.9%) were in classes III-IV. The mean ejection fraction was 40.6 ± 14.8%. The prevalences of hypertension and type 2 diabetes mellitus were 77.6% and 32.8% respectively.

Table 1 shows the patients' demographic and clinical characteristics, stratified according to heart failure etiology and survival. Among the patients with ischemic heart failure, there were no differences regarding age, ethnicity, New York Heart Association classification, ejection fraction, systolic and diastolic blood pressure, hemoglobin levels, diagnoses of hypertension, angina, atrial fibrillation or type 2 diabetes mellitus, or the number of drugs in use according to survival status (dead or alive). However, there was a trend for serum creatinine levels (P = 0.07). Among the individuals with non-ischemic heart failure, only hemoglobin levels were significantly lower in the group of patients who died (P = 0.01).

Regarding the main drugs used within the hospital setting, 101 patients (87.1%) were being treated with loop diuretics, 93 (80.2%) with beta blockers, 71 (61.2%) with platelet anticoagulant, 68 (58.6%) with angiotensin-converting-enzyme inhibitors, 55 (47.4%) with statins, 49 (42.2%) with potassium-sparing diuretics, 46 (39.7%) with digitalis, 39 (33.6%) with oral anticoagulants, 18 (15.5%) with angiotensin receptor blockers and 13 (11.2%) with calcium channel blockers.

The adiposity indexes according to heart failure etiology and survival status are described in **Table 2**. There were no significant differences in most of the anthropometric parameters, except for the visceral adiposity index and a trend for lipid accumulation product index values among individuals with ischemic heart failure, which were both higher among survivors than among patients who died (3.60 ± 3.71 versus 1.48 ± 1.58; P = 0.04 for visceral adiposity index; and 70.49 ± 50.73 versus 35.49 ± 32.62; P = 0.08 for lipid accumulation product index).

Survival analyses performed using the Cox regression model showed that higher values for the visceral adiposity index (> 1.21) were related to better prognosis (HR 0.12; 95% CI: 0.02-0.67;

Table 1. Demographic and clinical characteristics of the sample [mean ± SD or n (%)]

	Ischemic (n = 20)			Non-ischemic (n = 96)		
	Died (n = 6)	Survived (n = 14)	P	Died (n = 18)	Survived (n = 78)	P
Age (years)	71.7 ± 13.2	67.1 ± 10.9	0.43	57.2 ± 11.5	61.2 ± 12.1	0.20
Ethnicity						
White	5 (33.3)	10 (66.7)	0.57	14 (20.6)	54 (79.4)	0.47
Nonwhite	1 (20.0)	4 (80.0)		4 (14.3)	24 (85.7)	
New York Heart Association Functional Class						
I-II	2 (50.0)	2 (50.0)	0.55	4 (16.7)	20 (83.3)	0.76
III-IV	4 (25.0)	12 (75.0)		14 (19.4)	58 (80.6)	
Ejection fraction (%)	46.0 ± 15.3	40.8 ± 12.9	0.44	34.9 ± 13.9	41.7 ± 15.3	0.10
Systolic blood pressure (mmHg)	137.8 ± 23.4	123.3 ± 13.7	0.13	120.0 ± 19.0	120.5 ± 17.8	0.91
Diastolic blood pressure (mmHg)	85.0 ± 18.7	74.7 ± 11.1	0.17	74.1 ± 12.8	73.9 ± 10.3	0.94
Hemoglobin (mg/dl)	12.5 ± 1.5	12.6 ± 2.5	0.92	11.9 ± 2.3	13.3 ± 1.9	0.01
Creatinine (mg/dl)	1.15 ± 0.46	1.70 ± 0.62	0.07	1.41 ± 0.94	1.25 ± 0.43	0.28
Type 2 diabetes mellitus						
Yes	1 (10.0)	9 (90.0)	0.14	5 (17.9)	23 (82.1)	0.86
No	5 (50.0)	5 (50.0)		13 (19.4)	54 (80.6)	
Hypertension						
Yes	5 (27.8)	13 (72.2)	0.52	13 (18.1)	59 (81.9)	0.76
No	1 (50.0)	1 (50.0)		5 (21.7)	18 (78.3)	
Angina						
Yes	1 (33.3)	2 (66.7)	0.89	6 (20.0)	24 (80.0)	0.83
No	5 (29.4)	12 (70.6)		12 (18.2)	54 (81.8)	
Atrial fibrillation						
Yes	2 (66.7)	1 (33.3)	0.28	1 (6.2)	15 (93.8)	0.36
No	4 (23.5)	13 (76.5)		5 (83.3)	1 (16.7)	
Number of drugs in use	6.7 ± 1.9	6.3 ± 1.3	0.59	5.6 ± 1.9	5.6 ± 1.4	0.97

SD = standard deviation.

Table 2. Anthropometric characteristics of the sample (mean ± SD)

	Ischemic (n = 20)			Non-ischemic (n = 96)		
	Died (n = 6)	Survived (n = 14)	P	Died (n = 18)	Survived (n = 78)	P
Arm fat area (cm^2)	29.5 ± 14.7	29.1 ± 14.2	0.95*	28.6 ± 14.7	34.4 ± 25.4	0.74†
Neck circumference (cm)	36.7 ± 6.8	38.6 ± 2.2	0.34*	37.4 ± 3.7	38.1 ± 4.4	0.56*
Waist circumference (cm)	96.3 ± 13.2	97.1 ± 5.6	0.87*	98.8 ± 11.7	99.7 ± 17.3	0.83*
Body mass index (kg/m^2)	27.4 ± 4.9	26.8 ± 2.6	0.78*	27.9 ± 5.3	29.1 ± 7.4	0.50*
Body surface area (m^2)	1.79 ± 0.16	1.80 ± 0.14	0.96*	1.87 ± 0.21	1.89 ± 0.28	0.76*
Body adiposity index (%)	30.5 ± 8.5	29.1 ± 4.9	0.66*	30.4 ± 7.5	31.9 ± 7.9	0.46*
Lipid accumulation product index (LAP, cm.mmol.l)	35.49 ± 32.62	70.49 ± 50.73	0.08†	57.66 ± 71.64	58.22 ± 53.42	0.72†
Visceral adiposity index	1.48 ± 1.58	3.60 ± 3.71	0.04†	2.77 ± 2.63	2.69 ± 2.43	0.71†

SD = standard deviation. *Student's t test (parametric variables), taking P < 0.05 (5%) to be significant; †Wilcoxon and Mann-Whitney tests (nonparametric variables) for comparison, taking P < 0.05 (5%) to be significant.

P = 0.02) among patients with ischemic heart failure etiology. Kaplan-Meier survival curves plotted for patients with ischemic heart failure etiology showed that survival prognosis improved with visceral adiposity index values > 1.21 (P = 0.005 detected by means of the log-rank test) (**Figure 1**). Other anthropometric data did not show any relationship with prognosis in survival analyses.

DISCUSSION

To our knowledge, this is the first study on patients with heart failure to have evaluated non-traditional adiposity indexes as prognostic markers. Moreover, our study showed an inverse relationship between the visceral adiposity index as an abdominal obesity index and mortality, among patients with ischemic heart failure, and we were unable to find associations between general adiposity indexes (body adiposity index and body mass index) and survival in our population.

The obesity paradox has been widely studied, but controversy remains with regard to the best adiposity index for making the prognosis among individuals with ischemic and non-ischemic heart failure. A meta-analysis that evaluated nine studies and included 28,209 individuals with heart failure showed that overweight (body mass index 25-29.9 kg/m^2) and obesity (body mass index ≥ 30 kg/m^2) detected by means of body mass index cutoff points were associated with lower cardiovascular and all-causes mortality.[8] Among individuals with heart failure and type 2 diabetes mellitus, body mass index 25-30 kg/m^2 was also correlated with lower mortality.[31] On the other hand, obesity class III (body mass index ≥ 40 kg/m^2) was correlated with higher mortality rates.[32,33]

Although higher body mass index (≥ 25 kg/m^2) has been commonly correlated with a favorable prognosis, a meta-analysis including 12 studies and 6,142 individuals with acute decompensated heart failure showed that higher body mass index was associated with lower mortality, particularly among very elderly people (age > 75 years), individuals with reduced ejection fraction (< 50%), people without type 2 diabetes mellitus and cases of newly diagnosed heart failure. This suggested that aging, heart failure severity/chronicity and metabolism might explain the obesity paradox regarding use of the body mass index as an adiposity measure.[34] Moreover, among obese individuals with heart failure, symptoms may appear earlier, and therefore the diagnosis and treatment can take place at earlier stages of heart failure.

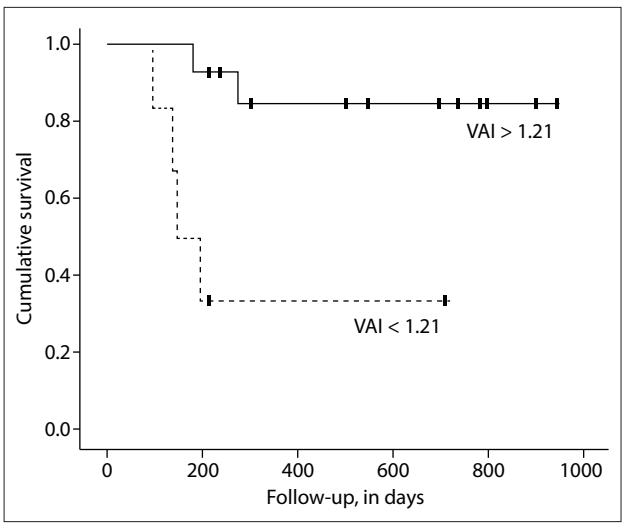

Figure 1. Kaplan-Meier survival curves plotted for patients with ischemic heart failure etiology (VAI: visceral adiposity index).

We were unable to find any association with body mass index and mortality according to heart failure etiology, and other authors have shown conflicting results. Among chronic heart failure patients, after a mean follow up of 30.7 months (annual event rate 6.0%), the ischemic heart failure overweight subgroup had a lower survival rate than the non-ischemic heart failure overweight subgroup; however, normal weight and obese individuals showed no difference regarding mortality and heart failure etiology.[5] Among individuals with chronic heart failure classes II and III, the obesity paradox was only observed in patients with non-ischemic heart failure after adjustment for age, sex, New York Heart Association functional class, ejection fraction, comorbidities and treatment.[6] These studies were conducted among individuals who were in a stable condition and receiving outpatient treatment, whereas our patients were evaluated in a hospital setting and had higher prevalence of New York Heart Association functional classes III and IV. Thus, it can be suggested that heart failure severity strongly contributes towards mortality independently of heart failure etiology and body mass index values.

Using body mass index as a body fat measurement may lead to misclassification of around 40% of patients with heart failure[35] and the usefulness of body mass index for evaluating body composition in these subjects has been questioned.[10,12] Body mass index does not detect the total amount of body fat[12,35,36] and its classification does not vary according to sex, age or ethnicity.[36] As well as in cases of heart failure, the obesity paradox has been shown in other cardiovascular disorders such as coronary artery disease.[37] Grade I obesity has not been associated with mortality among the general population.[38] However, body mass index has a good correlation with lean mass among individuals with coronary artery disease[13] and also in the general population.[12] Thus, the poor diagnostic performance of body mass index in discriminating fat and lean mass and also in assessing body fat content could explain the obesity paradox. Furthermore, the prognostic value of the body mass index in specific situations, such as athletes and patients with fluid accumulation, is also limited.[12]

The effects of body fat detected through other assessment measurements such as cutaneous skinfolds, bioelectrical impedance analysis and DEXA on the obesity paradox have also being

studied, given the limitations of the body mass index. Increased body fat detected through DEXA has been correlated with lower mortality among cases of heart failure.[35] However, these techniques are not always available and may be expensive, thus restricting their use in clinical practice. The body adiposity index is a simple alternative to the body mass index for detecting percentage body fat; however, we were unable to detect any association between higher body adiposity index and survival among cases of heart failure. It has been suggested that using the body adiposity index as an indicator of overall adiposity is likely to produce biased estimates of percentage body fat, in which the errors may vary according to sex and level of body fatness. Thus, estimates based on the body adiposity index may not be more accurate than those based on the body mass index.[39]

The prognostic value of waist circumference as an abdominal obesity index has also been studied among patients with heart failure. Interestingly, higher waist circumference values have been shown to have a protective role regarding mortality rates among patients with heart failure.[7,14] Increased waist circumference and normal body mass index may reflect lower levels of physical activity among healthy individuals, with consequently higher levels of fat mass and lower lean mass.[40] However, waist circumference has been correlated with both lean and fat mass detected by means of bioelectrical impedance analysis among individuals with heart failure, thus suggesting that both compartments are associated with a better prognosis among these patients.[17]

Waist circumference cannot distinguish subcutaneous adipose tissue from visceral adipose tissue, but it seems to be more strongly associated with subcutaneous fat, especially among overweight individuals.[41] Higher levels of visceral fat have been correlated with higher levels of inflammatory cytokines such as tumor necrosis factor-α, which have catabolic effects on lean mass and may contribute towards cardiac cachexia.[42,43] However, tumor necrosis factor-α receptors are highly expressed in subcutaneous adipose tissue,[44] and heart failure patients with an enlarged waist may be protected from the negative impact of increased levels of tumor necrosis factor-α through production of higher levels of these receptors, compared with patients with normal weight or who are underweight.[7] Since weight lost is associated with lower survival in situations of heart failure,[45] patients with greater severity of heart failure who also have excess body fat (including both the subcutaneous and the visceral compartment) may have greater metabolic reserves and be more resistant to the increased catabolic burden.[46]

Obese individuals and patients with ischemic heart disease have higher levels of lipoproteins such as cholesterol and chylomicron, and this contributes towards higher bacterial lipopolysaccharide levels, thereby stimulating release of pro-inflammatory cytokines.[47,48] Anthropometric and serum lipid values are needed in order to calculate both the lipid accumulation product index and visceral adiposity index. However, the formula for the visceral adiposity index includes two biomarkers that relate to lipoproteins (HDL-cholesterol and triglycerides) and two obesity indexes that are associated with lean mass and subcutaneous fat (waist circumference and body mass index), thus suggesting that instead of being a "visceral adipose function" as proposed originally,[11] the visceral adiposity index might reflect an "excess of weight function" among patients with heart failure.

This exploratory study may have been influenced by the small sample size, which will have conferred higher variability, and it may have lacked power to detect some associations, such as the lipid accumulation product index and death. Thus, some of our results may have been due to chance. Furthermore, we did not construct an adjusted multivariable model to detect independent associations between anthropometric indexes (especially the visceral adiposity index) and mortality. Our patients were relatively young and were predominantly in New York Heart Association classes III-IV, without a preserved ejection fraction, which may have limited the extrapolation of our results. On the other hand, the data collection was prospective, which considerably improves data quality. In addition, important prognostic factors were considered in our analysis.

CONCLUSION

We suggest that the visceral adiposity index may be a good predictor of mortality among patients with ischemic heart failure. However, further studies are needed in order to confirm these results.

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Address for correspondence:
Aline Marcadenti
Department of Nutrition
Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA)
Rua Sarmento Leite, 245
Porto Alegre (RS) — Brasil
CEP 90050-170
Tel. (+55 51) 3303-8830
Fax. (+55 51) 3303-8810
E-mail: marcadenti@yahoo.com.br

Are normal-weight adolescents satisfied with their weight?

Adolescentes eutróficos estão satisfeitos com o seu peso?

Mariana Contiero San Martini[I], Daniela de Assumpção[II], Marilisa Berti de Azevedo Barros[III], Ana Maria Canesqui[IV], Antonio de Azevedo Barros Filho[V]

Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil

[I]MSc. Researcher, Department of Pediatrics, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil.

[II]PhD. Postdoctoral Researcher, Universidade Federal de São Paulo (Unifesp), São Paulo, and Researcher, Department of Public Health, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil.

[III]PhD. Titular Professor, Department of Public Health, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil.

[IV]PhD. Collaborating Associate Professor, Department of Public Health, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil.

[V]PhD. Titular Professor, Department of Pediatrics, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
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Imagem corporal.
Inquéritos epidemiológicos.

ABSTRACT

CONTEXT AND OBJECTIVE: The high prevalence of obesity has led to public policies for combating it. People with normal weight may gain greater awareness of this issue and change their perceptions of their weight. The aim of this study was to evaluate the prevalence of body weight dissatisfaction among normal-weight adolescents, according to demographic and socioeconomic variables, health-related behavior and morbidities.

DESIGN AND SETTING: Population-based cross-sectional study that used data from a health survey conducted in the city of Campinas, São Paulo, in 2008-2009.

METHODS: The prevalence and prevalence ratios of weight dissatisfaction were estimated according to independent variables, by means of simple and multiple Poisson regression.

RESULTS: 573 normal-weight adolescents aged 10 to 19 years (mean age 14.7 years) were analyzed. The prevalence of weight dissatisfaction was 43.7% (95% confidence interval, CI: 37.8-49.8). Higher prevalences of weight dissatisfaction were observed among females, individuals aged 15 to 19 years, those whose households had eight or more domestic appliances, former smokers, individuals who reported alcohol intake and those who had one or more chronic diseases. Lower prevalence of dissatisfaction was observed among adolescents living in substandard housing. Among the normal-weight adolescents, 26.1% wished to lose weight and 17.6% wished to gain weight.

CONCLUSION: The results from this study indicate that even when weight is seen to be within the normal range, a high proportion of adolescents express dissatisfaction with their weight, especially females, older adolescents and those of higher socioeconomic level.

RESUMO

CONTEXTO E OBJETIVO: Com a alta prevalência da obesidade levando a políticas públicas para o seu enfrentamento, pessoas com o peso adequado podem se sensibilizar e alterar sua percepção sobre o seu peso. Objetivou-se avaliar a prevalência de insatisfação com o peso corporal em adolescentes eutróficos, segundo variáveis demográficas e socioeconômicas, comportamentos relacionados à saúde e morbidades.

TIPO DE ESTUDO E LOCAL: Estudo transversal de base populacional que utilizou dados do Inquérito de Saúde realizado em Campinas (SP), em 2008/2009.

MÉTODOS: Foram estimadas as prevalências e as razões de prevalência de insatisfação com o peso segundo as variáveis independentes, por meio de regressão simples e múltipla de Poisson.

RESULTADOS: Analisaram-se 573 adolescentes eutróficos de 10 a 19 anos, com idade média de 14,7 anos. A insatisfação com o peso atingiu prevalência de 43,7% (intervalo de confiança de 95%, IC: 37,8-49,8). Maiores prevalências de insatisfação com o peso foram verificadas no sexo feminino, nos adolescentes de 15 a 19 anos, que possuíam oito ou mais equipamentos na residência, nos ex-fumantes, nos que relataram ingerir bebida alcoólica e nos que apresentavam uma ou mais doenças crônicas. Menor prevalência de insatisfação foi observada nos adolescentes que residiam em moradias com condições inadequadas. Dos adolescentes eutróficos, 26,1% queriam perder peso e 17,6% desejavam ganhar.

CONCLUSÕES: Os resultados apontam que, mesmo apresentando o peso dentro da faixa de normalidade, elevada proporção de adolescentes manifesta-se insatisfeita com o seu peso, em especial os indivíduos de sexo feminino, os mais velhos e os de melhor nível socioeconômico.

INTRODUCTION

Obesity is a major public health problem that contributes towards the development of other morbidities, such as cardiovascular diseases, hypertension and diabetes mellitus, thus impairing quality of life and increasing the risk of mortality.[1-3] The increasing prevalence of overweight raises concern regarding healthy eating and regular physical activity,[4,5] within a context permeated by the demand for an ideal body and slimness, especially among young people.[6,7] Paradoxically, advertising broadcast by the mass media encourages intake of foods of high energy density and low nutrient density, such as cookies, chips/crisps and fast-food snacks.[8-10]

Adolescence is marked by deep biological, cognitive and psychosocial changes and the experiences at this stage of life, such as the beginning of sexual activity, possible introduction of risky health-related behavior, expansion of autonomy from the family[11,12] and greater exposure to the media[6] may influence self-assessment of body image, possibly causing weight dissatisfaction and harmful health-related behavior.[13]

The ideal body varies according to sex among young people. Boys tend to have less concern about appearance, but desire an athletic body,[14-16] while girls want a leaner body.[15-17] Girls more frequently engage in diets and exercise to lose weight, may be more influenced by advertisements for weight loss products and believe that slim people are more popular and attractive.[18] The female susceptibility to influences relating to physical appearance leads to higher risk of developing eating disorders.[18]

A study on adolescents between 14 and 19 years of age in the city of São Paulo found a distortion between the nutritional status and self-perceived body image, according to sex. Among normal-weight adolescents, 38.8% of the girls and 19.2% of the boys saw themselves as overweight, 47.6% of the girls saw themselves as obese and 26.3% of the boys saw themselves as having normal weight.[19]

Weight satisfaction is an essential factor for self-acceptance among young people, and when current weight is not compatible with the desired body, this can trigger inappropriate attitudes, thereby affecting these individuals' growth and development.

OBJECTIVE

Considering the increasing prevalence of obesity and its health implications, along with the fact that correct self-perception of nutritional status is essential for adopting appropriate health promotion practices, the objective of this study was to evaluate the prevalence of dissatisfaction with body weight among normal-weight adolescents living in the city of Campinas, São Paulo, Brazil, according to demographic and socioeconomic variables, health-related behavior and morbidities.

METHODS

This was a cross-sectional population-based prevalence study that used data from a health survey conducted in the city of Campinas (ISACamp, 2008) by the Collaborating Center on Health Situation Analysis of the Department of Public Health, School of Medical Sciences, State University of Campinas (Faculdade de Ciências Médicas da Universidade Estadual de Campinas, FCM/Unicamp). Data were gathered between the months of February 2008 and April 2009.

The survey sample was determined by means of probability sampling procedures in two cluster stages: census tracts and households. In the first stage, 50 census tracts were drawn with probabilities proportional to size (number of households). The census tracts of the Brazilian Institute for Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, IBGE) were used, based on the demographic census of 2000. Taking into account the time that had elapsed, the household data of the 50 census tracts selected were updated. In the second stage, households were drawn.

The survey population comprised adolescents of 10 to 19 years. The sample size was defined as 1,000 individuals for each age domain, which allowed for an estimated prevalence of 50%, with a 95% confidence level and a sampling error of between 4 and 5 percentage points, considering a design effect of 2.

By taking the response rate to be 80%, the sample size was set at 1,250. To achieve the desired sample size, 2,150 households were independently drawn for interviews with adolescents.

Information was gathered through a questionnaire structured into 14 thematic blocks. The questionnaire had been tested in pilot studies, and it was applied by trained interviewers overseen by the researchers. The thematic block relating to dietary habits contained questions on self-reported weight and height, weight satisfaction and practices used for weight loss, among others.

In the present study, teenagers aged 10 to 19 years, of both sexes, who were not institutionalized and were living in the urban area of the city of Campinas were studied. The dependent variable was dissatisfaction with body weight, which was evaluated according to responses to the question: "Would you like to gain or lose weight?" If the respondents answered yes, they were asked how much they would like to weigh. If their desire was to lose weight, they were asked whether they were doing anything to lose weight, and if so, what practices they were using for weight loss.

The independent variables analyzed in this study were as follows.

Demographic and socioeconomic factors: sex, age (in years), self-reported race/skin color, monthly per capita household income (expressed as multiples of the minimum monthly wage), number of appliances in the household, occupational activity, education level of household head (in years of schooling), possession of

health insurance, school attendance (and whether the school was public or private), and housing conditions, categorized as adequate or inadequate (substandard). Housing was considered adequate when houses or apartments had an internal water supply network connected to the public system, internal sanitary installation connected to the public sewer system and electric lighting. In the absence of one or more of these conditions, the housing was characterized as inadequate.

Health-related behavior: dentist appointment within the past year, smoking status, alcohol intake, length of time exposed to a computer (hours per day) and leisure physical activity. Individuals were classified as active, if they were doing at least 60 minutes of physical activity every day on at least five days a week (individuals aged 10 to 17 years) or at least 150 minutes a week, distributed across a minimum of three days (individuals aged 18-19 years). They were classified as insufficiently active if they did physical activity below the levels above; or as inactive if they did not do any kind of recreational physical activity.[20]

Morbidities: self-reported number of chronic diseases among those included in the survey checklist, such as hypertension, diabetes or asthma/bronchitis; and number of health complaints reported in a different checklist, such as headaches/migraines, allergies or emotional problems, among others.

Nutritional status was assessed from the body mass index (BMI) [weight (kg)/height (m^2)], which was calculated using the reported height and weight information. The adolescents' nutritional status was classified according to the BMI cutoff points for age that are recommended by the World Health Organization:[21] underweight BMI < 3rd percentile; normal weight BMI ≥ 3rd percentile and ≤ 85th percentile; overweight BMI > 85th percentile and ≤ 97th percentile; and obese BMI > 97th percentile.

The prevalence of body weight dissatisfaction among normal-weight individuals was estimated according to the independent variables. The association was verified using the χ^2 test, taking the significance level to be 5%. The prevalence rates and 95% confidence intervals were calculated through simple Poisson regression. The multiple model using multiple Poisson regression was developed in two stages. In the first stage, the demographic and socioeconomic variables that presented $P < 0.20$ in bivariate analysis were introduced, and variables with $P < 0.05$ were kept in the model. In the second stage, health-related behavior and morbidity variables were added to the model when any category presented $P < 0.20$ in bivariate analysis and were kept in the model if $P < 0.05$ in one of the categories.

The data were entered using Epidata 3.1 (Epidata Association, Odense, Denmark) and statistical analyses were conducted in the svy module of the Stata 11.0 software (Stata Corp., College Station, Texas, United States), which enables analysis on data from complex samples.

This study was approved by the Research Ethics Committee of the School of Medical Sciences, State University of Campinas, under Certificate of Presentation for Ethics Assessment no. 39756314.6.0000.5404.

RESULTS

In this study, only normal-weight adolescents were analyzed, totaling a sample of 573 individuals, with an average age of 14.7 years (95% confidence interval, CI: 14.5-14.9). Therefore, out of the sample of 822 individuals with BMI assessments, 249 were excluded because they were underweight, overweight or obese.

It was observed that 56.2% (95% CI: 50.2-62.1) of the adolescents with appropriate nutritional status were satisfied with their body weight. Among those who were dissatisfied with their weight, 17.6% reported a desire to gain weight, 18.0% wished to lose less than 10% of their weight and 8.1% wished to lose 10% or more. Among the boys, 65.5% said that they were satisfied with their weight, 19.8% wanted to gain weight, 9.9% wanted to lose < 10% and 4.8% wanted to lose ≥ 10%. Among the girls, 48.0% were satisfied with their weight, 15.6% wanted to gain weight, 25.3% wanted to lose < 10% and 11.1% wanted to lose ≥ 10% of their current weight.

In **Table 1**, higher prevalence of weight dissatisfaction can be observed among females, individuals between the ages of 15 to 19 years, those with eight or more appliances in the home and those who did not attend school. On the other hand, those who reported not working and those living in substandard housing showed significantly lower prevalence of weight dissatisfaction.

Regarding health-related behavior and morbidities (**Table 2**), greater prevalence of weight dissatisfaction was seen among the adolescents who were former smokers, those who drank alcohol, those who used computers for three or more hours/day, those who reported having one or more chronic diseases and those who reported having three or more health complaints.

The results from the multiple Poisson regression (**Table 3**) revealed that there was higher prevalence of weight dissatisfaction in the age group of 15 to 19 years, among females and among individuals who belonged to the category with the greatest number of appliances in the home. Living in substandard housing was associated with lower prevalence of weight dissatisfaction. Former smokers, individuals who drank alcoholic beverages and those with one or more chronic diseases were more dissatisfied with their current weight.

Table 4 presents the prevalence of weight satisfaction and the wish to gain or lose weight, among adolescents with healthy weight. The highest rates of desire to lose weight were seen among female adolescents, among females in the 15-19 year age group and among individuals living in homes with eight or more appliances. The prevalence of the desire to gain weight was greater

among males between 15 and 19 years of age and among individuals living in homes with eight or more appliances.

DISCUSSION

This study demonstrated that despite these adolescents' appropriate weight, they manifested high prevalence of weight dissatisfaction, especially females, individuals between the ages of 15 and 19 years, those of higher socioeconomic level (as assessed according to the number of household appliances and adequacy of housing conditions), former smokers, individuals who were consuming alcohol and those who presented at least one chronic disease. Among the normal-weight adolescents, 43.7% presented weight dissatisfaction. In an analysis on a sample of 594 adolescents between the ages of 15 to 20 years who were enrolled in public schools in Caruaru, Pernambuco, Brazil, Santos et al.[22] found that 55.1% of the adolescents within the normal weight range were dissatisfied with their weight.

Compared with the boys, the girls presented higher prevalence of weight dissatisfaction, thus corroborating the findings of other authors. In an evaluation on 17,817 Palestinians between the ages of 12 and 18 years, Al Sabbah et al.[23] observed that 16.0% of the boys and 24.0% of the girls presenting healthy nutritional status were dissatisfied with their weight. Using silhouette scales among 4,325 individuals between the ages of 14 and 15 years,

Table 1. Prevalence of body weight dissatisfaction among normal-weight adolescents aged 10 to 19 years, according to demographic and socioeconomic variables. Health Survey of Campinas (ISACamp, 2008/2009)

Variables	n	% (95% CI)	PR (95% CI)
Sex		P = 0.0001*	
Male	268	34.54 (27.90-41.84)	1
Female	305	52.03 (44.38-59.59)	**1.50 (1.23-1.84)**
Total	573	43.76 (37.89-49.81)	
Age (in years)		P = 0.0024*	
10 to 14	262	36.09 (28.72-44.18)	1
15 to 19	311	50.24 (43.46-57.02)	**1.39 (1.11-1.73)**
Self-reported race/skin color		P = 0.6594*	
White	375	44.04 (37.76-50.51)	1
Black	49	42.63 (29.43-56.97)	0.96 (0.69-1.34)
Brown	139	39.83 (31.09-49.28)	0.90 (0.72-1.13)
Per capita household income (monthly minimum wages)		P = 0.0935*	
< 0.5	180	35.29 (25.64-46.31)	1
≥ 0.5 to ≤ 1	175	44.63 (34.84-54.86)	1.26 (0.87-1.82)
> 1	218	49.88 (41.60-58.17)	1.41 (1.00-1.99)
Number of appliances in the home		P = 0.0008*	
0 to 7	112	21.53 (12.18-35.17)	1
8 to 15	275	46.94 (40.01-53.98)	**2.18 (1.21-3.91)**
16 or more	185	52.12 (43.44-60.67)	**2.42 (1.38-4.23)**
Occupational activity		P = 0.0104*	
Works	117	53.57 (44.76-62.16)	1
Does not work	451	41.29 (35.00-47.88)	**0.77 (0.63-0.93)**
Education level of household head (years of schooling)		P = 0.2634*	
0 to 3	68	31.47 (18.56-48.06)	0.73 (0.43-1.23)
4 to 7	158	46.80 (39.45-54.29)	1.09 (0.83-1.42)
8 to 11	198	46.33 (36.83-56.11)	1.08 (0.81-1.42)
12 or more	144	42.91 (33.80-52.52)	1
Health insurance		P = 0.0567*	
Yes	210	50.06 (41.28-58.84)	1
No	361	39.84 (33.20-46.87)	0.79 (0.63-1.00)
School attendance		P = 0.0387*	
Yes, public	360	38.81 (31.76-46.37)	1
Yes, private	111	51.59 (40.31-62.70)	1.32 (0.99-1.77)
No	101	51.44 (41.41-61.36)	**1.32 (1.04-1.67)**
Housing conditions		P = 0.0000*	
Adequate housing	537	45.86 (40.59-51.23)	1
Inadequate housing	36	11.42 (5.30-22.98)	**0.25 (0.12-0.50)**

n = number of individuals in the unweighted sample; 95% CI = 95% confidence interval; PR = prevalence ratio. *P-value from chi-square test.

Dumith et al.[24] found that 58.2% of the girls and 43.9% of the boys within the normal weight range presented body dissatisfaction. Among the students at a public school in the city of São Paulo, 43.6% of the girls and 19.2% of the boys with appropriate weight considered themselves to be overweight.[19]

A longitudinal study conducted in Juiz de Fora, Minas Gerais, among 358 students aged 11 to 14 years, showed that the prevalence of body dissatisfaction increased among girls as they grew up, while the opposite was observed among boys.[25] In an evaluation on 3,096 Irish students of healthy weight, Kelly et al.[26] found significantly greater prevalence of weight dissatisfaction among the oldest individuals, females and individuals who belonged to categories of higher socioeconomic level, as assessed through their parents' occupations.

The present study found higher prevalence of dissatisfaction with weight both among former smokers and among individuals who consumed alcohol. Similar results were found by Xie et al.[27] among females who smoked and drank five or more doses of alcohol on a single occasion. Among a sample of 4,746 adolescents in Minnesota, United States, Crow et al.[28] found that there was a significantly higher proportion of alcohol intake (42.5%) and tobacco use (38.9%) among girls who dieted to lose weight, compared with those who did not do any dieting. Okeke et al.[29] observed that adolescents who had never smoked had a more

Table 3. Poisson multiple regression model in two stages. Health Survey of Campinas (ISACamp, 2008/2009)

Variables	First stage PR* (95% CI)	Second stage PR† (95% CI)
Sex		
Male	1	1
Female	**1.51 (1.24-1.84)**	**1.57 (1.29-1.91)**
Age (in years)		
10 to 14	1	1
15 to 19	**1.40 (1.13-1.73)**	1.25 (0.98-1.60)
Number of appliances in the household		
0 to 7	1	1
8 to 15	**2.00 (1.20-3.30)**	**2.10 (1.25-3.52)**
16 or more	**2.16 (1.32-3.53)**	**2.30 (1.40-3.77)**
Housing conditions		
Adequate housing	1	1
Inadequate housing	**0.34 (0.14-0.79)**	**0.37 (0.16-0.88)**
Smoking		
Never smoked		1
Former smoker		**1.68 (1.22-2.31)**
Smoker		1.04 (0.64-1.70)
Alcohol intake		
No		1
Yes		**1.34 (1.04-1.72)**
Number of chronic diseases		
0		1
1 or more		**1.32 (1.07-1.63)**

95% CI = 95% confidence interval. *Prevalence ratio adjusted for demographic and socioeconomic variables; †Prevalence ratio adjusted for all variables in the table.

Table 2. Prevalence of body weight dissatisfaction among normal-weight adolescents aged 10 to 19 years, according to health-related behavior and morbidity variables. Health Survey of Campinas (ISACamp, 2008/2009)

Variables	n	% (95% CI)	PR (95% CI)
Dentist appointment within the last year		P = 0.0468*	
Yes	349	48.01 (41.19-54.89)	1
No	223	37.19 (28.84-46.38)	0.77 (0.59-1.00)
Smoking		P = 0.0170*	
Never smoked	544	42.64 (36.72-48.79)	1
Former smoker	12	82.79 (51.73-95.58)	**1.94 (1.44-2.61)**
Smoker	17	51.57 (29.10-73.42)	1.20 (0.75-1.92)
Alcohol intake		P = 0.0038*	
No	464	40.03 (33.81-46.60)	1
Yes	106	59.39 (48.14-69.74)	**1.48 (1.16-1.89)**
Leisure physical activity		P = 0.1109*	
Active	113	47.28 (38.59-56.14)	1
Insufficiently active	269	38.87 (30.45-48.01)	1.02 (0.79-1.33)
Inactive	191	48.61 (40.88-56.41)	0.82 (0.63-1.06)
Computer usage (hours/day)		P = 0.0354*	
0 to 1	376	41.35 (34.51-48.54)	1
2	71	36.30 (24.95-49.41)	0.87 (0.60-1.28)
3 or more	123	55.44 (44.62-65.76)	**1.34 (1.03-1.72)**
Number of chronic diseases		P = 0.0019*	
0	461	39.86 (33.70-46.36)	1
1 or more	111	59.50 (48.26-69.82)	**1.49 (1.18-1.87)**
Number of health complaints		P = 0.0097*	
0	198	38.76 (30.94-47.20)	1
1	196	43.79 (34.22-53.86)	1.12 (0.85-1.49)
2	115	40.15 (30.12-51.08)	1.03 (0.76-1.39)
3 or more	64	65.35 (52.73-76.12)	**1.68 (1.28-2.21)**

n = number of individuals in the unweighted sample; 95% CI = 95% confidence interval; PR = prevalence ratio. *P-value from chi-square test.

Table 4. Distribution of desire to change weight among normal-weight adolescents, according to sociodemographic strata

Strata	n	Wishes to lose weight (95% CI)	Does not wish to change (95% CI)	Wishes to gain weight (95% CI)	P-value
Sex					
Male	268	14.70 (10.51-20.18)	65.46 (58.16-72.10)	19.84 (15.02-25.73)	
Female	305	36.42 (29.79-43.62)	47.97 (40.41-55.62)	15.61 (11.70-20.52)	0.0000*
Total	573	26.15 (21.97-30.82)	56.24 (50.19-62.11)	17.61 (13.96-21.97)	
Female					
10 to 14 years	140	29.10 (20.20-39.97)	56.09 (45.16-66.46)	14.81 (9.22-22.93)	0.0553*
15 to 19 years	165	42.69 (34.61-51.17)	41.02 (31.90-50.81)	16.29 (11.66-22.30)	
Male					
10 to 14 years	122	16.58 (10.47-25.24)	72.76 (62.92-80.79)	10.66 (6.26-17.55)	0.0018*
15 to 19 years	146	13.14 (8.38-19.98)	59.38 (50.68-67.53)	27.48 (20.94-35.16)	
Female					
0 to 7 home appliances	60	16.80 (8.43-30.69)	73.02 (52.73-86.78)	10.18 (2.76-31.15)	
8 to 15 home appliances	150	36.82 (28.49-46.01)	44.65 (35.51-54.18)	18.53 (13.06-25.61)	0.0175*
16 or more home appliances	95	47.54 (36.06-59.28)	38.02 (25.60-52.24)	14.44 (8.96-22.44)	
Male					
0 to 7 home appliances	52	5.74 (1.60-18.58)	84.70 (71.49-92.43)	9.56 (4.24-20.13)	
8 to 15 home appliances	125	13.97 (9.14-20.76)	63.02 (54.17-71.07)	23.01 (16.88-30.55)	0.0207*
16 or more home appliances	90	20.60 (13.73-29.74)	57.99 (47.77-67.56)	21.41 (12.44-34.31)	

95% CI = 95% confidence interval; n: number of subjects in unweighted sample. *P-value from chi-square test.

positive perception of body image. They concluded that many individuals believed that cigarettes acted as a means of weight control[29] or weight loss and that they regulated emotions, whereas in reality body dissatisfaction had the power to trigger the start of smoking.[30]

Individuals who reported the presence of one or more chronic diseases had greater prevalence of weight dissatisfaction. A study showed that young people with chronic diseases reported greater body dissatisfaction and engagement in inappropriate weight loss practices.[31] Another study conducted among 9,584 adults over the age of 20 years found that people who were dissatisfied with their weight, independent of BMI, presented higher risk of developing type 2 diabetes.[32] The finding of greater dissatisfaction among adolescents with normal weight and chronic disease, requires further studies in order to understand this association.

Exposure to the media can contribute towards body dissatisfaction among adolescents between 14 and 16 years of age.[33] Hargreaves and Tiggemann[14] suggested that male adolescents do not express their body dissatisfaction because they believe that this is a feminine topic. On the other hand, among young women, the type of media exposure (television and magazines) can influence body dissatisfaction differently.[34]

Another communication medium that deserves attention is the internet, which is used about four times more than magazines. The internet is the only means of providing instant access to diversified content and images, while magazines depict specific and limited subjects. In the United States, similar associations regarding body dissatisfaction were found among undergraduate women exposed to television and the internet.[35] Tiggemann and Slater[36] found that use of social media, like Facebook, caused greater desire to lose weight, greater attention towards the body and greater internalization of the notion of slimness among female users of social media than among nonusers.

It has been highlighted that there is a tendency in contemporary society to value a beauty paradigm characterized by slim women and muscular men. The idea that this physical appearance is considered ideal and should be sought at any cost has been disseminated. Nonetheless, this may cause harm to adolescents' health and contribute towards development of eating disorders.[19,28,37]

Brazilian studies that have taken a qualitative approach have suggested that the body can be treated as capital (physical, symbolic, economic or social). It is also an important means of access to the labor market, sexual activity, marriage and social ascension towards prestige positions, success and money. Contemporary culture recommends that the body should always be displayed as young, sexy and in good shape.[38,39] According to Goldenberg,[38,39] clothing is an instrument to promote and expose the body, which is displayed, shaped, produced and worked. In order to achieve the desired physical form, discipline, dedication and great investments are necessary, which can result in significant dissatisfaction with one's appearance.[39] Another qualitative study, carried out in Santa Catarina, pointed out that the female body should display beauty, slimness, power of attraction and seduction towards the opposite sex, while the male body should be endowed with physical strength, power and virility.[40]

Among the limitations of the present study, it is important to highlight that self-reported information was used to ascertain adolescent weight and height. This is an especially important point at this stage of life, at which there are great changes to measurements caused by rapid growth and physical development. However, several studies have indicated that there is good agreement between reported and assessed height and weight measurements among adolescents, and have therefore considered that it is valid to use this information in epidemiological studies.[41-43]

It is important to mention that very few studies have evaluated the factors associated with body weight dissatisfaction, especially among normal-weight individuals, even though body image and body dissatisfaction are topics greatly explored in the literature.

The results from this study draw attention to the high percentage of adolescents who do not identify their nutritional status correctly. Healthy eating and health promotion programs, along with the fight against obesity, should take this point into account.

CONCLUSIONS

In this study, 43.8% of normal-weight adolescents were dissatisfied with their body weight. Greater prevalence of body weight dissatisfaction was found among girls, individuals aged 15 to 19 years, those at a higher socioeconomic level, former smokers, individuals who drank alcohol and those who reported having a chronic disease.

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Address for correspondence:
Mariana Contiero San Martini
Departamento de Pediatria/Centro de Investigação em Pediatria (CIPED)/UNICAMP
Rua Tessália Vieira de Camargo, 126
Cidade Universitária Zeferino Vaz
Campinas (SP) — Brasil
CEP 13083-887
Tel. (+55 19) 3241-5886
Cel. (+55 19) 99325-6784
E-mail: mari_martini08@yahoo.com.br

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Disability due to maternal common mental disorders (CMDs) as a risk factor for chronic childhood malnutrition: cross-sectional study

Incapacidade decorrente de transtornos mentais comuns (TMC) maternos como fator de risco para desnutrição crônica infantil: estudo transversal

Jorge Lopes Cavalcante-Neto[I], Cristiane Silvestre de Paula[II], Telma Maria de Menezes Toledo Florêncio[III], Claudio Torres de Miranda[IV]

Universidade Federal de Alagoas (UFAL), Maceió, AL, Brazil

[I]MSc. Assistant Professor, Universidade do Estado da Bahia (UNEB), Salvador, BA, Brazil.
[II]PhD. Adjunct Professor, Postgraduate Program on Developmental Disorders, Universidade Presbiteriana Mackenzie, São Paulo, SP, Brazil.
[III]PhD. Associate Professor II, School of Nutrition, Universidade Federal de Alagoas (UFAL), Maceió, AL, Brazil.
[IV]MD, PhD. Associate Professor I, School of Medicine, Universidade Federal de Alagoas (UFAL), Maceió, AL, Brazil.

KEY WORDS:
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Serviços de saúde materna.
Pobreza.

ABSTRACT

CONTEXT AND OBJECTIVE: The disability associated with maternal common mental disorders (CMDs) is among the possible explanations for the association between chronic childhood malnutrition and CMDs. CMDs may impair the mother's ability to perform her role, particularly in deprived environments. The present study aimed to evaluate whether disability relating to CMDs could be part of the pathway of the association between childhood malnutrition and maternal CMDs.

DESIGN AND SETTING: Cross-sectional study conducted in two institutions: one for malnourished children and another for eutrophic children living in a low-income community in the state of Alagoas, Brazil.

METHOD: The cases consisted of 55 malnourished children aged from 12 to 60 months who were attending a nutritional rehabilitation center, with height-for-age z-scores < 2. The controls were 70 eutrophic children of the same age who were attending a day care center in the same area as the cases. The Self-Report Questionnaire made it possible to identify likely cases of maternal CMD. The Sheehan Disability Scale enabled evaluation of the associated disability.

RESULTS: Chronic childhood malnutrition was significantly associated with maternal disability relating to CMDs (OR = 2.28; 95% CI: 1.02-5.1). The best logistic regression model using chronic childhood malnutrition as the dependent variable included the following independent variables: higher number of people living in the household; absence of the biological father from the household; and maternal disability relating to CMDs.

CONCLUSIONS: If confirmed, the association between chronic childhood malnutrition and maternal disability relating to CMDs may be useful in helping to identify the causal chain between childhood malnutrition and maternal CMDs and to indicate environmental risk factors associated with chronic childhood malnutrition.

RESUMO

CONTEXTO E OBJETIVO: A incapacidade associada aos transtornos mentais comuns (TMCs) maternos está entre as explicações possíveis para a associação entre a desnutrição infantil crônica e os TMCs. Os TMCs podem comprometer a capacidade materna de desempenhar seu papel, especialmente em ambientes precários. O presente estudo objetivou avaliar se a incapacidade relacionada com TMCs pode fazer parte do processo de associação entre a desnutrição infantil e TMCs maternos.

DESENHO E LOCALIZAÇÃO: Estudo transversal realizado em duas instituições, uma para crianças desnutridas e outra para crianças eutróficas vivendo em uma comunidade de baixa renda no estado de Alagoas, Brasil.

METODOLOGIA: Casos foram 55 crianças desnutridas de 12 a 60 meses atendidas num centro de recuperação nutricional com escore z da idade para altura < 2. Controles foram 70 crianças eutróficas da mesma idade que frequentavam uma creche na mesma área do que os casos. O "Self-Report Questionnaire" permitiu identificar casos prováveis de TMCs maternos; a "Sheehan Disability Scale" possibilitou a avaliação de incapacidade associada.

RESULTADOS: A desnutrição crônica infantil e a incapacidade maternal relacionada ao TMCs mostraram-se associados (OR = 2.28, IC 95% 1.02-5.1). O melhor modelo de regressão logística utilizando desnutrição crônica infantil como variável dependente incluiu um maior número de residentes na casa, ausência do pai biológico na residência e incapacidade materna relacionada ao TMCs como variáveis independentes.

CONCLUSÕES: Se confirmada, a associação entre desnutrição crônica infantil e incapacidade materna relacionada a TMCs pode ser útil para ajudar a identificar a cadeia causal entre a desnutrição infantil e os TMCs maternos e indicar fatores de risco ambientais associados com a desnutrição crônica infantil.

INTRODUCTION

Prospective studies have investigated the direction of associations of maternal common mental disorders (CMDs) and depression with chronic childhood malnutrition. In different cultures, these studies have shown that such disorders tend to be a risk factor for chronic childhood malnutrition.[1-3]

In 2011, Surkan et al.[1] conducted a meta-analysis to investigate the association of childhood malnutrition with maternal CMDs and maternal depression worldwide. They analyzed 17 studies that included case-control, cross-sectional and cohort designs. The results showed that maternal CMDs including depression were associated with early childhood underweight and stunting.[4,5]

These studies reported that the association remained positive after adjustment for several possible confounders such as paternal and maternal education, maternal age, birth weight, infant physical health, breastfeeding practices, number of children and socioeconomic status.[1,2,6-8] On the basis of the results from the meta-analysis, it was unlikely that the results were a chance finding. The adjustment for confounding factors diminished the importance of these factors, because of other explanations. The consistency of the prospective studies suggested that a temporal relationship existed, and perhaps a relationship that was less likely to be subject to information bias. The evidence for a positive association was strong.[1,2]

According to the World Health Organization (WHO) Report on Disability, the concept of disability (i.e. limitation of opportunities to take part in society on an equal level with other individuals because of social and environmental barriers) includes the impairment (loss or difference of physiological or psychological function) that may lead to this disability.[9] Therefore, by using the concept of "disability relating to CMDs" instead of CMDs alone, the possibility of including variables relating to the environment (such as socioeconomic and cultural variables), as risk factors, can be improved. These variables may constitute an additional resource for understanding and managing the specific consequences of such mental health problems that may or may not be associated with disabilities in different environments. Furthermore, the results from a study conducted in 15 countries showed that impairments due to psychiatric illnesses may directly affect social disability (e.g. occupational role functioning, social contacts, parenting and partner role), and that the disability originating from mental disorders has a greater impact than the disability stemming from physical disorders.[10]

The concept of psychosocial care includes talking to the child, telling stories, having frequent physical contact with the child and providing a safe environment that exerts a protective effect on the child. Lack of such care may affect the child's nutritional status negatively.[8] The quality of mother/child interaction may indicate the quality of psychosocial care. Miranda et al. found a positive association between low interaction of the mother with the malnourished child and maternal CMDs.[11]

OBJECTIVE

Starting from the hypothesis that maternal CMDs may be a risk factor for childhood malnutrition, the present study aimed to evaluate whether disability relating to CMDs could be part of the pathway of the association between childhood malnutrition and maternal CMDs.

METHODS

Design and subjects

This cross-sectional study involved a sample coming from two institutions located in the same low-income neighborhood in the city of Maceió, Brazil. The sample consisted of mother-child dyads, in which the child was between 12 and 60 months of age. Dyads were selected until the calculated minimum sample size was reached. One of the institutions was a nutritional rehabilitation center. Fifty-five children attending this institution had height-for-age z-scores ≤ -2 standard deviations (SDs), measured according to the WHO guidelines.[12] The second institution was a day care center located in the same low-income neighborhood as the rehabilitation center. Seventy mother-eutrophic child dyads belonging to the latter institution were included in this study. These children were also aged between 12 and 60 months. Data collection took place from October 2009 to April 2010.

Before administering any data-gathering instruments, the interviewers obtained written informed consent from the mothers. The Research Ethics Committee of the Federal University of Alagoas approved this study under procedural number 012090/2009-79.

Measurements

Nutritional status

Nutritional status was assessed with the aid of height-for-age (H/A) z-scores. H/A z-scores ≤ -2 SDs were taken in accordance with the WHO reference standard. For this analysis, the Anthro 2007 software was used for children aged up to five years and the Plus Anthro software for children older than five years.[12]

Psychiatric assessment

To identify mothers with probable CMDs, the Self-Report Questionnaire (SRQ-20) was used. This consists of 20 closed questions with two alternatives for the answers (yes/no). The results from a Brazilian validation study that obtained a cutoff ≥ 8 positive responses was used to identify probable cases of CMDs (sensitivity = 83% and specificity = 80%).[13]

Disability relating to CMDs

Mothers with an SRQ-20 score of 8 or higher were evaluated for disability by means of the Sheehan Disability Scale (SDS). SDS is

a scale used not only in psychiatry but also in other chronic medical conditions. This scale has been translated into 21 languages, including Portuguese. SDS assesses three areas: occupational, social and family life. Each area is given a score from 0 to 10.[14] In this study, SDS was considered positive whenever any kind of disability was identified. Any score higher than 0 was considered positive. On the basis of the meta-analysis study described earlier,[5] disability was assumed to be associated with maternal CMDs and depression.

Demographic status

The following variables were examined: demographic data, socioeconomic data, mother's age, child's age, maternal education, family income, number of children, number of people living in the household, work activity of both parents and presence of the child's biological father in the household.

Social class

Social class was defined in accordance with the five classes proposed by the Brazilian Association of Polling Companies (Associação Brasileira de Empresas de Pesquisa, ABEP). It was dichotomized by bringing together the higher classes (A, B, and C) and the lower classes (D and E). The low-income population studied here consisted of individuals belonging to classes C, D and E. Thus, we divided them as class C versus classes D + E.

Covariables

Covariables were dichotomized based on the following *a priori* criteria: (a) children's ages ≤ 36 months versus > 36 months, because children up to 36 months of age required more maternal attention; (b) mother's age ≤ 29 years versus > 29 years, because of the perceived change in the social role of older women; (c) working versus non-working mothers, because of the importance of the woman as a household provider; (d) working versus non-working father or substitute, for the same reason; (e) number of rooms ≤ 3 or > 3, because of the importance of space and privacy; (f) number of people living in the household ≤ 4 or > 4, because the latter condition was considered to represent a risk of an overcrowded environment; (g) mother's educational status, in which low was defined as < 4 and high as ≥ 4, because four years corresponds to the first phase of the Brazilian elementary school system; (h) number of children, in which low was defined as 1 and high as ≥ 2, because it was assumed that low-income families with ≥ 2 children would have additional difficulties, such as more severe financial constraints and less time to spend with each child; (i) absence of the child's biological father from the household (yes or no), because absence of the father could be a risk factor for the child; and (j) social class, in which "E" was considered low, because it lies below the poverty line. "Family Social Economic Status (SES) and head-of-household's educational status and occupation were taken as components of a five-level social class scale ranging from A, the highest, to E, the lowest".[15]

The dichotomization criteria followed the cutoff points of other studies, for comparative purposes.[6,16-21]

Statistical analyses

Odds ratios (OR) were used to compare differences between cases and controls in the bivariate analysis. To investigate the variables associated with child nutritional status and to model potential interactions, a sequence of bivariate analyses were performed on each variable, with controlling for potential confounders by all other variables ("maternal disability relating to CMDs", "number of people living in the household", "absence of biological father from the household" and "husband/partner in the labor market"). All the variables with P-values lower than 0.15 at this stage of the analysis were selected for the initial multivariate model. The final model retained the variables with P-values ≤ 0.05. P-values between 0.05 and 0.10 were interpreted as having borderline statistical significance.

The Hosmer-Lemeshow test was used to assess goodness-of-fit. Multicollinearity was verified by calculating the variance inflation factor; a cutoff > 10 was considered to be an indicator of collinearity. All the analyses were conducted by using the Statistical Package for the Social Sciences (SPSS), version 20.

Sample size

The sample size planned for the study consisted of 45 cases and 45 controls. This number was based on an alpha error of 0.05, beta error of 0.2 (i.e. lower than 80%), prevalence of disability relating to depression among the mothers of eutrophic children of 20% and OR of 4.0, as measurements of clinical importance.

RESULTS

Table 1 shows the distribution of the study variables among the mothers of malnourished and eutrophic children. Malnourished children were those with height-for-age z-scores ≤ –2 SDs, measured in accordance with the WHO guidelines. Eutrophic children were those with z-scores > –1 and < 1. Only the disability associated with CMDs was investigated. Mothers of malnourished children presented twice as much chance of presenting disability as did mothers of eutrophic children (OR = 2.28; 95% CI = 1.02-5.1). The cases and controls were similar with regard to most SES factors, including the mother's age, mother in the labor market, mother's educational status, number of rooms in the household, number of children in the household and social class. On the other hand, in the families with malnourished children, the percentage with a working father or substitute was lower than in the families of the controls (50% versus 70%; OR = 0.44; 95% CI = 0.21-0.93). At the same time, the number of people living in the household

Table 1. Association between child nutritional status (cases and controls) and disability associated with maternal common mental disorders (CMDs) and selected covariables

Variables	Malnourished children (n = 55) n (%)	Eutrophic children (n = 70) n (%)	OR (95% CI)	P-value
Main risk factor				
Maternal disability relating to CMD				
Positive	20 (36.7)	14 (20.0)	2.28 (1.02-5.1)	0.04
Negative	35 (63.7)	56 (80.0)		
Covariables				
Mother's age				
≤ 29 years	25 (45.5)	33 (47.1)	0.93 (0.43-2.02)	0.86
> 29 years	30 (54.5)	37 (42.9)		
Mother in the labor market				
Works	18 (32.7)	14 (20.0)	1.95 (0.80-4.75)	0.15
Doesn't work	37 (67.3)	56 (80.0)		
Husband/partner in the labor market				
Yes	28 (50.1)	49 (70.0)	0.44 (0.21-0.93)	0.04
No	27 (49.9)	21 (30.0)		
Number of rooms				
≤ 3 rooms	24 (43.6)	30 (42.9)	1.03 (0.48-2.24)	0.93
> 3 rooms	31 (56.4)	40 (57.1)		
Number of people living in the household				
≤ 4 people	15 (27.3)	35 (50.0)	0.38 (0.18-0.80)	0.01
> 4 persons	40 (62.7)	35 (50.0)		
Mother's educational status				
< 4 years of study	35 (63.6)	40 (57.1)	1.31 (0.60-2.89)	0.58
≥ 4 years of study	20 (36.4)	30 (42.9)		
Number of children				
< 2 children	32 (58.2)	30 (42.9)	1.86 (0.83-4.04)	0.10
≥ 2 children	23 (41.8)	40 (57.1)		
Absence of the child's biological father from the household*				
Yes	13 (21.0)	7 (12.5)	3.14 (1.12-8.8)	0.047
No	29 (79.0)	49 (87.5)		
Social class				
C	47 (85.5)	64 (91.4)	0.55 (0.16-1.90)	0.44
D + E	8 (14.5)	6 (08.6)		

*n = 98 instead of 125; OR = odds ratio; CI = confidence interval.

was higher in families with malnourished children than in families with eutrophic children (OR = 2.67; 95% CI = 1.17-6.11). Finally, absence of the biological father from the household was more common in families with malnourished children than in families with eutrophic children (OR = 3.14; 95% CI = 1.12-8.76).

Construction of a logistic regression model to include the four factors singly associated with childhood malnutrition in **Table 1** helped in ascertaining whether the combination of disability associated with maternal CMDs and sociodemographic factors had any effect on the risk that a child would be malnourished. In constructing the logistic regression, the following independent variables were included: maternal disability relating to CMDs; husband/partner in the labor market; number of people living in the household; absence of the child's father from the household; and number of children. The final and most parsimonious model identified three independent correlates (**Table 2**): (1) children with malnutrition were more than twice as likely to have a mother presenting disability associated with maternal CMDs (OR = 2.446; 95% CI = 1.059-5.649; P = 0.036); (2) children whose biological father was absent from the household were twice as likely to have a mother with disability associated with childhood malnutrition (OR = 2.097; 95% CI = 0.958-4.589; P = 0.064); and (3) smaller numbers of people living in the household had a protective effect (OR = 0.778; 95% CI = 0.631-0.959; P = 0.018).

Table 2. Initial and final multiple logistic regression model on factors associated with childhood malnutrition (n = 125)

Likelihood of associated factors	B	OR (95% CI)	P-value	-2 log
Final model				155.639
Maternal disability relating to CMDs	0.895	2.446 (1.059-5.649)	0.036	
Absence of biological father from the household	0.740	2.097 (0.958-4.589)	0.064	
Number of people living in the household	-0.251	0.778 (0.631- 0.959)	0.018	

CMDs = common mental disorders; OR = odds ratio; CI = confidence interval.

DISCUSSION

The present study found that three factors were associated with childhood malnutrition: disability associated with maternal CMDs; higher numbers of people living in the household, which points towards overcrowding; and absence of the biological father, which suggests that the lack of social support matters.

To the best of our knowledge, no other studies have addressed the association between childhood malnutrition and disability relating to the mother's mental health. The cohort study of Rahman et al. examined the association between maternal depression and infant malnutrition and assessed maternal disability among both depressed and non-depressed mothers.[2] These authors reported that depressed mothers had higher scores for disability than did non-depressed mothers. They also stated that the disability presented by these mothers could prevent them from taking proper care of their children, and therefore it constituted a risk factor for impairment of the infants' nutritional status.[2] A review study on the consequences of maternal CMDs for child development (including nutritional status) listed maternal depression, social support and overcrowding as risk factors. However, this study did not specify the maternal disability.[6]

The findings from the present investigation are in agreement with those from previous studies: higher numbers of people living in the same household (overcrowding), in comparison with

controls constituted another variable that was associated with childhood malnutrition. In a cross-sectional study conducted in Pakistan, Shah et al. found that children younger than three years of age who were living in more crowded households were more likely to present stunting.[20] Similarly, in a case control study on Mexican children (aged 0 to 2 years), Sandoval-Priego et al. found that overcrowding was one of the risk factors for chronic malnutrition (in a logistic regression model).[21] Absence of the biological father from the household was the third variable in the logistic regression that was associated with childhood malnutrition. Similarly, another case-control study conducted in an urban area in southeastern Brazil found a positive association between childhood malnutrition and the absence of the biological father, regardless of per capita income and maternal educational status. The authors of that study hypothesized that the presence of the biological father was the most important component of social support for the mother in that environment. They elaborated on this topic by stating, "with the progressive extinction of the extended family, the father may be considered to be the mother's main supplier of emotional and material support in caring for the child".[22]

The present study presents some limitations. Although the SDS is an instrument that can provide quantitative disability measurements, disability was only classified as present or absent in this study, because of the small sample size. The cross-sectional data collection procedure, which was conducted for operational reasons, and also the dichotomization of continuous variables, formed limitations. The study would have benefited from a larger number of available cases of stunted children.

The association between chronic childhood malnutrition and maternal CMD-related disability may help to identify the causal chain between childhood malnutrition and maternal CMDs and to point out social and environmental risk factors associated with chronic childhood malnutrition. According to the "World report on disability", this disability not only involves mental impairment, but also includes social and environmental barriers.[9] Therefore, adequate management of CMD-related disability might help to overcome internal and external factors that are linked to the mother's lack of autonomy.[23]

CONCLUSIONS

Overall, it is possible to assume that the disability associated with maternal CMDs may vary according to the environment in which the person lives. In addition to the relationship with CMDs, this disability also relates to the interaction between the subject and the environment. This interaction will be important for defining the social support that should be made available.

Further prospective studies are necessary, in order to ascertain the association between chronic childhood malnutrition and maternal disability relating to CMDs.

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Address for correspondence:
Claudio Torres de Miranda
Av. Dr. José Sampaio Luz, 878 — apto 101
Ponta Verde — Maceió (AL) — Brasil
CEP 57035-260
Tel. (+55 82) 3325-1697
Cel. (+ 55 82) 9993-8278
E-mail: mirandaclaudio@gmail.com

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The effect of red grape seed extract on serum paraoxonase activity in patients with mild to moderate hyperlipidemia

O efeito do extrato de semente de uva vermelha na atividade do soro paraoxonase em doentes com hiperlipidemia leve a moderada

Hassan Argani[I], Amir Ghorbanihaghjo[II], Hamid Vatankhahan[III], Nadereh Rashtchizadeh[IV], Sina Raeisi[V], Hadi Ilghami[VI]

Tabriz University of Medical Sciences, Tabriz, Iran

[I]MD. Professor, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[II]MSc, PhD. Professor, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[III]MSc. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[IV]MSc, PhD. Professor, Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[V]MSc. Doctoral Student, Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[VI]MSc. Doctoral Student, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

KEY WORDS:
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PALAVRAS-CHAVE:
HDL-colesterol.
Apolipoproteína A-I.
Flavonoides.
LDL-colesterol.
Colesterol.

ABSTRACT

CONTEXT AND OBJECTIVE: Red grape seed extract (RGSE) contains oligomeric proanthocyanidin complexes as a class of flavonoids. These compounds are potent antioxidants and exert many health-promoting effects. This study aimed to determine the effects of RGSE on serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein AI (apo-AI) levels and paraoxonase (PON) activity in patients with mild to moderate hyperlipidemia (MMH).

DESIGN AND SETTINGS: A randomized double-blind placebo-controlled clinical trial was conducted at Shahid-Modarres Hospital (Tehran, Iran) and Tabriz University of Medical Sciences. Seventy MMH patients were randomly assigned to receive treatment (200 mg/day of RGSE) or placebo for eight weeks.

RESULTS: Significant elevation in serum levels of apo-AI (P = 0.001), HDL-C (P = 0.001) and PON activity (P = 0.001) and marked decreases in concentrations of TC (P = 0.015), TG (P = 0.011) and LDL-C (P = 0.014) were found in the cases. PON activity was significantly correlated with apo-AI (r = 0.270; P < 0.01) and HDL-C (r = 0.45; P < 0.001). Significant differences between the RGSE and control groups (before and after treatment) for TC (P = 0.001), TG (P = 0.001), PON (P = 0.03), apo-AI (P = 0.001) and LDL-C (P = 0.002) were seen.

CONCLUSION: It is possible that RGSE increases PON activity mostly through increasing HDL-C and apo-AI levels in MMH patients. It may thus have potential beneficial effects in preventing oxidative stress and atherosclerosis in these patients.

RESUMO

CONTEXTO E OBJETIVO: Extrato de semente de uva vermelha (RGSE) contém complexos de proantocianidinas oligoméricas como classe de flavonoides. Estes compostos são antioxidantes potentes e exercem muitos efeitos de promoção da saúde. Este estudo visou determinar os efeitos de RGSE nos níveis séricos de triglicérides (TG), colesterol total (TC), colesterol de lipoproteína alta-densidade (HDL-C), colesterol de lipoproteína baixa-densidade (LDL-C), apolipoproteína AI (apo-AI) e atividade de paraoxonase (PON) em pacientes com hiperlipidemia leve a moderada (MMH).

DESENHO E LOCAL: Estudo clínico randomizado duplo-cego controlado com placebo, realizado no Hospital Shahid-Modarres (Teerã, Irã) e na Universidade de Ciências Médicas de Tabriz. Setenta pacientes com MMH foram aleatoriamente designados para receber tratamento (200 mg/dia de RGSE) ou placebo durante oito semanas.

RESULTADOS: Elevação significativa nos níveis séricos de apo-AI (P = 0,001), HDL-C (P = 0,001) e atividade de PON (P = 0,001) e diminuição marcada nas concentrações de TC (P = 0,015), TG (P = 0,011) e LDL-C (P = 0,014) foram encontradas nos casos. Atividade de PON mostrou correlação significativa com apo-AI (r = 0,270; P < 0,01) e HDL-C (r = 0,45; P < 0,001). Diferenças significativas entre os grupos RGSE e controle (antes e após tratamento) para TC (P = 0,001), TG (P = 0,001), PON (P = 0,03), apo-AI (P = 0,001) e LDL-C (P = 0,002) foram observadas.

CONCLUSÃO: É possível que RGSE aumente atividade de PON principalmente através da elevação dos níveis de HDL-C e apo-AI em pacientes MMH. Ele pode, assim, ter efeitos benéficos potenciais na prevenção de estresse oxidativo e aterosclerose nesses pacientes.

INTRODUCTION

Hyperlipidemia and serum lipoprotein disorders have long been known as risk factors for progression of atherosclerosis and cardiovascular disease. Lipoprotein deposition in the intimal layer of arteries causes formation of atherosclerotic plaque.[1-3] Changes to serum lipid profile can induce formation of hydroperoxide and lysis of phospholipids, oxysterol and other lipids.[1] Oxidation of low-density lipoprotein cholesterol (LDL-C) in the arterial walls is accepted as an important mechanism for atherosclerosis. Many studies have focused on preventing LDL-C oxidation mechanisms.[4] It has also been shown that serum levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein-AI (apo-AI) are lower in atherosclerotic patients than in the healthy population. Decreased HDL-C levels also have an important role in increasing the risk of cardiovascular diseases.[5] Human paraoxonase (PON) is a calcium-dependent enzyme (hydrolase) with 354 amino acids and molecular weight of 43 kDa, which is produced by the liver and released into serum. It is mainly associated with the apo-AI that is located in HDL-C. *In vitro* experiments have shown that PON can inhibit procreation of oxidized LDL (ox-LDL).[6,7] Because of the key role of LDL-C oxidation in inducing atherosclerosis, reduced serum activity of PON may explain one of the essential mechanisms for increased risk of atherosclerosis and cardiovascular disease in hyperlipidemic patients.[8] Moreover, it has been shown that PON prevents peroxidation of cholesterol esters.[9] Therefore, interventions that can increase apo-AI levels, PON activity and HDL-C levels may decrease the progression of atherosclerosis.

Red grape seed extract (RGSE) contains oligomeric proanthocyanidin complexes (OPCs) as a class of flavonoids. These compounds are potent antioxidants and exert many health-promoting effects.[10] The antioxidant effect of OPCs is approximately 50 times greater than that of vitamin C and vitamin E.[11] The impact of RGSE on atherosclerosis has been studied, and some beneficial effects of these compounds against atherosclerosis have been reported.[12-14]

OBJECTIVE

Because only limited information about the effects of RGSE on PON activity and on its relationships with apo-AI and HDL-C levels is available, this study was carried out to determine the effects of RGSE supplementation on the lipid profile, apo-AI levels and PON activity in patients with mild to moderate hyperlipidemia (MMH).

METHODS

A randomized double-blind placebo-controlled clinical trial was carried out at Shahid-Modarres Hospital (Tehran, Iran) and at Tabriz University of Medical Sciences. The target population was adults aged 21–64 years, and individuals with MMH (triglycerides, TG > 150 mg/dl; and total cholesterol, TC > 200 mg/dl) were included in this study. Individuals with severe hyperlipidemia (TG > 300 mg/dl; TC > 250 mg/dl), diabetes mellitus, severe and/or poorly controlled hypertension (except mild hypertensive patients with acceptable blood pressure (BP) control through a low-salt diet alone), body mass index (BMI) > 30 kg/m^2, heart failure, chronic renal failure, chronic hepatic disease, malignancy, any lipid-lowering drug use, vegetarian diet, alcohol use and cigarette use were excluded from the study.

All the participants signed an informed consent and the study was registered in ClinicalTrials.gov (IRCT ID: IRCT138902073812N1). The grape seed capsules used in this study were prepared at the Drug Applied Research Center (Tabriz, Iran). The capsule ingredients were as follows: dicalcium phosphate, gelatin, microcrystalline cellulose and 100 mg of RGSE. Each capsule contained the equivalent of 5–8 grape seeds. In an analysis performed on these capsules, they were found to contain at least 95% proanthocyanidins and 80% other polyphenolic compounds.

The individuals thus recruited participated in a series of joint meetings to become acquainted with the aims and significance of the study. The participants were advised not to change their lifestyle, general nutritional habits or daily physical activity during the study period and were kept under observation throughout the study period. They were encouraged to continue consuming any dietary supplementation or medication that they were using before the study, but were asked to stop consumption of any grape product during the active phases. Informed consent was obtained from all patients after they had been given explanations about the stages and aims of the study. The study was approved by the ethics committee of Tabriz University.

The RGSE group received capsules containing 200 mg/day of RGSE for eight weeks, and the placebo group received similar-looking capsules (filled with starch and cellulose) for the same amount of time. The physical activity levels of the patients were assessed using the short form of the international physical activity questionnaire and daily nutritional intake was recorded using a food frequency questionnaire at the beginning and end of each round. The questionnaires allowed the interviewer to calculate the amount of each dietary intake category (protein, carbohydrate and fat), expressed as grams/day.

Initial blood samples were taken after overnight fasting at the beginning of the study and a second blood sample was collected at the end of the round. We collected venous blood samples of 10 ml after 12 hours of fasting at the beginning of the study and after eight weeks of treatment. The lipid parameters were determined from the fresh serum samples and these samples were then stored at -70 °C for PON activity measurement before the

biochemical analysis. The serum levels of apo-AI were assayed by means of commercially available immunoturbidimetric kits. Serum PON activity was determined spectrophotometrically using paraoxon (O,O-diethyl-O-P nitrophenylphosphate) as the substrate. TC, TG, and HDL-C levels were determined using commercial kits (Pars Azmoon, Tehran, Iran) with an automated chemical analyzer (Hitachi 917).

Sample size calculations were performed using the Power and Sample Size Calculation (PS) software, version 3.1.2. We studied a continuous-response variable from independent control and experimental subjects, with one placebo subject per RGSE subject. The calculation was based on information obtained from a pilot study, taking the response within each normally distributed subject group to be a true difference in the RGSE and control means of up to five subjects. Thus, the sample size was estimated to be nearly 30 subjects per group, in order to achieve an alpha of 5% and a power of 80%. The dropout rate was taken to be 15% and therefore the sample size was increased to 35 in each group. The SPSS 18 for Windows software was used to perform statistical analyses. We performed a per-protocol analysis. Results are presented as mean values ± standard deviation (SD). After determining the distribution of continuous variables using the Kolmogorov-Smirnov test, the paired Student's t test was used to assess the significance of intra-group changes during the intervention period and an independent-sample t test was applied to compare the results of the two groups. Correlations were evaluated using Pearson's test and the statistical significance level was set at $P < 0.05$.

RESULTS

Seventy-five patients were initially recruited. Five patients failed to complete the study period because of lack of cooperation and the analysis was performed on a total of 70 cases (43 females). The mean age of the subjects was 48.22 ± 9.07 years.

Although the daily protein and carbohydrate intake increased during RGSE intake, the mean daily fat and fiber intake did not show any meaningful change ($P > 0.05$) (**Table 1**). Data on HDL-C, LDL-C, TG, TC, body weight and systolic and diastolic blood pressures are shown in **Table 2**. The placebo and RGSE groups did not show any significant differences in the means of any of the variables at the beginning of the study (**Table 2**). The mean lipid profile, apo-AI levels and PON activity in the RGSE and control group after eight weeks are shown in Table 2. Significant differences between the RGSE and control groups (both before and after the treatment) were seen in relation to TC ($P = 0.001$), TG ($P = 0.001$), PON ($P = 0.03$), apo-AI ($P = 0.001$) and LDL-C ($P = 0.002$) (**Table 2**).

To determine the effects of RGSE and placebo on PON activity, the correlation between the degree of change in PON after two months (Δ PON) with the degrees of change in HDL-C (Δ HDL-C) and in apo-AI (Δ apo-AI) and also the correlation between Δ HDL-C and Δ apo-AI are shown in **Table 3**. Significant

Table 1. Characteristics of study groups at baseline

Variables	Placebo group (n = 35)	RGSE group (n = 35)	P-value
Age (years), mean (SD)	46.6 ± 8.4	47.3 ± 9.3	0.76*
Sex (male/female) n	11/24	12/23	0.8†
Baseline protein intake (g/d), mean (SD)	73.7 ± 17.2	75.5 ± 22.2	0.71*
Baseline fat intake (g/d), mean (SD)	74.5 ± 14.8	67.6 ± 17.0	0.07*
Baseline carbohydrate intake (g/d), mean (SD)	271.9 ± 85.5	272.2 ± 80.7	0.98*
Physical activity			
Low, n (%)	17 (48.6)	16 (45.7)	-
Moderate, n (%)	18 (51.4)	19 (54.3)	-
Systolic pressure (mmHg)	114.6 ± 9.8	113.1 ± 10.5	0.6*
Diastolic pressure (mmHg)	72.0 ± 9.0	71.1 ± 9.8	0.7*

RGSE = red grape seed extract; SD = standard deviation.
*Performed using independent-sample t test; †Performed using chi-square test.

Table 2. Lipid profile, apo-AI levels and PON activity in the RGSE and control groups

Parameters	Before treatment n = 35 (12 males; 23 females) (mean ± SD)	After treatment n = 35 (11 males; 24 females) (mean ± SD)	Difference in results before and after treatment (mean ± SD)
TC (mg/dl) RGSE	226.9 ± 14.5	212.1 ± 17.1	-14.8 ± 19.7
TC (mg/dl) control	229.7 ± 15.5	239.8 ± 25.0	10.1 ± 24.9
P-value*			0.001
TG (mg/dl) RGSE	200.0 ± 47.2	180.6 ± 55.2	-19.4 ± 42.4
TG (mg/dl) control	184.6 ± 34.0	195.9 ± 38.8	11.3 ± 23.7
P-value*			0.001
PON (IU/l) RGSE	112.7 ± 21.3	117.2 ± 22.3	4.5 ± 7.7
PON (IU/l) control	103.2 ± 20.1	102.6 ± 19.8	-0.6 ± 11.5
P-value*			0.03
Apo-AI (mg/dl) RGSE	114.1 ± 15.4	123.4 ± 21.5	9.3 ± 11.7
Apo-AI (mg/dl) control	109.4 ± 9.4	103.3 ± 18.0	-6.1 ± 18.2
P-value*			0.001
LDL-C (mg/dl) RGSE	142.1 ± 15.5	129.0 ± 19.9	-13.1 ± 20.6
LDL-C (mg/dl) control	137.8 ± 30.6	150.9 ± 27.1	13.1 ± 42.4
P-value*			0.002
HDL-C (mg/dl) RGSE	44.9 ± 6.5	47.0 ± 6.0	2.1 ± 3.7
HDL-C (mg/dl) control	55.0 ± 29.5	49.7 ± 13.5	-5.3 ± 35.7
P-value*			0.23

apo-AI = apolipoprotein AI; PON = paraoxonase; RGSE = red grape seed extract; SD = standard deviation; TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein. Values are reported as mean ± SD. *Comparison of the differences (before and after treatment) between RGSE and control groups (independent-sample t test).

Table 3. Correlations between the changes in PON activity, HDL-C and apo-AI levels after two months of RGSE (red grape seed extract) or placebo treatment

	Placebo group				RGSE group			
	Δ PON		Δ apo-AI		Δ PON		Δ apo-AI	
	r	P-value	r	P-value	r	P-value	r	P-value
Δ PON	–	–	0.06	0.75	–	–	0.43	0.01
Δ HDL-C	-0.04	0.81	0.11	0.54	0.67	0.001	0.41	0.02

PON = paraoxonase; HDL-C = high-density lipoprotein cholesterol, apo-AI = apolipoprotein AI; Δ PON = PON (after two months of RGSE or placebo treatment) minus PON (baseline); Δ HDL-C = HDL-C (after two months of RGSE or placebo treatment) minus HDL-C (baseline); Δ apo-AI = apo-AI (after two months of RGSE or placebo treatment) minus apo-AI (baseline); Pearson's test was used to determine correlations between Δ PON, Δ apo-AI and Δ HDL-C.

correlations between Δ PON and Δ HDL-C ($P < 0.001$; $r = 0.672$) and between Δ PON and Δ apo-AI ($P < 0.01$; $r = 0.427$) were seen in the RGSE group. In addition, Δ apo-AI significantly correlated with Δ HDL-C ($P < 0.05$; $r = 0.408$) in the RGSE group.

DISCUSSION

Clinical and epidemiological studies have over the years established that dyslipidemia constitutes the main risk factor for atherosclerosis.[15] Although hyperlipidemia is a major factor for atherosclerosis, lipid peroxidation also has an important role in this process.[16] Oxidative damage plays a key role in accelerated atherosclerosis and is involved in cardiovascular disease among hyperlipidemia patients who are at risk of increased oxidative stress.[17] There is a growing body of evidence demonstrating that reduced activity of the HDL-associated enzyme PON is predictive of vascular disease in humans. This evidence includes the results from prospective studies.[18] It has been suggested that some drugs with lipid-lowering properties can alter lipid peroxidation products.[19,20] Unlike drugs, which are associated with some toxic effects, natural antioxidants have beneficial effects.[21-23] RGSE, which is now available as a dietary supplement, contains a number of polyphenols, including procyanidins and proanthocyanidins, which are powerful free radical scavengers.[24,25] The exact chemical characteristics and the mechanism of action of RGSE have not yet been completely understood and the experimental findings are inconsistent.

It has been demonstrated that serum concentrations of apo-AI are a better indicator of coronary heart disease (CHD) than serum lipid and lipoprotein levels.[26,27] The results from this study indicate that RGSE administration can significantly increase the apo-AI levels in MMH patients. Some studies, such as those by Ignea et al.[28] and El-Alfy et al.,[29] showed that RGSE can increase the activity of antioxidant enzymes and can prevent lipid peroxidation. In this study, we showed that two months of RGSE administration can lead to increased PON activity in MMH patients. Furthermore, we previously showed that RGSE administration can improve lipid profiles and lead to decreased ox-LDL.[30] Human PON is a calcium-dependent esterase closely associated with HDL. It contains apo-AI, which has been reported to confer antioxidant properties on HDL-C through decreasing the accumulation of lipid peroxidation products.[31,32] HDL-C protects against atherosclerosis by returning excess cholesterol from peripheral tissues back to the liver for reuse or excretion into the bile. Several reports have suggested that HDL may have an antioxidative function, which may contribute towards its anti-atherogenic activity.[33-35]

In the study by Song et al.,[36] 28 days of administration of grape seed powder was found to be capable of reducing the levels of serum lipids (TC, TG and LDL-C) and preventing occurrences of fatty liver among rats. In confirmation of this study, we demonstrated that RGSE had the capacity to significantly increase the concentration of HDL-C apo-AI and lead to decreased TC, TG and LDL-C levels, in relation to the pre-treatment values. Moreover, significant correlations of PON with both apo-AI and HDL-C were found. A significant correlation between the changes in HDL-C and apo-AI levels following two months of RGSE administration was also noticed.

The major limitations of the present study included the short duration of the study, the use of only a single dosage of RGSE and the lack of measurements on the levels of apo-J, superoxide dismutase, catalase, glutathione peroxidase activity and antioxidant capacity. However, to the best of our knowledge, this was the first study to attempt to show the effect of RGSE on PON in people with MMH.

CONCLUSIONS

It seems that RGSE administration increases PON activity through increasing the HDL-C and apo-A1 levels and/or cooperative increases in the concentrations of both factors in MMH patients. In conclusion, RGSE administration in MMH patients has beneficial effects on the lipid and apolipoprotein profiles. It increases PON activity mostly through increasing HDL-C and apo-AI levels. It may have potential beneficial effects on oxidative stress and can exert an anti-atherosclerotic effect in MMH patients, which is effected by increased PON activity. Studies involving a larger population size should be conducted in order to confirm these hypotheses.

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Address for correspondence:
Amir Ghorbanihaghjo
Drug Applied Research Center
Tabriz University of Medical Sciences
Daneshgah Street
Tabriz — Iran
Tel. 0098-413-3364666
Fax. 0098-413-3364666
Email: ghorbaniamir@hotmail.com

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Frequency of cholecystectomy and associated sociodemographic and clinical risk factors in the ELSA-Brasil study

Frequência de colecistectomia e fatores de risco sociodemográficos e clínicos associados no estudo ELSA-Brasil

Kamila Rafaela Alves[I], Alessandra Carvalho Goulart[II], Roberto Marini Ladeira[III], Ilka Regina Souza de Oliveira[IV], Isabela Martins Benseñor[V]

Center for Clinical and Epidemiological Research, University Hospital, Universidade de São Paulo (USP), São Paulo, SP, Brazil

[I]BSc. Postgraduate Student, Department of Medicine, Education and Health, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

[II]MD, PhD. Clinical Epidemiologist and Researcher, Center for Clinical and Epidemiological Research, University Hospital, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

[III]MD, PhD. Attending Physician at Hospital Foundation of the State of Minas Gerais, Epidemiologist in the Municipal Health Department of Belo Horizonte, MG, Brazil and Director of the Longitudinal Study of Adult Health (Estudo Longitudinal de Saúde do Adulto, ELSA-Brasil), Belo Horizonte, MG, Brazil.

[IV]MD, PhD. Professor, Department of Radiology, School of Medicine, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

[V]MD, PhD. Professor, Department of Internal Medicine, and Director of Center for Clinical and Epidemiological Research, University Hospital, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
Colecistectomia.
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ABSTRACT

CONTEXT AND OBJECTIVE: There are few data in the literature on the frequency of cholecystectomy in Brazil. The frequency of cholecystectomy and associated risk factors were evaluated in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).

DESIGN AND SETTING: Cross-sectional study using baseline data on 5061 participants in São Paulo.

METHODS: The frequency of cholecystectomy and associated risk factors were evaluated over the first two years of follow-up of the study and over the course of life. A multivariate regression analysis was presented: odds ratio (OR) and 95% confidence interval (95% CI).

RESULTS: A total of 4716 individuals (93.2%) with information about cholecystectomy were included. After two years of follow-up, 56 had undergone surgery (1.2%: 1.7% of the women; 0.6% of the men). A total of 188 participants underwent cholecystectomy during their lifetime. The risk factors associated with surgery after the two-year follow-up period were female sex (OR, 2.85; 95% CI, 1.53–5.32), indigenous ethnicity (OR, 2.1; 95% CI, 2.28–15.85) and body mass index (BMI) (OR, 1.10; 95% CI, 1.01–1.19 per 1 kg/m^2 increase). The risk factors associated over the lifetime were age (OR, 1.03; 95% CI, 1.02–1.05 per one year increase), diabetes (OR, 1.92; 95% CI, 1.34–2.76) and previous bariatric surgery (OR, 5.37; 95% CI, 1.53–18.82). No association was found with parity or fertile age.

CONCLUSION: Female sex and high BMI remained as associated risk factors while parity and fertile age lost significance. New factors such as bariatric surgery and indigenous ethnicity have gained importance in this country.

RESUMO

CONTEXTO E OBJETIVO: Há escassez de dados na literatura sobre a frequência de colecistectomia no Brasil. Avaliou-se a frequência de colecistectomia e os fatores de risco associados no Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil).

DESENHO E LOCAL: Estudo transversal com dados da linha de base de 5061 participantes em São Paulo.

MÉTODOS: Avaliou-se a frequência de colecistectomia e fatores de risco associados nos dois primeiros anos de seguimento do estudo e ao longo da vida. Apresentou-se regressão logística [razão de chances (RC); intervalo de confiança de 95% (IC 95%)] multivariada.

RESULTADOS: Um total de 4716 (93,2%) indivíduos com informação sobre colecistectomia foi incluído. Após 2 anos de seguimento, 56 participantes tinham sido operados (1,2%: 1,7% nas mulheres; 0,6% nos homens), totalizando 188 participantes com colecistectomia durante a vida. Os fatores de risco associados à cirurgia após dois dois anos de seguimento foram sexo feminino (RC, 2,85; IC 95%, 1,53-5,32), etnia indígena (RC, 2,1; IC 95%, 2,28-15,85) e índice de massa corpórea, IMC (RC, 1,10; IC 95%, 1,01-1,19 por aumento de 1 kg/m^2); e, ao longo da vida: idade (RC, 1,03; IC 95%, 1,02-1,05 por um ano de aumento), diabetes (RC, 2,10; IC 95%, 1,34-2,76) e cirurgia bariátrica prévia (RC, 5.37; IC 95%, 1,53-18,82). Não se observou associação com paridade ou idade fértil.

CONCLUSÃO: Sexo feminino e IMC elevado permanecem sendo fatores de risco associados à colecistectomia, mas paridade e idade fértil perderam significância. Novos fatores de risco, como cirurgia bariátrica prévia e etnia indígena, ganharam relevância no país.

INTRODUCTION

Gallstones have been recognized since antiquity, and have been found in Egyptian mummies.[1] Today, they are a frequent problem in developed countries, affecting 10 to 15% of the adult population in the United States,[2] while in Europe, the prevalence ranges from 5.9% to 21.9%.[3] In Brazil, few studies have evaluated the frequency of gallstones and associated risk factors. Coelho et al.[4] screened 1000 individuals in two shopping centers in the city of Curitiba, Brazil, using ultrasound, and found that the frequency of gallstones was 6.4%. The direct and indirect costs of this ailment have been progressively increasing over recent years, as a consequence of increased numbers of surgical procedures in recent decades, and especially since the introduction of laparoscopic cholecystectomy in 1989.[5,6]

Multiple factors are responsible for cholelithiasis. Advancing age is one important risk factor for gallstones, and female sex is also a known risk factor.[2] Estrogen seems to play a critical role in this increased risk, because pregnancy, high parity and estrogen replacement therapy increase the risk of gallstones.[7] Obesity is another risk factor for gallstone development, likely caused by increased hepatic secretion of cholesterol.[1] The risk is especially high among women and increases linearly with increasing body mass index.[8] Ironically, rapid weight loss is also a risk factor for gallstone development, such that gallstones occur in 25% to 30% of patients who undergo bariatric surgery.[2] Although most people with gallstones are asymptomatic, about 20% present symptoms at some point and 7% require surgical intervention.[9]

The prevalence of cholecystectomy in the United States is higher among women than among men, and it varies widely according to race.[10] Maclure et al.[8] in the Nurses' Health Study reported that the frequency of cholecystectomy was 0.49%,[8] while Liu et al.[11] reported a frequency of 1.3% in Taiwan. Except for the study by Coelho et al.,[4] in which a frequency of 2.9% was reported from ultrasound screening of people in a shopping center in Curitiba, few studies on the epidemiology of cholecystectomy and associated risk factors have been conducted in Brazil.

OBJECTIVE

The aim of this study was to conduct a cross-sectional investigation of the frequency of cholecystectomy and associated sociodemographic and clinical risk factors using data from all participants in the ELSA-Brasil research center of São Paulo who had baseline information about previous cholecystectomy.

METHODS

Study design and population

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a prospective cohort study designed to investigate the incidence of cardiovascular diseases and diabetes, along with their biological and social determinants. The study originally included 15,105 subjects aged 35-74 years from six cities located in three different regions of Brazil: Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, São Paulo and Vitória.[12-14] In our analysis, we included baseline (cross-sectional) information from the first examination, which took place between August 2008 and December 2010. Only the participants evaluated at the São Paulo Research Center for whom data about cholecystectomy was available were included in this analysis.

Data were gathered from participants in two phases. The first, which lasted for approximately one hour, consisted of obtaining informed consent and conducting the initial interview at the participant's work site. The second comprised additional interviews and examinations, lasted for approximately six hours and was conducted at the study clinic.[15] ELSA-Brasil was approved by the Institutional Review Board at the University Hospital of the University of São Paulo, and all participants signed an informed consent statement.

Cholecystectomy information

Information about the previous cholecystectomy was obtained from the baseline study questionnaire, and was confirmed by means of an ultrasound evaluation that focused on screening for hepatic steatosis, which was also performed at baseline. As part of a multicenter protocol, all of the liver images were obtained in the same position in relation to four anatomical landmarks, one of which was viewing of the gallbladder. More details about the liver ultrasound examination have been published elsewhere.[16] Participants who did not report having had any previous cholecystectomy or did not undergo hepatic ultrasound were excluded from the analysis.

Sociodemographic characteristics

We analyzed sociodemographic characteristics such as sex, age (years), self-reported race/skin color (white, mixed, black, East Asian or indigenous), years of schooling (< 11 years, 11–15 years or > 15) years, mean net family income (≤ US$ 1245, US$ 1246-3319 or ≥ US$ 3320), partner status (single or married) and previous history of bariatric surgery (yes or no).[17] Local currency [Brazilian reais (BRL)] was converted to U.S. dollars (USD) at a rate of BRL 2.00 = USD 1.00 in December 2008.

Anthropometric and blood pressure measurements

Anthropometric and blood pressure measurements were made on all participants. Weight, height and waist circumference were measured following standard techniques.[18] Body mass index (BMI) was calculated as weight (in kilograms) divided by squared height (in meters).[19] Abdominal obesity was defined as a

waist circumference > 88 cm among women or > 102 cm among men.[19] Resting blood pressure was measured three times in a seated position after five minutes of rest, using a validated oscillometric device. The averages of the second and third measurements were taken to be the systolic and diastolic blood pressures in the analyses.[20]

Cardiovascular risk factors

Hypertension was defined as use of medication to treat hypertension, or systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg. Diabetes was defined as a previous medical history of diabetes, or use of medication to treat diabetes, or fasting serum glucose ≥ 126 mg/dl, or two-hour oral glucose tolerance test ≥ 200 mg/dl, or HbA1c levels ≥ 6.5%. Dyslipidemia was defined as LDL-cholesterol > 130 mg/dl or current use of cholesterol-lowering medication. Patients were categorized according to smoking status and alcohol consumption as never, past or current users.

Psychiatric disorders

Mental diagnoses were assessed by trained interviewers using the validated Portuguese version of the Clinical Interview Schedule – Revised (CIS-R). The CIS-R is a structured interview for diagnosing and measuring non-psychotic psychiatric morbidity in the community. This short and straightforward questionnaire was developed in 1992 by Lewis et al.[21] to be used specifically within community and primary care. Additionally, diagnoses of specific disorders were obtained by applying algorithms based on the International Classification of Diseases (ICD)-10 diagnostic criteria.[22]

Surveillance

Surveillance is being conducted through annual telephone interviews, through a second examination four years after the baseline assessment at the ELSA-Brasil research centers, and through linkage to national databases, such as the National Mortality Information System. Annual telephone calls are made to verify the overall state of the participants' health, including new diagnoses, deaths, hospitalizations and emergency department visits over the first two years of follow-up. All diagnoses noted in hospital discharge summaries or hospital records are recorded. Full hospital information is abstracted by trained personnel if the diagnoses include any ICD codes that relate to ELSA-Brasil endpoints.[23] For this analysis, we used all information about gallbladder procedures from the first two years of follow-up data.

Statistical analysis

Categorical variables are presented as proportions and were compared using the chi-square test. Continuous variables are presented as means (with standard deviations) and were compared using one-way ANOVA with the Bonferroni post-hoc test.

A logistic regression model was built using sociodemographic characteristics and cardiovascular risk factors as the independent variables, and cholecystectomy after the baseline measurements as the dependent variable. Odds ratio were presented as crude values, adjusted for age and sex, and with multivariate adjustment. For sociodemographic risk factors, we did not present multivariate adjustment because the only sociodemographic characteristics with $P < 0.20$ in **Table 1** were age and sex. For clinical variables, multivariate adjustment was done in accordance with sociodemographic and clinical characteristics with $P < 0.20$ in **Table 1**, which were age, sex, body mass index, waist measurement, hypertension, diabetes, dyslipidemia, major depressive disorders and previous bariatric surgery. Other logistic models were produced considering all cases of cholecystectomy (before and after the baseline assessment).

A logistic regression model was also built using sociodemographic characteristics and cardiovascular risk factors as the independent variables, and all lifetime cholecystectomy procedures as the dependent variable. For sociodemographic risk factors, multivariate adjustment was done for other sociodemographic characteristics with $P < 0.20$ in **Table 1**: age, sex, race, marital status and having private health insurance. For clinical variables, multivariate adjustment was done for all sociodemographic and clinical risk factors with $P < 0.20$ in **Table 1**: age, sex, body mass index, waist measurement, marital status, health insurance plan, hypertension, diabetes, dyslipidemia, smoking, alcohol intake, major depressive disorders and bariatric surgery.

The significant level was set at $P < 0.05$. All the analyses were performed using the SPSS software, version 22.0.

RESULTS

Overall, there were 5,061 participants (53.9% women) at the São Paulo research center. No information about previous cholecystectomy or liver imaging was available for 345 participants, and these were excluded from the analysis. Consequently, 4,716 participants remained in the study. There were 132 cases of cholecystectomy before enrollment in ELSA-Brasil, 56 cases over the first two years of follow-up and, thus, 188 cases over these individuals' lifetimes.

The prevalence of cholecystectomy before enrollment was 2.8%: 3.6% among women and 1.8% among men. Excluding these cases that underwent cholecystectomy before enrollment, 4,584 participants with full information about cholecystectomy (clinical questionnaire and ultrasound data) over the first two years of follow-up remained in the sample. In this subset, the prevalence of cholecystectomy was 1.2%: 1.7% among women and 0.6% among men. The mean age was 51.4 years (± 8.9) and 76.7% of the total sample were women. The lifetime prevalence of cholecystectomy was 4.0%: 5.3% among women and 2.4% among men.

Table 1. Sociodemographic and clinical characteristics of participants according to cholecystectomy during the first two years of follow-up

	Cholecystectomy				
	No	Yes After baseline	P-value	Yes Lifetime	P-value
	n = 4528 (%)	n = 56 (%)		n = 188 (%)	
Age* (years)	51 (9.0)	52 (9.4)	0.45	54 (9.6)	0.0001
Age strata (%)					
35-44.9	1109 (24.7)	18 (13.6)	0.0001	31 (16.5)	0.0001
45-54.9	1882 (41.1)	53 (40.2)		76 (40.4)	
55-64.9	1167 (25.5)	34 (25.8)		46 (24.5)	
65-74	426 (9.3)	27 (20.6)		35 (18.6)	
Female (%)	2442 (53.9)	43 (76.8)	0.001	136 (72.3)	0.0001
Body mass index* (kg/m^2)	27 (4.9)	30 (6.4)	0.0001	29 (5.7)	0.0001
Waist circumference* (cm)	94 (13.1)	90 (12.7)	0.047	94 (13.3)	0.0001
Race (%)					
White	2647 (59.2)	33 (62.3)	0.35	123 (67.6)	0.03
Mixed	976 (21.8)	9 (17.0)		31 (17)	
Black	606 (13.6)	10 (18.9)		24 (13.2)	
East Asian	195 (4.4)	0 (0.0)		1 (0.5)	
Indigenous	46 (1.0)	1 (1.8)		3 (1.6)	
Education (years) (%)					
< 11	701 (15.5)	7 (12.5)	0.57	32 (17)	0.77
11 to 15	1795 (39.6)	26 (46.4)		76 (40.4)	
> 15	2032 (44.9)	23 (41.1)		80 (42.6)	
Mean family income (US$) (%) BRL 2.00 = USD 1.00					
≤ 1245	1414 (31.4)	16 (28.6)	0.90	53 (28.3)	0.63
≥ 1246 to 3319	1918 (42.6)	25 (44.6)		81 (43.3)	
≥ 3320	1173 (26.0)	15 (26.8)		53 (28.3)	
Not single (%)	3033 (67.0)	34 (60.7)	0.32	109 (58)	0.01
Health insurance (%)	1612 (35.6)	20 (35.7)	0.99	77 (41)	0.13
Hypertension (%)	1145 (31.9)	23 (41.1)	0.15	71 (37.8)	0.09
Diabetes (%)	909 (20.1)	16 (28.6)	0.12	65 (34.6)	0.0001
Dyslipidemia (%)	2555 (56.8)	27 (48.2)	0.20	89 (47.6)	0.01
Smoking (%)					
Never	2390 (52.8)	33 (58.9)	0.31	92 (48.9)	0.09
Past	1394 (30.8)	18 (32.1)		72 (38.3)	
Current	744 (16.4)	5 (8.9)		24 (12.8)	
Alcohol intake (%)					
Never	530 (11.7)	8 (14.3)	0.84	32 (17)	0.09
Past	920 (20.3)	11 (19.6)		37 (19.7)	
Current	3076 (68)	36 (66.1)		119 (63.3)	
Physical activity (%)					
Mild	3431 (78.6)	44 (83)	0.71	148 (82.7)	0.35
Moderate	577 (13.2)	6 (11.3)		21 (11.7)	
Vigorous	358 (8.2)	3 (5.7)		10 (5.6)	
Depressive disorder (%)	189 (4.2)	5 (8.9)	0.08	13 (6.9)	0.07
Previous bariatric surgery (%)	11 (0.2)	1 (1.8)	0.03	4 (2.1)	0.0001
Female participants only					
Previous pregnancy (%)					
No	495 (20.3)	5 (11.6)	0.16	20 (14.7)	0.11
Yes	1945 (79.7)	38 (88.4)		116 (85.3)	
Parity (%)					
0	128 (6.6)	3 (7.9)	0.41	3 (2.6)	0.20
1–3	1578 (81.1)	33 (86.8)		96 (82.8)	
≥ 4	240 (12.3)	2 (5.3)		17 (14.7)	
Fertile age category (%)					
≤ 49 years	1134 (46.5)	20 (46.5)	0.99	54 (39.7)	0.12
> 49 years	1305 (53.5)	23 (53.5)		82 (60.3)	

*Mean (standard deviation).

Table 1 compares several sociodemographic and clinical risk factors among participants who underwent cholecystectomy over the first two years of follow-up, in relation to participants who did not. Additionally, it also includes a comparison of all participants who underwent cholecystectomy at any point during their lifetimes (before or after the baseline) with those who did not.

Participants who underwent cholecystectomy after the baseline were more likely to be women and have higher BMI and waist measurement values, along with higher frequency of previous bariatric surgery, compared with participants who did not undergo surgery (**Table 1**). In the analysis that included lifetime cases of cholecystectomy (before and after baseline assessment), patients who underwent surgery were older and had higher BMI. The proportions of white and indigenous individuals and of singles and diabetics were higher, while the proportion of the patients with dyslipidemia was lower than the proportion who did not undergo cholecystectomy.

Table 2 presents logistic models for sociodemographic and clinical risk factors for all participants who underwent cholecystectomy during the first two years of follow-up. After multivariate adjustment, we found that there were positive associations with female sex (OR, 2.85; 95% confidence interval, 95% CI, 1.53-5.32) and indigenous ethnicity (OR, 2.10; 95% CI, 2.28-15.85). We also detected a positive OR of 1.10 (95% CI, 1.01-1.19) per 1 kg/m^2 increase in BMI, which remained significant after multivariate adjustment.

Table 3 shows the same associations, taking into consideration all the participants who underwent cholecystectomy (before and after the baseline, combined). After multivariate adjustment, we found that there were positive associations with age (for each one year increase in age: OR, 1.03; 95% CI, 1.02-1.05), female sex (OR, 2.35; 95% CI, 1.65-3.33), diabetes (OR, 1.92; 95% CI, 1.34-2.76) and previous bariatric surgery (OR, 5.37; 95% CI, 1.53-18.82), which remained significant after multivariate adjustment. East Asian ethnicity (OR, 0.09; 95% CI, 0.01-0.65) and dyslipidemia (OR, 0.62; 95% CI, 0.45-0.85) were protective factors against cholecystectomy. We did not find that previous pregnancy, parity or the age stratum from 35 to 49 years (stratum of fertile age) showed any association with cholecystectomy in this subset of the ELSA-Brasil participants.

Table 4 describes the frequencies of elective and laparoscopic surgery according to sex. Most surgeries were laparoscopic and performed as elective procedures for women and as an emergency for men. However, these differences about the number of elective surgery according to sex were not statistically significant.

Table 2. Odds ratio (OR) and 95% confidence intervals (95% CI) showing sociodemographic and clinical risk factors associated with cholecystectomy during the first two years after baseline examination among ELSA-Brasil participants at the São Paulo research center

	Risk factors		
Sociodemographic	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‡**
Age* (years)	1.01 (0.98-1.04)	1.01 (0.98-1.04)†	
Age strata (%)			
35-44.9	1.0 (Reference)	1.0 (Reference)†	
45-54.9	1.04 (0.53-2.07)	1.04 (0.53-2.07)†	
55-64.9	0.88 (0.40-1.93)	0.87 (0.40-1.92)†	
65-74	1.61 (0.66-3.92)	1.81 (0.74-4.40)†	
Sex (%)			
Male	1.0 (Reference)	1.0 (Reference)	
Female	2.83 (1.52-5.27)	2.85 (1.53-5.32)§	
Race (%)			
White	1.0 (Reference)	1.0 (Reference)	
Mixed	0.74 (0.35-1.55)	0.81 (0.38-1.70)	
Black	1.32 (0.65-2.70)	1.30 (0.63-2.65)	
East Asian	---------	----------	
Indigenous	1.74 (0.23-13.02)	2.10 (2.28-15.85)	
Education (years) (%)			
< 11	1.0 (Reference)	1.0 (Reference)	
11 to 15	1.45 (0.63-3.36)	1.40 (0.59-3.34)	
> 15	1.13 (0.48-2.65)	1.00 (0.42-2.36)	
Mean family income (US$) (%) BRL 2.00 = USD 1.00			
≤ 1245	1.0 (Reference)	1.0 (Reference)	
≥ 1246 to 3319	1.15 (0.61-2.17)	1.06 (0.56-1.99)	
≥ 3320	1.13 (0.56-2.30)	1.04 (0.50-2.17)	

Continue...

Table 2. Continues...

	Risk factors		
Sociodemographic	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‡**
Marital status (%)			
Single	1.0 (Reference)	1.0 (Reference)	
Not single	0.76 (0.44-1.31)	1.03 (0.59-1.81)	
Health insurance (%)			
No	1.0 (Reference)	1.0 (Reference)	
Yes	1.00 (0.58-1.74)	0.90 (0.51-1.56)	
Clinical	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‖**
Body mass index* (kg/m^2)	1.09 (1.05-1.14)	1.09 (1.04-1.13)†	1.10 (1.01-1.19)
Waist circumference* (cm)	1.02 (1.0-1.04)	1.03 (1.01-1.05)‡	0.99 (0.95-1.03)
Hypertension (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.49 (0.87-2.54)	1.64 (0.93-2.89)	1.28 (0.70-2.33)
Diabetes (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.59 (0.89-2.86)	1.73 (0.94-3.17)	1.28 (0.67-2.45)
Dyslipidemia (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	0.71 (0.42-1.20)	0.68 (0.39-1.16)	0.65 (0.37-1.12)
Smoking (%)			
Never	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Past	0.94 (0.53-1.67)	1.04 (0.58-1.86)	1.01 (0.56-1.81)
Current	0.49 (0.19-1.25)	0.52 (0.20-1.35)	0.56 (0.22-1.45)
Alcohol intake (%)			
Never	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Past	0.79 (0.32-1.98)	1.02 (0.41-2.57)	0.98 (0.39-2.50)
Current	0.80 (0.37-1.72)	1.05 (0.48-2.28)	1.14 (0.52-2.50)
Physical activity (%)			
Mild	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	0.81 (0.34-1.91)	0.87 (0.37-2.05)	0.99 (0.42-2.36)
Vigorous	0.65 (0.20-2.12)	0.74 (0.23-2.39)	0.87 (0.27-2.87)
Depressive disorder (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	2.25 (0.89-5.70)	1.93 (0.76-4.93)	1.56 (0.60-4.04)
Generalized anxiety disorder (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	0.90 (0.39-2.12)	0.82 (0.35-1.92)	0.79 (0.33-1.89)
Bariatric surgery (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	7.46 (0.95-58.81)	6.09 (0.76-48.54)	3.97 (0.48-33.07)
Female participants only			
Previous pregnancy (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.48 (0.91-2.40)	1.46 (0.90-2.37)	1.33 (0.80-2.23)
Parity (%)			
0	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
1–3	260 (0.81-8.31)	2.67 (0.83-8.56)	2.09 (0.64-6.79)
≥ 4	3.02 (0.87-10.51)	2.61 (0.75-9.13)	1.74 (0.48-6.36)
Fertility age category (%)			
≤ 49 years	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
> 49 years	1.32 (0.93-1.88)	0.75 (0.46-1.23)	0.91 (0.47-1.77)

*Mean (standard deviation); †Adjusted for sex; ‡Multivariate adjustment was not presented because only age and sex had P < 0.20; §Adjusted for age; ‖Multivariate adjustment for age, sex, race, education, mean net family income, marital status, health insurance, BMI, waist circumference, hypertension, diabetes, dyslipidemia, smoking, alcohol intake, physical activity, depressive disorder and bariatric surgery.

Table 3. Odds ratio (OR) and 95% confidence intervals (95% CI) showing sociodemographic and clinical risk factors associated with lifetime occurrence of cholecystectomy among ELSA-Brasil participants at the São Paulo research center

	Risk factors		
Sociodemographic	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‖**
Age* (years)	1.03 (1.02-1.05)	1.04 (1.02-1.05)†	1.03 (1.02-1.05)
Sex (%)			
Male	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Female	2.23 (1.61-3.09)	2.31 (1.67-3.20)§	2.35 (1.65-3.33)
Race (%)			
White	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Mixed	0.68 (0.46-1.02)	0.78 (0.52-1.16)	0.80 (0.53-1.21)
Black	0.85 (0.55-1.33)	0.87 (0.56-1.36)	0.89 (0.56-1.41)
East Asian	0.11 (0.02-0.79)	0.09 (0.01-0.66)	0.09 (0.01-0.65)
Indigenous	1.40 (0.43-4.58)	1.58 (0.48-5.21)	1.63 (0.49-5.41)
Education (years) (%)			
< 11	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
11 to 15	0.93 (0.61-1.42)	1.03 (0.66-1.60)	0.93 (0.59-1.47)
> 15	0.86 (0.57-1.31)	0.82 (0.53-1.25)	0.65 (0.39-1.08)
Mean family income (US$) (%) BRL 2.00 = USD 1.00			
≤ 1245	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
≥ 1246 to 3319	1.13 (0.79-1.60)	1.05 (0.73-1.50)	1.05 (0.72-1.54)
≥ 3320	1.21 (0.82-1.78)	1.00 (0.64-1.43)	0.87 (0.53-1.44)
Marital status (%)			
Single	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Not single	0.68 (0.51-0.92)	0.89 (0.66-1.22)	0.93 (0.67-1.28)
Health insurance (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.25 (0.93-1.69)	1.08 (0.80-1.46)	1.11 (0.80-1.53)
Clinical	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‖**
Body mass index* (kg/m^2)	1.07 (1.05-1.10)	1.07 (1.04-1.10)†	1.04 (0.99-1.11)
Waist circumference* (cm)	1.02 (1.01-1.03)	1.03 (1.02-1.04)§	1.01 (0.98-1.03)
Hypertension (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.49 (0.87-2.54)	1.64 (0.93-2.89)	1.28 (0.70-2.33)
Diabetes (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	2.10 (1.54-2.87)	2.05 (1.48-2.83)	1.92 (1.34-2.76)
Dyslipidemia (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	0.69 (0.52-0.93)	0.61 (0.45-0.83)	0.62 (0.45-0.85)
Smoking (%)			
Never	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Past	1.34 (0.98-1.84)	1.41 (1.02-1.94)	1.38 (0.98-1.93)
Current	0.84 (0.53-1.32)	0.90 (0.57-1.42)	1.03 (0.64-1.66)
Alcohol intake (%)			
Never	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Past	0.67 (0.41-1.08)	0.85 (0.52-1.38)	0.75 (0.44-1.26)
Current	0.64 (0.43-0.96)	0.82 (0.54-1.23)	0.79 (0.51-1.22)
Physical activity (%)			
Mild	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	0.84 (0.53-1.34)	0.85 (0.53-1.35)	0.92 (0.56-1.50)
Vigorous	0.65 (0.34-1.24)	0.70 (0.37-1.35)	0.71 (0.35-1.42)
Depressive disorder (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.71 (0.95-3.05)	1.58 (0.88-2.84)	1.18 (0.63-2.21)

Continue...

Table 3. Continues...

	Risk factors		
Clinical	**Crude**	**Adjusted for age and sex**	**Multivariate adjusted‖**
Bariatric surgery (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	8.92 (2.82-28.29)	7.99 (2.46-25.93)	5.37 (1.53-18.82)
Female participants only			
Previous pregnancy (%)			
No	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Yes	1.48 (0.91-2.40)	1.46 (0.90-2.37)	1.33 (0.80-2.23)
Parity (%)			
0	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
1–3	2.60 (0.81-8.31)	2.67 (0.83-8.56)	2.09 (0.64-6.79)
≥ 4	3.02 (0.87-10.51)	2.61 (0.75-9,13)	1.74 (0.48-6.36)
Fertility age category (%)			
≤ 49 years	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
> 49 years	1.32 (0.93-1.88)	0.75 (0.46-1.23)	0.91 (0.47-1.77)

*Mean (standard deviation); †Adjusted for sex; ‡Multivariate adjustment for all sociodemographic variables; §Adjusted for age; ‖Multivariate adjustment for age, sex, race, education, mean net family income, marital status, health insurance, BMI, waist circumference, hypertension, diabetes, dyslipidemia, smoking, alcohol intake, physical activity, depressive disorder, generalized anxiety disorder and bariatric surgery.

Table 4. Type of cholecystectomy during the first two years of follow-up according to sex.

Cholecystectomy	Females n = 43 (%)	Males n = 13 (%)	P-value
Elective	35 (81.4)	5 (38.5)	0.14
Emergency	8 (18.6)	8 (61.5)	
Laparoscopic	42 (97.7)	11 (84.6)	0.07
Open	1 (2.3)	2 (15.4)	

DISCUSSION

In summary the prevalence of cholecystectomy in the baseline examination was 2.8% (3.6% among women and 1.8% among men). The frequency of cholecystectomy over the first two years of follow-up was 1.2% (1.7% among women and 0.6% among men). The lifetime prevalence of cholecystectomy was 4% (5.3% among women and 2.4% among men) We found that female sex, indigenous ethnicity and high BMI values were associated with cholecystectomy performed during the first two years of follow-up. For lifetime cholecystectomy (cholecystectomy before and after follow-up), positive associations were found with age, female sex, diabetes and bariatric surgery. East Asian ethnicity and dyslipidemia were protective factors against cholecystectomy among the ELSA-Brasil participants. No association was found between cholecystectomy and previously known risk factors for women such as previous pregnancy, parity or age within the fertile stratum (35 to 49 years), with regard to either lifetime cholecystectomy or its occurrence during the first two years of follow-up.

Some studies conducted in other countries have investigated the frequency of cholecystectomy in apparently healthy populations. A cross-sectional study in Taiwan evaluated 2,386 healthy adults and reported that the prevalence of cholecystectomy was 1.3%.[11] A cross-sectional analysis within the German Study of Health in Pomerania (SHIP) cohort evaluated 4,202 subjects aged 20-79 years and found that the prevalence of previous cholecystectomy before enrollment in the study cohort was 11.1%.[24] In Italy, Barbara et al.[25] evaluated 1,911 people aged 18 to 65 in the township of Sirmione and found that the prevalence of previous cholecystectomy was 4.1%. A German study with 2,147 participants aged 10 to 65 years found a prevalence of 3.9%.[26] Only one Brazilian study evaluated the frequency of cholecystectomy, in a sample of 1,000 apparently healthy men (479) and women (521), and it reported a frequency of 2.9%,[4] i.e. similar to the frequency of cholecystectomy in our sample before enrollment in ELSA-Brasil. The results from the present study were similar to those of this previous Brazilian study, but were higher than those of the Taiwan study and lower than the prevalence in Europe, especially compared with the SHIP study, which yielded significantly higher prevalence than the other studies. Considering the lifetime prevalence of cholecystectomy, our results are similar to the European data, except for the SHIP study.

Although data on the prevalence of cholecystectomy worldwide is available, data on its incidence is very scarce. Maclure et al.[8] evaluated the incidence of cholecystectomy in women in the Nurses' Health Study and found 433 new cases in a 4-year follow-up among 88,837 women aged 34 to 59 years.[8] As we did not yet have the estimate of person-years from the ELSA-Brasil survey, in this present study we chose to use logistic models and the frequency of cholecystectomy over the first two years of follow-up, rather than the Cox proportional hazards model, to calculate the incidence of cholecystectomy in the sample.

One important limitation in our analysis is that we did not have information about the presence of gallstones, since in the

ELSA-Brasil survey, we did not perform complete ultrasound scans with decubitus changes in order to look for gallstones. Our main objective was to evaluate the presence of signs of fatty liver disease in the sample, and our protocol focused on including the gallbladder, whenever possible, as one anatomical landmark in the liver images acquired. In this way, we gathered information on whether the gallbladder was present or not when we acquired the image of the right liver lobe, in the same position for all participants who underwent ultrasound measurements.

As stated previously by Pedersen et al.,[27] the prevalence of cholecystectomy does not exactly reflect the prevalence of gallstones. There are also other factors involved, such as increased frequency of symptomatic gallstones, better access to health services, availability of ultrasound examination or a lowered clinical threshold for gallbladder surgery, which could affect this relationship.

Another limitation of the present study in relation to calculating the incidence of cholecystectomy was that the length of follow-up was too short to evaluate the risk of new cases of cholecystectomy based on risk factors identified at the baseline examination.

Our results showed that the main risk factors associated with cholecystectomy after the baseline examination of this study were female sex, indigenous ethnicity and high BMI. On analyzing all the cases of cholecystectomy together, we also found associations with age, higher frequency of being diabetic and previous bariatric surgery.

Aging is associated with gallstones in all racial groups,[28] and in both sexes.[25,29] Most studies also showed higher frequencies of gallstones and cholecystectomy among women than among men.[25,29] Everhart et al.[10] used data from the NHANES III study to evaluate the frequencies of gallstones and surgery to treat gallstones among men and women according to race. They found that among men, the frequency of surgery was around 25 to 35% of the total frequency of gallstones, while among women, this frequency was around 40 to 50%, and neither of these results was related to race. However in the same sample, among Mexican-Americans, more than 50% of the women underwent surgery.[10] A possible explanation for the condition that women are more affected by gallstones than men may be related to the use of estrogen. It has been demonstrated that parity is an important risk factor for gallbladder disease.[30]

Interestingly, in our sample, we did not find any relationship between cholecystectomy and previous pregnancy, parity or the fertile period (from 35 to 49 years of age among women). It is likely that these risk factors have lost their importance over recent years. Fertility rates are progressively declining in Brazil, especially in the state of São Paulo. It is possible that a progressive decline in fertility rates could decrease the impact of parity on gallbladder disease and cholecystectomy in this state. However, Walcher et al.[26] did not find any association between parity and cholecystectomy in Germany in 2005.

Even the association with age does not seem as important now as it once was. In relation to the lifetime prevalence of cholecystectomy, there was a positive association, but if the analysis was restricted to the first two years of follow-up, we found that there was no association between cholecystectomy and aging. However, it is possible that we did not have sufficient statistical power because of the low number of cases.

Ethnicity is another very important risk factor. The highest prevalence of cholelithiasis occurs among North American Indians, such that 73% of female Pima Indians over the age of 30 years are affected,[31] with high rates among other American and Canadian Indians.[32] Although few studies have been published, South American Indians have a similarly high prevalence of gallbladder disease. In Chile, the native Mapuche Indians demonstrate this elevated occurrence: 49.4% among women and 12.6% among men (> 60% among women in their fifties). The frequency is lower among Chilean Hispanics with lesser degrees of American Indian admixture: 36.7% among women and 13.1% among men.[33] The prevalence of gallstones among Mexican-Americans is also a direct function of the degree of Amerindian admixture. White Americans have somewhat lower prevalence.[10] This is the first Brazilian study to show an association between indigenous ethnicity and higher risk of cholecystectomy, thus showing that this high prevalence among Indians is also valid for Brazil. In our sample, we also observed that East Asian ethnicity was a protective factor against cholecystectomy. This is corroborated by the study by Liu et al.[11] in Taiwan, which found a very low prevalence of 1.3%.

It is interesting that presence of diabetes was also a significant factor when considering all the cases of cholecystectomy. There is no clear explanation in the literature for a higher frequency of gallstones among diabetics. However, gallbladder disease and diabetes have a very important common risk factor, obesity,[34] and its effect is stronger among women than among men. Diabetes, abdominal obesity and gallstones appear to be linked through metabolic syndrome.[35,36] Liu et al.[11] also reported an association between cholecystectomy and type 2 diabetes.

In our study, dyslipidemia was protective against gallbladder surgery. This is probably because our definition of dyslipidemia included LDL-cholesterol < 130 mg/dl and/or use of statins. Use of statins was described in a previous study as a protective factor against cholelithiasis.[37] Interestingly, we found that the association between depressive disorders and cholecystectomy showed borderline significance (P = 0.08) among participants undergoing surgery after baseline, and P = 0.07 for all participants who had cholecystectomy. It is possible that we did not have sufficient statistical power for the analysis, but this can be investigated in the future with a longer follow-up period.

Previous bariatric surgery is a factor associated with cholecystectomy that has emerged more recently and is gaining importance, due to the increasing frequency of both obesity and bariatric surgery in Brazil and worldwide. Rapid weight loss on low-calorie diets or after bariatric surgery is a major risk

factor for cholesterol gallstone formation.[1] Sludge and gallstones develop following bariatric surgery on extremely obese individuals in as many as 25–35% of the cases,[2] usually during the first six weeks after surgery, when the weight loss is most profound.[38]

In the present study, laparoscopic surgery was the main method used for cholecystectomy and most of the operations were elective procedures. Laparoscopic cholecystectomy has been the current surgical standard for most diseases of the gallbladder over the past three decades.[39,40] It was no surprise that we found results similar to previous findings[39,40] in a sample with better access to health services than the general population in Brazil. A study at the University of Michigan Medical Center investigated 772 patients who underwent cholecystectomy and found that the frequency of laparoscopic surgery (77.8%) was higher than that of open cholecystectomy (5.7%).[41] A study using the database of the Health Care Utilization Project — Nationwide Inpatient Sample (HCUP-NIS) analyzed 358,091 patients who underwent cholecystectomy procedures from 1999 to 2006. The authors of this study reported that laparoscopic surgery was performed more frequently than open cholecystectomy, and that there was a gradual increase in laparoscopic surgery across all age groups (≥ 18 years) during this period.[42]

Our study has some strengths. It had a large sample of apparently healthy men and women with detailed information about the risk factors that may be associated with cholecystectomy. Only one Brazilian study evaluated the frequency of cholecystectomy worldwide assessed samples that did not have previous complaints associated with gallbladder stones.[4] Our study also has some limitations. It was a cross-sectional analysis and therefore only allowed assessment of associations, rather than causality. We only had information about the frequency of cholecystectomy, without any information about cholelithiasis, unlike other studies that evaluated both of these factors.

CONCLUSIONS

In conclusion, the frequency of lifetime cholecystectomy was similar to values that have previously been published in studies worldwide. As in other countries, most of the surgeries were laparoscopic procedures. Female sex and high BMI values continue to be risk factors, but others, such as parity and fertile age, lost significance, while new factors such as bariatric surgery and indigenous Brazilian ethnicity are gaining importance.

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Address for correspondence:
Isabela Martins Bensenor
Hospital Universitário — Universidade de São Paulo
3º andar — Centro de Pesquisa Clínica e Epidemiológica
Avenida Lineu Prestes, 2.565
São Paulo — São Paulo — Brazil
CEP 05508-000
Tel. (+55 11) 3091-9300
Fax. (+55 11) 3091-9241
E-mail: isabensenor@gmail.com

Prevalence of stunting and overweight/obesity among Brazilian children according to different epidemiological scenarios: systematic review and meta-analysis

Prevalências de déficit de estatura e de sobrepeso/obesidade em crianças brasileiras, segundo diferentes cenários epidemiológicos: revisão sistemática com metanálise

Carolina Pereira da Cunha Sousa[I], Ricardo Alves de Olinda[II], Dixis Figueroa Pedraza[III]

Universidade Estadual da Paraíba (UEPB), Campina Grande, PB, Brazil

[I]MSc. Postgraduate Public Health Program, Universidade Estadual da Paraíba (UEPB), Campina Grande, PB, Brasil.

[II]PhD. Professor in the Department of Statistics, Universidade Estadual da Paraíba (UEPB), Campina Grande, PB, Brasil.

[III]PhD. Professor in the Department of Nursing and Postgraduate Public Health Program, Universidade Estadual da Paraíba (UEPB), Campina Grande, PB, Brasil.

KEY WORDS:
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PALAVRAS-CHAVE:
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ABSTRACT

CONTEXT AND OBJECTIVE: Within the Brazilian nutritional panorama, coexistence of antagonistic nutritional disorders can be seen, especially the increasing prevalence of overweight and the persistence of significant rates of chronic malnutrition in vulnerable groups of the population. Because these are major public health problems, this study aimed to ascertain the prevalence of stunting and overweight/obesity among Brazilian children according to different epidemiological scenarios.

DESIGN AND SETTING: This was a systematic review of prevalence studies, developed at the State University of Paraíba.

METHODS: The SciELO, Lilacs and PubMed databases were searched for articles, using specific keywords. Articles published between 2006 and 2014 were selected. The review was conducted by two reviewers who worked independently. A systematic review with meta-analysis was conducted, for which the studies were grouped within different epidemiological settings.

RESULTS: Among the 33 articles recovered, 9 involved samples from daycare centers, 4 had samples from public healthcare services or social registers, 5 related to populations in situations of social inequity and 15 were population-based. Higher chances of stunting were found in populations in situations of social inequity and in those at public healthcare services or on social registers, in relation to reference populations. For overweight/obesity, none of the scenarios had a higher chance than the reference.

CONCLUSION: Among Brazilian children, stunting continues to be a socially determined public health problem that mainly affects marginalized populations. This problem coexists with significant rates of overweight/obesity affecting all social groups.

RESUMO

CONTEXTO E OBJETIVO: O panorama nutricional brasileiro revela a coexistência de agravos nutricionais antagônicos, com destaque para as prevalências crescentes de excesso de peso e a persistência de taxas expressivas de desnutrição crônica em grupos vulneráveis da população. Por constituírem importantes problemas de saúde pública, este artigo teve por objetivo verificar as prevalências de déficit de estatura e de sobrepeso/obesidade em crianças brasileiras segundo diferentes cenários epidemiológicos.

TIPO DE ESTUDO E LOCAL: Revisão sistemática de estudos de prevalência, desenvolvida na Universidade Estadual da Paraíba.

MÉTODOS: Os artigos foram pesquisados nos bancos de dados SciELO, Lilacs e PubMed, usando-se palavras-chave específicas. Selecionaram-se artigos publicados entre 2006 e 2014. A revisão foi conduzida por dois revisores de forma independente. Realizou-se revisão sistemática com metanálise, para a qual os estudos foram agrupados em diferentes cenários epidemiológicos.

RESULTADOS: Dentre os 33 artigos recuperados, 9 envolveram amostras obtidas em creches, 4 de serviços públicos de saúde ou de cadastros sociais, 5 foram referentes a populações com iniquidades sociais e 15 tinham base populacional. Maiores chances de déficit de estatura foram constatadas nas populações com iniquidade social e naquelas provenientes de serviços públicos de saúde ou de cadastros sociais, em relação às populações de referência. Para o sobrepeso/obesidade, nenhum cenário apresentou maior risco do que a referência.

CONCLUSÕES: Nas crianças brasileiras, o déficit de estatura continua representando um problema de saúde pública de determinação social que atinge, principalmente, populações marginalizadas. Essa problemática coexiste com taxas expressivas de sobrepeso/obesidade que afetam todos os grupos sociais.

INTRODUCTION

Within the current Brazilian nutritional panorama, a declining trend of malnutrition followed by a rapid increase in the prevalence of childhood overweight and obesity can be seen.[1] These paradoxical data are explained by the process of nutritional transition, characterized by inversion of the distribution patterns of nutritional problems.[2]

It has been observed over recent years that the prevalence of overweight/obesity among Brazilian children exceeds the prevalence of weight deficit, with similar behavior in all regions of the country.[3] The exponential increase in overweight/obese rates serves as a warning of the epidemic nature of this disease.[4]

On the other hand, stunting remains an alarming public health problem in Brazil. Children of low socioeconomic status are more vulnerable to this and its prevalence varies within and between regions.[5-7] This context, with uneven advances in terms of nutritional status, indicates that the prevalence of chronic malnutrition in vulnerable groups remains high, especially among children in indigenous populations (26%) and *quilombo* populations (descendants of escapees from slavery; 16%), as well as among those who are beneficiaries of income transfer programs (15%). In relation to excess weight, rapid growth in all age and income groups has been observed.[3]

Stunting during childhood can have undesirable consequences such as increased incidence and severity of infectious diseases, increased infant mortality rates, delayed psychomotor development, lower school performance, emergence of chronic diseases and reduced production capacity in adulthood, with losses in economic growth and social development of the country. Female children of short stature present higher risk that, in adulthood, they will have children with low birth weight, which will have negative effects on nutritional status and morbidity and mortality.[8]

If present early in childhood, obesity is associated with development of chronic diseases in adulthood, decreased quality of life and high healthcare costs. Obesity early in life is a risk factor for respiratory problems, type 2 diabetes mellitus, hypertension, dyslipidemia, metabolic syndrome, atherosclerosis, acute myocardial infarction and stroke. In addition, it is related to psychosocial complications due to social withdrawal consequent to discrimination.[9-11]

OBJECTIVE

Because of the importance that stunting still presents among children, as a public health problem coexisting with greater overweight/obesity, this study aimed to determine the prevalence of stunting and overweight/obesity among Brazilian children according to different epidemiological settings.

METHODS

This was a systematic review with meta-analysis on scientific studies on stunting and overweight/obesity conducted in Brazil that included preschool children and/or those younger than five years of age. Articles of interest were identified by two reviewers who searched the SciELO, Lilacs and PubMed databases independently. The search was limited to title words or abstracts, using the following combination of descriptors in Portuguese and English respectively: (*estado nutricional/nutritional status* OR *crescimento/growth* OR *antropometria/anthropometry* OR *desnutrição/malnutrition* OR *déficit de estatura/stunting* OR *baixa estatura/short stature* OR *sobrepeso/overweight* OR *obesidade/obesity*) AND (*criança/child* OR *pré-escolar/preschool children*).

Articles published between 2006 and the search date (February 26, 2015) were taken into consideration for the purpose of this review. We chose a period beginning in 2006 since this was the year of publication of the new growth curves adopted by the World Health Organization and the year of the last National Survey of Demographics and Health of Children and Women.[12]

All articles identified in the databases were entered into an Excel spreadsheet, with the objective of detecting repetitions of documents in the same database and duplicates in different databases. These procedures were used with the intention of ensuring greater accuracy and reliability of the review results. This spreadsheet was used later on to extract data from selected studies, and it brought together information about the author, publication date, study type, age of participants and outcomes of interest.

The criteria used for including articles in this review were that these needed to be observational studies with representative randomly selected samples; studies with descriptions of stunting prevalence estimated using the anthropometric index of height for age and/or overweight/obesity; studies with estimates using the anthropometric indexes of weight for height or body mass index for age; and studies including preschool Brazilian children and/or those younger than five years of age.

Regarding exclusion criteria, the following studies were considered to be unsuitable for the proposed objectives: review articles; intervention studies; theses; letters to the editor; editorials; correspondence; qualitative studies; studies including deaths; studies conducted outside of Brazil; studies from secondary data (subject to bias); studies based on secondary analysis on population-based nationwide data or specific populations (from which the results were already known and thus their use would diverge from the aim constructed here or would introduce bias); population-based studies on some kind of illness or preexisting nutritional deficit; studies that did not include preschool children and/or those under the age of five years; studies with unrepresentative samples and/or non-random selection (case studies, reports on experiences, clinical cases or case series); studies that lacked data on the outcomes of interest; studies that did not show the prevalence of stunting and/or overweight/obesity among children aged 0-60 months; studies using reference populations that differed

from the ones used by the World Health Organization; and studies that did not report the cutoff points for the nutritional diagnosis or that used inadequate parameters.

The cutoff point that was considered to be appropriate for making a diagnosis of stunting was height for age < -2 or ≤ -2 Z-scores. For overweight/obesity, cutoff points for weight-for-height or body mass index for age > 2 or ≥ 2 Z-scores were considered appropriate. Although the cutoff points that are suitable for diagnosing stunting and overweight/obesity are height-for-age < -2 Z-scores and weight-for-height or body mass index for age > 2 Z-scores, respectively, values ≤ -2 Z-scores for stunting and ≥ 2 Z-scores for overweight/obesity were also considered adequate because these have been used by some authors.

Titles and abstracts were read in order to identify review articles, intervention studies, theses, letters to the editor, editorials, correspondence and qualitative studies. The method or the full study was read in order to identify other selection criteria.

Subsequently, the lists of references of articles already included in the review were reviewed in order to try to identify additional articles. Articles thus identified then underwent the same processes as used for articles that had earlier been identified in bibliographic databases, and were included in this review if it was possible to confirm compliance with the selection criteria.

Discrepancies between the reviewers in the literature search, study selection and classification of articles as included or excluded were resolved by reaching a consensus. This observation was also valid for articles identified in the list of references. Articles not fully available online were purchased.

The articles classified as meeting the selection criteria, and thus included in this review, were characterized according to the following parameters: source, study site, age group, sample size, anthropometric indexes and results (prevalence of stunting and/or overweight/obesity). Furthermore, the quality of the studies was assessed using the critical appraisal tool for prevalence studies that was developed and tested by Munn et al.[13] This tool consists of 10 questions on the adequacy and accuracy of the study, relating to the validity of the methods, interpretation and applicability of the results. Each item was rated with one point when the answer was yes or not applicable, half a point when the answer was unclear and zero points when the answer was no, thus generating a maximum score of 10 points. The score for each article was used to classify the articles into three quality categories: 8 to 10 (high quality); 5 to 7 (average quality); and 0 to 4 (low quality).

The articles were also grouped into four categories according to where their respective samples came from: daycare centers; public healthcare services or social registers; populations in situations of social inequity; and population-based studies representing cities, regions or states. These categories represented epidemiological study scenarios.

In order to produce a synthesis of the data, the results from the studies were systematized by considering the variations in the prevalence of stunting and overweight/obesity according to the epidemiological scenarios adopted. For each scenario, the average prevalence weighted according to sample size and range was calculated. To make statistical syntheses on the first three scenarios, odds ratios for 95% confidence intervals regarding stunting and overweight/obesity were calculated, taking the prevalences found in the National Survey of Demographics and Health of Children and Women[12] and in the population-based studies systematized in this review as the reference data. The significance of differences ($P < 0.05$) among the frequencies found was also ascertained using the chi-square test. The software used in statistical analyses was Rv2.10.0.

RESULTS

Through the above procedures, 1,481 records were identified: 402 in SciELO (of which 199 were repeated), 593 in Lilacs (of which 101 were repeated) and 486 in PubMed. A total of 328 records were replicated among the databases and, thus, 853 documents were identified, after excluding those that were repeated or duplicated. After applying the selection criteria, 823 documents were excluded and 30 articles were considered suitable for the study purposes. Consultation of the reference lists of these 30 articles produced another three articles that met the selection criteria, and therefore a total of 33 articles were read and systematized. Nine of these involved samples from daycare centers, four had samples from primary healthcare units or social registers, five had samples from populations in situations of social inequity and 15 used representative population-based samples from cities, regions and states in Brazil. **Figure 1** shows the flowchart used for identification and selection of studies.

In assessing the quality of articles, six were categorized as medium quality and the other 24, as high quality. The quality criterion for which the articles showed greatest limitation was, remarkably, identification of confounding factors. Given that the estimated quality of all the articles was average or high and that the main risks of bias within the analysis related to confounding factors, without impairing the review of goals, it was decided to systematize all the studies.

Prevalence of stunting and overweight/obesity in samples from daycare centers

Nine articles[14-22] with sample sizes ranging from 189[19] to 676[22] were included. The prevalence of stunting and overweight/obesity ranged from 3.3%[21] to 20.5%[19] and from 2.3%[21] to 7.5%,[15] respectively. The average prevalence of stunting weighted for sample size was 9.11%, and the average prevalence of overweight/obesity weighted for sample size was 5.37% (**Table 1**).

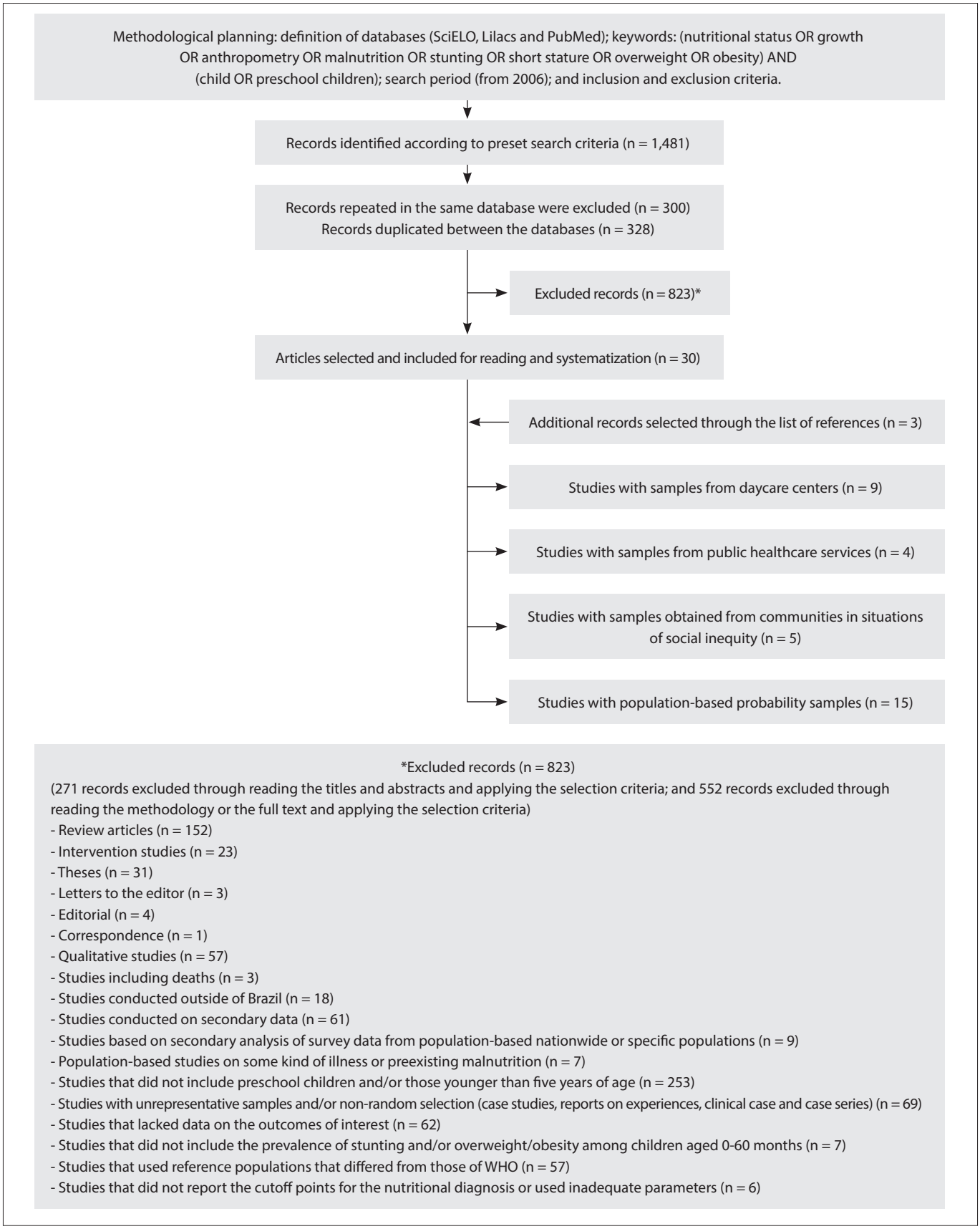

Figure 1. Flowchart used for identification and selection of studies on stunting and/or overweight/obesity among children that were conducted in Brazil and published between 2006 and 2014.

Prevalence of stunting and overweight/obesity in samples obtained from primary healthcare units or social registers

Four articles[23-26] involving sample sizes ranging from 155[23] to 443[25] children were included. Stunting was evaluated in only two studies and showed prevalence rates of 6.3%[25] and 9.7%.[26] According to the body mass index for age, overweight/obesity ranged from 5.2%[25] to 17.9%.[24] The average prevalence of stunting weighted for sample size was 7.25% for stunting and the average prevalence of overweight/obesity weighted for sample size was 10.97% (diagnosed using the body mass index for age) (**Table 2**).

Table 1. Prevalence of stunting and overweight/obesity, according to studies published between 2006 and 2014, involving samples taken from daycare centers located in Brazil

Source	Study site	Age group (months)	Sample size	Anthropometric indexes used	Prevalence (%)*		
					Stunting (H/A < -2 or ≤ -2 Z-scores)	Overweight/obesity	
						W/H > 2 or ≥ 2 Z-scores	BMI/A > 2 or ≥ 2 Z-scores
Figueroa Pedraza et al.[14]	Public daycare centers in Paraíba (PA)	12-72	240	H/A and W/H	5.8	3.8	-
Oliveira et al.[15]	Public daycare centers in the city of Recife (PE)	6-30	321	H/A, W/H and BMI/A	13.4	7.5	10.6
Souzaet al.[16]	Public daycare centers in the city of João Pessoa (PB)	< 60	250	H/A and W/H	7.6	6.4	-
Figueroa Pedraza et al.[17]	Public daycare centers in Paraíba (PA)	6-72	235	H/A	7.7	-	-
Sousa et al.[18]	Public daycare centers in Paraíba (PA)	6-72	365	H/A and W/H	7.4	6.2	-
Martino et al.[19]	Educational centers in Alfenas (MG)	16-82	189	H /A and W/H	20.5	4.0	-
Azevedo et al.[20]	Public daycare centers in the city of Recife (PE)	24-60	344	H/A and W/H	8.6	3.9	-
Camillo et al.[21]	Public daycare centers in the city of Guaxupé (MG)	6-72	211	H/A and W/H	3.3	2.3	-
Bueno et al.[22]	Public daycare centers in the city of São Paulo (SP)	24-84	676	W/H	-	6.2	-
Average weighted for sample size	-	-	314.55	-	9.11	5.37	10.6

H/A = height-for-age; W/H = weight-for-height; BMI/A = body mass index for age.
*Although the cutoff points suitable for making the diagnoses of stunting and overweight/obesity are the H/A values < -2 Z-scores and W/H or BMI/A > 2 Z-scores, respectively, values ≤ -2 Z-scores for stunting and ≥ 2 Z-scores for overweight/obesity were also considered adequate, because they have been used by some authors.

Table 2. Prevalence of stunting and overweight/obesity among children, according to studies published between 2006 and 2014, involving samples from primary healthcare units or social registers in Brazil

Source	Study site	Age group (months)	Sample size	Anthropometric indexes used	Prevalence (%)*		
					Stunting (H/A < -2 or ≤ -2 Z-scores)	Overweight/obesity	
						W/H > 2 or ≥ 2 Z-scores	BMI/A > 2 or ≥ 2 Z-scores
Ferreira-Marim et al.[23]	PHU linked to Ribeirão Preto Medical School (SP)	13-72	155	BMI/A	-	-	9.0%
Momoi et al.[24]	Santo Eduardo PHU and Santa Emília PHU, municipality of Embu (SP)	< 12	414	H/A, W/H and BMI/A	-	-	17.9
Oliveira et al.[25]	Municipality of Paula Cândido (MG) (families registered in the BFP)	6-84	443	H/A, W/H and BMI/A	6.3	2.9	5.2
Barros et al.[26]	PHUs in the municipalities of Vespasiano and Santa Luzia (5 PHUs in each municipality) (MG)	6-24	173	H/A, W/H and BMI/A	9.7	-	-
Average weighted for sample size	-	-	296.25	-	7.25	2.9	10.97

PHU = primary healthcare unit; BFP = Bolsa Família program; H/A = height-for-age; W/H = weight-for-height; BMI/A = body mass index for age.
*Although the cutoff points suitable for making the diagnoses of stunting and overweight/obesity are the H/A values < -2 Z-scores and W/H or BMI/A > 2 Z-scores, respectively, values ≤ -2 Z-scores for stunting and ≥ 2 Z-scores for overweight/obesity were also considered adequate, because they have been used by some authors.

Prevalence of stunting and overweight/obesity in samples obtained from populations in situations of social inequity

Five articles[27-31] involving sample sizes ranging from 99[27] to 973[28] children were included. For stunting, prevalences from 11.5%[28] to 45.3%[29] were found. For the diagnosis of overweight/obesity, all the studies used weight-for-height, while only two[29,30] used the body mass index for age. The minimum and maximum prevalences were 2.1[29] and 7.1%,[28] according to weight-for-height, and they were 5.9%[29] and 6.4%,[30] according to the body mass index for age. The average prevalence of stunting weighted for sample size was 21.42% and the average prevalence of overweight/obesity weighted for sample size was 5.64% and 6.04%, respectively from the weight-for-height and body mass index for age indexes (**Table 3**).

Prevalence of stunting and overweight/obesity in studies representative of cities, regions or states of Brazil

Fifteen articles[32-46] with sample sizes ranging from 164[37] to 6,397[33] children were included. The lowest prevalence of stunting found was 5.0%[32] and the highest was 16.5%.[38] The prevalence of overweight/obesity ranged from 3.2%[37] to 12.5%[39], according to weight-for-height and from 6.3%[38] to 11.2%,[34] according to the body mass index for age. The average prevalence of stunting weighted for sample size was 10.02%. For overweight/obesity, the weighted average prevalence was 10.18%, according to weight-for-height and 7.70%, according to the body mass index for age (**Table 4**).

Prevalence of stunting and overweight/obesity among Brazilian children according to different epidemiological scenarios

Table 5 shows the results found for the analysis categories. It was observed that the highest prevalence of stunting was found among populations in situations of social inequity (21.42%). Taking the prevalence of stunting observed in population-based studies in cities, regions or states in Brazil (10.02%) as a reference, it was found that the likelihood that a child belonging to a population in a situation of social inequity would show stunting was 2.38 times greater (95% CI: 1.03-6.01). Samples from public healthcare services were also likely to show stunting: 2.37 (95% CI: 1.01-6.03). Similar findings were observed when the results from the National Survey of Demographics and Health of Children and Women were taken as the reference.[12] The odds ratios described above were not observed in relation to children at daycare centers or samples from public healthcare services or social registers. With regard to overweight/obesity, it was observed that none of the categories under review (daycare centers, public healthcare units and social registers, and populations in situations of social inequity) had odds ratios higher than the outcome in relation to reference populations.

Table 3. Prevalence of stunting and overweight/obesity among children, according to studies published between 2006 and 2014, involving samples obtained from populations in situations of social inequity located in Brazil

Source	Study site	Age group (months)	Sample size	Anthropometric indexes used	Prevalence (%)*		
					Stunting (H/A < -2 or ≤ -2 Z-scores)	Overweight/obesity	
						W/H > 2 or ≥ 2 Z-scores	BMI/A > 2 or ≥ 2 Z-scores
Pereira et al.[27]	Plak-Ôe village and Terra Nova settlement, in the municipality of São Sebastião (AL)	6-59	99	H/A and W/H	15.6	6.2	-
Ferreira et al.[28]	Remaining *quilombo* communities in the state of Alagoas (AL)	6-59	973	H/A and W/H	11.5	7.1	-
Orellana et al.[29]	Indigenous populations of the Amazon region: Suruí, Xavánte and Wari	0-59	Total: 336 Suruí: 153 Wari: 60 Xavánte: 123	H/A, W/H and BMI/A	Indigenous populations: 45.3 Suruí: 38.6 Xavánte: 42.3 Wari: 68.3	Indigenous populations: 2.1 Suruí: 3.9 Xavánte: 0.8 Wari: 0.0	Indigenous populations: 5.9 Suruí: 5.4 Xavánte: 9.5 Wari: 0.0
Kühl et al.[30]	Indigenous land of Mangueirinha (PR)	0-60	141	H/A, W/H and BMI/A	24.8	3.6	6.4
Pícoli et al.[31]	Kaiowá and Guarani indigenous people of the indigenous area of Caarapó (MS)	0-59	137	H/A	34.1	-	-
Average weighted for sample size	-	-	337.20	-	21.42	5.64	6.04

H/A = height-for-age; W/H = weight-for-height; BMI/A = body mass index for age.

*Although the cutoff points suitable for making the diagnoses of stunting and overweight/obesity are the H/A values < -2 Z-scores and W/H or BMI/A > 2 Z-scores, respectively, values ≤ -2 Z-scores for stunting and ≥ 2 Z-scores for overweight/obesity were also considered adequate, because they have been used by some authors.

DISCUSSION

While nationwide population surveys have shown prevalences of 6.0% of stunting among children younger than five years of age[47] and 7.0%,[12] similar works focusing on populations in situations of social inequity have shown prevalences of 15.0% in populations living in *quilombos*[48] and 25.7%[49] in indigenous populations. In addition, temporal analyses have indicated that there have been sharp declines in the prevalence of stunting among Brazilian children.[50,51] However, it is possible that different behavior has been conditioned, for example, by the length of time that individuals have been benefiting from income transfer programs.[52] Analysis according to income group has indicated that the risk of childhood malnutrition in Brazil is strongly determined by family income.[43] Moreover, analyses on the institutionalization

Table 4. Prevalence of stunting and overweight/obesity among children, according to studies published between 2006 and 2014, involving samples representative of cities, regions or states in Brazil

Source	Study site	Age group (months)	Sample size	Anthropometric indexes used	Prevalence (%)*		
					Stunting (H/A <-2 or ≤-2 Z-scores)	Overweight/obesity	
						W/H> 2 or ≥ 2 Z-scores	BMI/A> 2 or ≥ 2 Z-scores
Jesus et al.[32]	Large cities in the state of Bahia (BA)	< 48	793	H/A	5.0	-	-
Müller et al.[33]	100 municipalities in the five geopolitical regions	< 60	6397	W/H	-	12.0	-
Crispim et al.[34]	Municipality of Goiânia (GO)	< 60	276	BMI/A	-	-	11.2%
Chagas et al.[35]	State of Maranhão (six largest municipalities)	< 60	1214	H/A and W/H	8.5	6.7	-
Queiroz et al.[36]	Municipalities of Laje and Mutuípe (BA)	0-12	489	H/A	5.2	-	-
Souza et al.[37]	Municipalities of Acrelândia and Assis Brasil (AC)	< 60	667	H/A and W/H	9.9	-	-
Moreira et al.[38]	Semiarid region of the state of Alagoas (AL)	< 60	963	W/H	-	8.6	-
Menezes et al.[39]	State of Pernambuco (state, Metropolitan Region of Recife, urban area and rural area) (PE)	0-59	954	BMI/A	-	-	Pernambuco: 8.1 MRR: 9.0 Urban area: 9.7 Rural area: 6.8
Garcia et al.[40]	Urban area of the municipality of Acrelândia (AC)	6-24	164	H/A and W/H	12.0	3.2	-
Oliveira et al.[41]	Municipality of Gameleira (municipality, urban area and rural area) (PE)	< 60	697	H/A, W/H and BMI/A	Gameleira: 16.5 Urban area: 14.9 Rural area: 17.9	Gameleira: 5.6 Urban area: 4.4 Rural area: 6.6	Gameleira: 6.3 Urban area: 4.4 Rural area: 8.0
Jesus et al.[42]	Municipality of Feira de Santana (BA)	< 48	793	W/H	-	12.5	-
Ferreira et al.[43]	Semiarid region of the state of Alagoas (AL)	12-60	716	H/A and W/H	11.5	6.3	-
Ferreira et al.[44]	State of Alagoas (AL)	< 60	1386	H/A and W/H	10.3	9.7	-
Oliveira et al.[45]	Municipality of São João do Tigre (municipality, urban area and rural area) (PB)	< 60	558	H/A and BMI/A	São João do Tigre: 14.6 Urban area: 12.9 Rural area: 16.8	-	São João do Tigre: 7.0 Urban area: 7.6 Rural area: 6.3
Barroso et al.[46]	Municipality of Duque de Caxias (second district) (RJ)	6-30	402	H/A	8.6	-	-
Average weighted for sample size	-		1097.93		10.02	10.18	7.70

H/A = height-for-age; W/H = weight-for-height; BMI/A = body mass index for age.

*Although the cutoff points suitable for making the diagnoses of stunting and overweight/obesity are the H/A values < -2 Z-scores and W/H or BMI/A > 2 Z-scores, respectively, values ≤ -2 Z-scores for stunting and ≥ 2 Z-scores for overweight/obesity were also considered adequate, because they have been used by some authors.

Table 5. Odds ratios and 95% confidence intervals for stunting and overweight/obesity among Brazilian children according to different epidemiological scenarios considering the studies published between 2006 and 2014, taking the reference data to be the prevalence found in the NSDH[12] and the population-based studies on cities, regions or states in Brazil

Sample origin	Prevalence of nutritional disorders (%) (range)	Reference			
		Population-based study on Brazil (NSDH, 2006)		Population-based studies on cities, regions or states in Brazil	
		Odds ratio (95% CI)	P-value (χ^2)	Odds ratio (95% CI)	P-value (χ^2)
Stunting (H/A < -2 or ≤ -2 Z-scores)					
Population-based study on Brazil[12]	7.0	1	-	-	-
Population-based studies on cities, regions or states in Brazil	10.02 (16.5-5.0 = 11.5)	1.47 (0.48; 4.77)	0.61	1	-
Daycare centers	9.11 (20.5-3.3 = 17.2)	1.31 (0.42; 4.34)	0.79	0.89 (0.30; 2.57)	0.99
Populations in situations of social inequity	21.42 (45.3-11.5 = 33.8)	3.52 (1.35; 10.31)	0.01	2.38 (1.03; 6.01)	0.04
Public healthcare services and social registers	7.25 (9.7-6.3 = 3.4)	1.00 (0.28; 3.48)	0.01	0.68 (0.21; 2.07)	0.08
Overweight/obesity (W/H > 2 or ≥ 2 Z-scores)					
Population-based study on Brazil[12]	6.6	1	-	-	-
Population-based studies on cities, regions or states in Brazil	10.18 (12.5-3.2 = 9.3)	1.47 (0.48; 4.77)	0.61	1	-
Daycare centers	5.37 (7.5-2.3 = 5.2)	0.70 (0.16; 2.69)	0.77	0.47 (0.12; 1.60)	0.28
Populations in situations of social inequity	5.64 (7.1-2.1 = 5.0)	0.85 (0.25; 3.07)	0.99	0.57 (0.16; 1.83)	0.43
Public healthcare services and social registers	2.90 (2.9-0.0 = 2.9)	0.41 (0.06; 1.87)	0.33	0.28 (0.05; 1.33)	0.08
Overweight/obesity (BMI/A > 2 or ≥ 2 Z-scores)					
Population-based study on Brazil[12]	-		-	-	-
Population-based studies on cities, regions or states in Brazil	7.70 (11.2-6.3 = 4.9)		-	1	-
Daycare centers	10.60 (10.6-0.0 = 10.6)		-	1.42 (0.49; 4.27)	0.63
Populations in situations of social inequity	6.04 (6.4-5.9 = 0.5)		-	0.73 (0.20; 2.52)	0.78
Public healthcare services and social registers	10.97 (17.9-5.2 = 12.7)		-	1.41 (0.49; 4.26)	0.63

NSDH = National Survey of Demographics and Health of Children and Women; CI = confidence interval; H/A = height-for-age; W/H = weight-for-height; BMI/A = body mass index for age.

of children in daycare centers have suggested that daycare attendance has a protective effect on children's growth.[53,54] The results from this study showed that populations in situations of social inequity are more likely to present stunting than reference populations, unlike the results from studies in which the samples were from daycare centers.

Thus, it was observed that, despite evidence of reductions in the prevalence of stunting among children below the age of five years, this condition still remains a public health problem associated with social inequalities. These findings show that there is a need for measures to prevent stunting, including actions within the socioeconomic, health and educational spheres focusing on socially and economically vulnerable populations. In this regard, care provided through specific programs such as daycare centers and within the context of the Bolsa Família program (an income supplementation program), with assurances regarding the conditions attached to such programs, seems to offer continued assistance for protecting the nutritional status and health of vulnerable children.

The importance of daycare centers in relation to children's nutritional status comes from the fact that they are long-term care institutions that provide virtually all daily meals and constitute an ideal environment for implementation of health promotion strategies and actions, which are conditions that directly affect the nutritional status and growth of children.[2,53] Thus, daycare centers are an important instrument for ensuring food and nutrition safety, and their role has gradually been expanding such that they are becoming a public policy proposal for the education, nutrition and health sectors.[15,20]

Despite the relevance of actions such as providing benefits through the Bolsa Família program, which has been a timely initiative for improving the living conditions of low-income families, it has been suggested that this action alone does not satisfactorily ensure adequate food and nutritional safety levels.[55] This argument is justified with regard to determining children's growth from the perspective of social and economic inequality, as well as in relation to child health inequities. In this context, inadequate conditions within the social and economic environment produce deprivation of the basic necessities of life, with restrictions on food intake, poor health conditions and high morbidity rates, which negatively influence children's growth potential.[56-58] Thus, the Bolsa Família program, which is based on fulfillment of healthcare-related conditional factors, goes beyond income transfer to inclusion of the population involved, in healthcare actions and primary healthcare services that minimize inequities, thereby adding

quality consistent with the potential relating to nutritional status, as explained above.[52]

In relation to the context of public healthcare services, two situations have been suggested: i) there may be greater use of these services among disabled people or those suffering from diseases, who have higher predisposition towards nutritional deficiencies; and ii) healthcare may have a positive effect on nutritional status,[59,60] which cannot be indicated through the results from the meta-analysis shown in this work. In this regard, it is important to highlight that the lack of effect of the Bolsa Família program on indicators relating to children's health may be related to the characteristics of program implementation.[61] This situation indicates that inclusion of input from nutritionists and adequacy of food and nutrition actions are important conditions relating to compliance with the principles of comprehensiveness, universality and problem resolution within healthcare.[62,63]

Regarding overweight/obesity, the results from this study did not indicate any odds ratios indicative of risk or protection in relation to the population-based rates that were used as a reference, for any of the epidemiological scenarios considered. These data may indicate that the distribution of overweight/obesity is equitable among Brazilian children, without any differences in vulnerability. These findings are consistent with the prevalence of overweight/obesity of 5.4% that was found among children in *quilombo* communities in Brazil,[48] as well as the results that systematize this problem among Brazilian children,[64,65] including those institutionalized in daycare centers.[66] In addition to the high frequency of overweight/obesity, the findings from this review provide a warning in relation to progressive increases in rates,[64,65] equitable distribution across social classes[64] and the nutritional transition process and its related factors, in determining the problem.[64-66] Thus, there is a need for preventive measures aimed towards the coexistence of nutritional deficits and excesses, with a view towards ensuring food and nutritional security and the human right to food.

The results presented here show that, despite the undoubted importance of population-based studies nationwide, they do not discriminate between specific situations and, thus, differences prevail according to different epidemiological contexts. In turn, analyses that enable such differentiation make it viable to ascertain the distribution of nutritional disorders and social inequalities within healthcare and therefore to identify the need for specific and differentiated nutritional and health actions.[57,67]

This review, in particular, had some limitations, particularly its inclusion of articles identified in just three bibliographic databases, which restricted the analysis spectrum. Nevertheless, consultation of the reference lists of articles previously included in the review was adopted as a methodological strategy that could minimize this limitation. Despite this restriction, the relevance of the results needs to be highlighted, considering their exceptional nature, given that sources characterized by their quality were used in this review. In this regard, the results presented here demonstrate the challenge faced in developing research and public policies to deal with a nutritional problem among Brazilian children that encompasses stunting in marginalized segments and overweight/obesity in all population groups.

CONCLUSION

The results from this study show the social determinants of stunting among Brazilian children. Its prevalence in populations in situations of social inequity and in populations that use public healthcare services or social registers remains a matter for concern. These populations also present significant overweight/obesity rates. Thus, there is need for appropriate public policies focusing on these realities, while recognizing the possible obstacles implicit in structures and processes that might compromise the effectiveness of these actions.

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Address for correspondence:
Dixis Figueroa Pedraza
Universidade Estadual da Paraíba
Av. das Baraúnas, 351 — Campus Universitário
Bodocongó — Campina Grande (PB) — Brasil
CEP 58109-753
Tel. (+55 83) 3315-3300
E-mail: dixisfigueroa@gmail.com

Paracoccidioidomycosis in the spine: case report and review of the literature

Paracoccidioidomicose na coluna vertebral: relato de caso e revisão da literatura

José Alexandre Lopes da Silva Alvarenga[I], Délio Eulálio Martins[II], Michel Kanas[I], Hugo Gustavo Kunzle Elizeche[III], Adriana Macêdo Dell'Aquila[IV], Eloy De Avila Fernandes[V], Marcelo Wajchenberg[VI], Eduardo Barros Puertas[VII]

Universidade Federal de São Paulo (Unifesp), São Paulo, Brazil

[I]MD. Spine Resident, Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.
[II]MD, PhD. Attending Physician, Department of Orthopedics and Traumatology, Universidade Federal de Sao Paulo (Unifesp), São Paulo, SP, Brazil.
[III]MD. Spine Fellow, Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.
[IV]MD, PhD. Infectious Disease Specialist, Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.
[V]MD, PhD. Affiliated Professor, Department of Imaging Diagnostics, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.
[VI]MD, PhD. Affiliated Professor, Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.
[VII]MD, PhD. Full Professor, Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil.

KEY WORDS:
Paracoccidioides.
Paracoccidioidomycosis.
Spine.
Discitis.
Osteomyelitis.

PALAVRAS-CHAVE:
Paracoccidioides.
Paracoccidioidomicose.
Coluna vertebral.
Discite.
Osteomielite.

ABSTRACT

CONTEXT: Paracoccidioidomycosis is a systemic form of mycosis that spreads hematogenously, secondarily to reactivation of lung infection or infection at another site or to new exposure to the causative agent. Few cases of bone involvement have been reported in the literature and involvement of the spine is extremely rare.

CASE REPORT: We describe a case of a 68-year-old male patient with spondylodiscitis at the levels L4-L5 caused by presence of the fungus *Paracoccidioides brasiliensis,* which was diagnosed through percutaneous biopsy. The patient was treated with sulfamethoxazole and trimethoprim for 36 months, with complete resolution of the symptoms.

CONCLUSION: Spondylodiscitis caused by the fungus *Paracoccidioides brasiliensis* is uncommon. However, in patients with chronic low-back pain who live or used to live in endemic regions, this infection should be considered as a possible differential diagnosis.

RESUMO

CONTEXTO: Paracoccidioidomicose é uma micose sistêmica de disseminação hematogênica, secundária a reativação de uma infecção pulmonar ou de outro sítio, ou a uma nova exposição ao agente causador. Poucos casos de envolvimento ósseo são relatados na literatura, e o acometimento da coluna vertebral é extremamente raro.

RELATO DE CASO: Descrevemos o caso de um paciente masculino de 68 anos, apresentando espondilodiscite no nível L4-L5, causada pela presença do fungo *Paracoccidioides brasiliensis,* diagnosticada após biópsia percutânea. O paciente foi tratado com sulfametoxazol e trimetoprim por 36 semanas, com resolução completa dos sintomas.

CONCLUSÃO: A espondilodiscite causada pelo fungo *Paracoccidioides brasiliensis* é incomum, mas, em pacientes portadores de lombalgia crônica que viveram ou vivem em regiões endêmicas, deve ser considerada como um possível diagnóstico diferencial.

INTRODUCTION

Paracoccidioidomycosis or South American blastomycosis is a granulomatous systemic disease caused by the fungus *Paracoccidioides brasiliensis*.[1,2] It presents geographic distribution limited to Latin America, with higher incidence observed in Brazil, where its prevalence and clinical and epidemiological characteristics vary according to the region of this country.[2-4] Contamination occurs through inhalation of the fungus, which is followed by lymphatic or hematogenous spreading. The infective process is usually asymptomatic and tends to come to an end spontaneously, but remaining residual lesions may contain viable fungus for years.[5] The most commonly affected areas are the skin, mucous membranes and lungs. However, bone involvement is unusual and involvement of the spine is rare.[4,6,7]

CASE REPORT

A 68-year-old male patient, a former farm laborer, presented with low-back pain that had started four months earlier. Over the preceding two years, he had had three episodes of pneumonia and lost 20 kg. He had systemic arterial hypertension, which was controlled through use of antihypertensive drugs. There was no diabetes, thyroid diseases or any other metabolic cause for weight loss. He was a smoker (50 pack-years) and moderate alcohol user.

The symptoms became worse during trunk flexion and there was painful low-back muscle palpation. There were no abnormalities on neurological examination, no fever and no night pain. No signs of consumptive syndrome were noted.

Laboratory tests revealed increased levels of C-reactive protein (CRP) of 118.5 mg/dl (normal, 0.05 mg/dl) and erythrocyte sedimentation rate (ESR) of 53 mm/h (normal < 15 mm/h). The white blood cell count was 6-7 k/mm^3 (normal, 4.0-11.0 k/mm^3). A sputum culture was negative for tuberculosis. Chest radiography and computed tomography (CT) scan did not reveal any signs of tumor or infection.

Radiography of the lumbosacral spine showed diffuse degenerative changes, irregular vertebral endplates of L4 and L5, and reduction of disc spaces L4-L5 and L5-S1 (**Figure 1A**).

Lumbar spine magnetic resonance imaging (MRI) demonstrated spondylodiscitis of L4-L5, with subligamentous abscess anterior to the vertebral bodies, reduced disc height of L4-L5 and L5-S1 and spinal stenosis from L2 to S1 (**Figures 1B** and **1C**).

After analyzing MRI data, main hypotheses for this case were the presence of a tumor, tuberculosis or pyogenic spondylodiscitis. However, transpedicular biopsy of L4 revealed infection with *Paracoccidioides brasiliensis* (**Figure 2**).

Since the patient was not presenting any mechanical or neurological instability, clinical treatment was implemented. Therapy with itraconazole (200 mg/day) was started, but the patient presented an adverse reaction to this drug (worsening of liver function), and therefore this was replaced with sulfamethoxazole and trimethoprim (20 mg/kg) for the rest of the treatment. After 36 months of treatment with sulfamethoxazole and trimethoprim (20 mg/kg), the patient became asymptomatic and the inflammatory blood tests (CRP and ESR) returned to normal.

A control radiograph showed ligament ossification of L4-L5, subchondral sclerosis and reduction of disc space. Control MRI demonstrated reduction of edema and abscess in the paraspinal soft tissues (**Figure 3**).

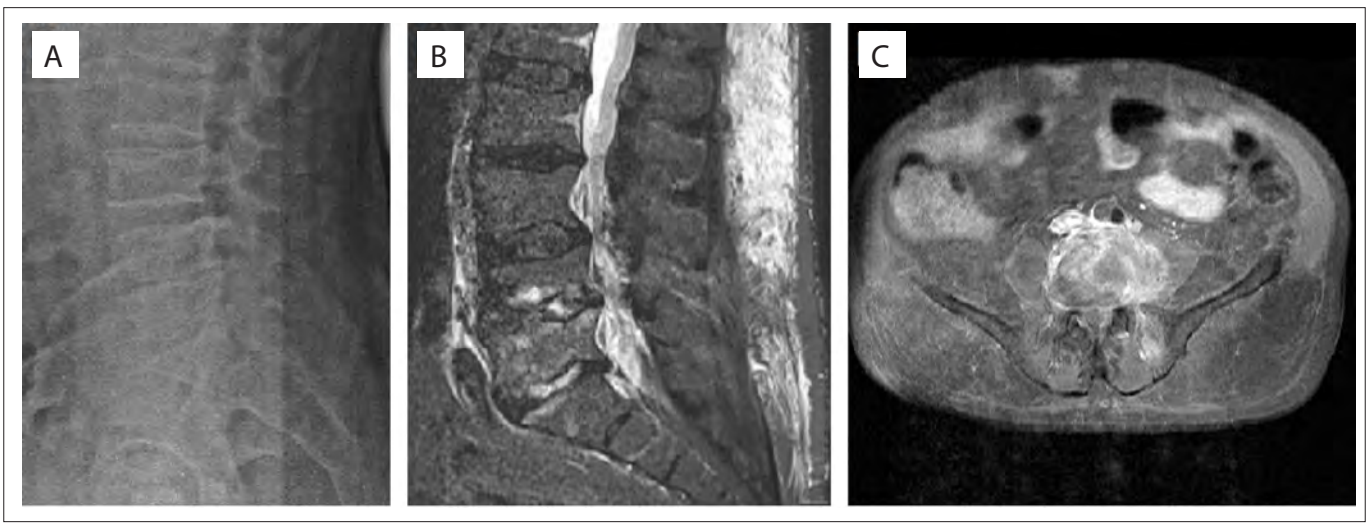

Figure 1. Radiography and magnetic resonance imaging (MRI) of lumbosacral spine. (A) Lateral radiograph showing irregular vertebral plates of L4 and L5, and reduction of disc spaces L4-L5 and L5-S1. (B) MRI in sagittal view with T1 fat-saturation contrast, showing spondylodiscitis of L4-L5, with subligamentous inflammatory tissue and reduced disc height of L4-L5 and L5-S1. (C) MRI in axial view with T1 gadolinium contrast, showing inflammatory tissue involving L4 and left psoas muscle.

DISCUSSION

Paracoccidioidomycosis was originally described in 1908 by Lutz.[8] The infection is caused by inhaling particles of the dimorphic fungus *Paracoccidioides brasiliensis*.

The major risk factor for acquiring this infection are activities involving close contact with soil, given that the soil is contaminated with this fungus in endemic areas. It is primarily acquired during the first two decades of life, with peak incidence between the ages of 10 and 20 years.[4,5,7]

Individuals who become infected may develop two main clinical forms: the acute or subacute form (juvenile type), with severe involvement of internal organs and mononuclear phagocyte system; or the chronic form (adult type), which represents 90% of the cases, with insidious evolution and involvement of the lungs that can leave residual damage (latent foci) with fungus that remains viable for years. In the unifocal presentation, the mycosis is restricted to one organ, which is rare. Most cases affect multiple organs simultaneously.[4,9]

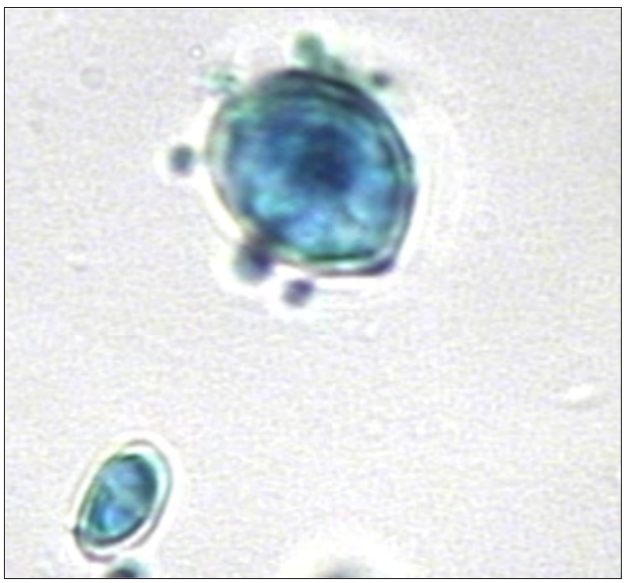

Figure 2. Histological slide of L4, stained with lactophenol cotton blue, showing fungal spores of *Paracoccidioides brasiliensis* (400 X).

Once the fungus has become established in the body, it can affect any organ or tissue, but most commonly it affects the skin, mucous membranes and lungs.[4,6,7] Osteoarticular involvement is unusual, with an incidence that ranges from 5.9 to 23%. This is generally associated with lung infection that spreads to bones through lympho-hematogenous dissemination.[4,7,10-13]

Chest wall bones are the ones most affected by the disease, including ribs, sternum, scapula and acromion.[13] Spinal involvement is rare.

A search in the main electronic databases (PubMed, Embase and Lilacs) using the key words: "Paracoccidioidomycosis" and "Spine" was conducted (**Table 1**). Only four case reports were found,[7,14-16] and only one of them described infection in the vertebral body. The three other reports comprised one of sternal infection,[14] one of spinal cord blastomycotic granuloma[15] and one of vertebral infection caused by *Blastomyces dermatitidis*.[16]

Our case is the first in the literature to show the MRI characteristics of the vertebral body during infection by *Paracoccidioides brasiliensis,* and after antibiotic treatment.

The diagnosis can be made by isolating and identifying the fungus; by direct mycological examination of sputum; by histopathological, cytopathological or cytological examination after puncture biopsy or culturing; or through serological techniques.[17]

In this study, the patient presented the clinical and epidemiological characteristics of the disease, which included: male gender, farm laborer in an endemic area and age over 40 years.[6-13,17-19] The patient needed to have become infected during his second decade of life, when he worked in the fields for 10 years in an

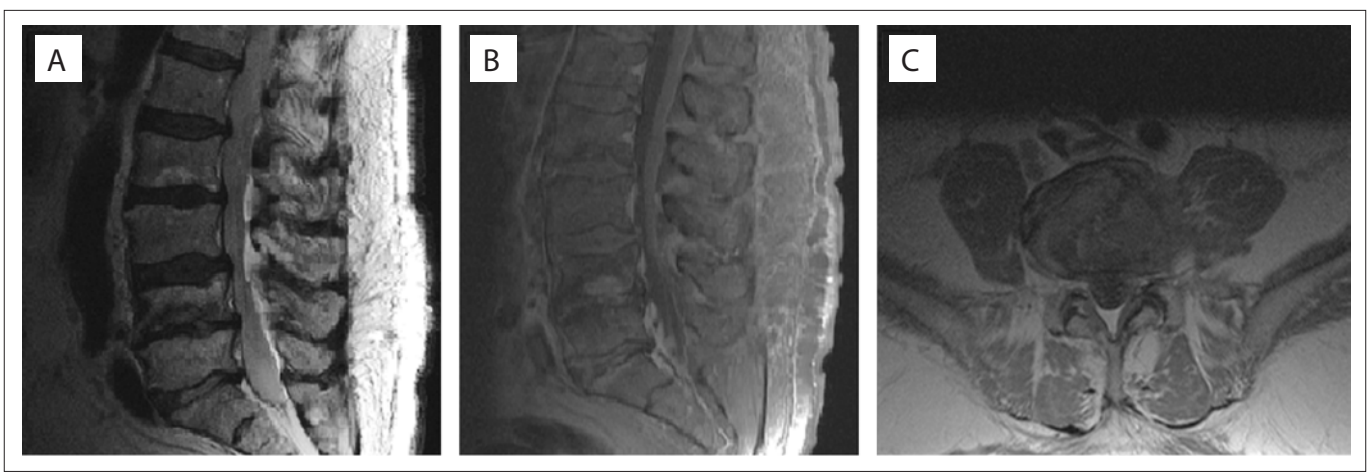

Figure 3. Magnetic resonance imaging (MRI) after 36 months of antibiotic therapy showing reduction of paraspinal soft-tissue edema and abscess. (A) Lateral T2 view. (B) Lateral T1 gadolinium view. (C) Axial T1 gadolinium view.

Table 1. Database search results for Paracoccidioidomycosis and Spine on December 29, 2015

Database	Search Strategies	Papers found	Papers related
Medline (via PubMed)	"Paracoccidioidomycosis"[MeSH] AND "Spine"[MeSH] or "Intraspinal" and "Paracoccidioidomycosis"	2	None with the vertebral body as the main structure affected
Embase (via Elsevier)	"Paracoccidioidomycosis" AND "Spine"	0	0
Lilacs	"Paracoccidioidomycosis" AND "Spine"	2	1

endemic area of the disease. After more than 40 years of incubation, the focus was reactivated.

Fungal infections in the spine are rare, and generally occur in patients with an impaired immune system.[20] The infectious agent usually reaches the vertebral body through small metaphyseal arteries arising from larger periosteal arteries that are branches of spinal arteries. Another proposed route is retrograde flow of venous blood via the Batson plexus.[21,22]

In this case, we could not detect the way in which the infection arrived at the vertebral body (arterial or venous route). However, the patient's medical history of three recent bouts of pneumonia and his weight loss, smoking and alcohol use were likely to have contributed towards compromising his immune system and making him susceptible to reactivation of latent fungal infection.

It was not possible to confirm that the previous repeated episodes of pneumonia were manifestations of paracoccidioidomycosis, since no yeast cells were isolated previously. Although chest radiography and CT scan did not show any signs of infection or scars, the patient did not present any other episode of pneumonia after the antibiotic treatment for spinal infection, over the subsequent two-year follow-up period.

After 36 months, the patient was seen to be clinically asymptomatic, and his blood inflammatory markers were within the normal range. These parameters are considered to be the most important healing criteria.[14]

Control radiographs showed L4-L5 ankylosis, subchondral sclerosis and reduction of disc space. MRI showed regression of paraspinal soft-tissue edema and abscess. We considered that the better-quality imaging provided by MRI, was important for certifying remission of the infection[14] (**Figure 3**).

CONCLUSION

Spondylodiscitis caused by the fungus *Paracoccidioides brasiliensis* is uncommon, but in patients with chronic low-back pain who live or used to live in endemic regions, this infection should be included as a possible diagnosis. In the case presented, the patient had a satisfactory evolution with appropriate treatment.

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Address for correspondence:
Michel Kanas
Rua Borges Lagoa, 783 — 5º andar
São Paulo (SP) — Brasil
CEP 04038-031
Tel. (+55 11) 5571-6621
E-mail: michelkanas@gmail.com

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Pneumonia caused by *Bordetella bronchiseptica* in two HIV-positive patients

Pneumonia causada por *Bordetella bronchiseptica* em dois pacientes HIV-positivos

Roberta Filipini Rampelotto[I], Andreas Hörner[II], Christine Hörner[III], Roselene Righi[IV], Rosmari Hörner[V]

Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

[I]MSc. Doctoral Student, Postgraduate Pharmaceutical Sciences Program, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

[II]Undergraduate Student, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

[III]Undergraduate Student, Universidade do Extremo Sul Catarinense (UNESC), Criciúma, SC, Brazil.

[IV]MSc. Pharmacist, Bacteriology Laboratory, Hospital Universitário de Santa Maria (HUSM), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

[V]PhD. Associate Professor, Department of Clinical and Toxicological Analyses, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

KEY WORDS:
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Case reports [publication type].
Humans.

PALAVRAS-CHAVE:
Bordetella bronchiseptica.
HIV.
Pneumonia.
Relatos de casos.
Humanos.

ABSTRACT

CONTEXT AND OBJECTIVE: *Bordetella bronchiseptica* (BB) is a Gram-negative coccobacillus responsible for respiratory diseases in dogs, cats and rabbits. Reports on its development in humans are rare. However, in immunosuppressed patients, especially in those with the immunodeficiency virus (HIV), BB can cause severe pulmonary infections. We report on two cases of pneumonia caused by BB in HIV-positive male patients in a university hospital.

CASE REPORT: The first case comprised a 43-year-old patient who was admitted presenting chronic leg pain and coughing, with suspected pneumonia. BB was isolated from sputum culture and was successfully treated with trimethoprim/sulfamethoxazole in association with levofloxacin. The second case comprised a 49-year-old patient who was admitted presenting fever, nausea, sweating and a dry cough, also with suspected pneumonia. BB was isolated from sputum culture, tracheal secretions and bronchoalveolar lavage. The disease was treated with ciprofloxacin but the patient died.

CONCLUSION: BB should be included in the etiology of pneumonia in immunodeficient HIV patients. As far as we know, these two were the first cases of pneumonia due to BB to occur in this university hospital.

RESUMO

CONTEXTO E OBJETIVO: *Bordetella bronchiseptica* (BB) é um cocobacilo Gram-negativo responsável por causar doenças no trato respiratório de cães, gatos e coelhos. São raros os relatos do desenvolvimento desse microrganismo em seres humanos. Porém, em pacientes imunodeprimidos, especialmente nos portadores do vírus da imunodeficiência humana (HIV), a BB pode causar infecções pulmonares graves. Nós relatamos dois casos de pneumonia por BB em pacientes do sexo masculino, HIV-positivos em um hospital universitário.

RELATO DE CASO: No primeiro caso, o paciente de 43 anos foi internado apresentando dor crônica nos membros inferiores e tosse com suspeita de pneumonia. Na cultura de escarro, foi isolado BB, e a infecção foi tratada com sucesso com a associação de sulfametoxazol/trimetroprima e levofloxacino. No segundo caso, o paciente de 49 anos foi internado apresentando febre, náuseas, sudorese e tosse seca, também com suspeita de pneumonia. Das culturas de escarro, secreção traqueal e lavado bronco-alveolar, foi isolado BB, infecção tratada com ciprofloxacino: porém, o paciente foi a óbito.

CONCLUSÃO: BB deve ser incluído na etiologia de pneumonia em pacientes imunocomprometidos com HIV. Pelo que é de nosso conhecimento, estes dois relatos foram os primeiros casos de pneumonia por BB que ocorreram neste hospital universitário.

INTRODUCTION

Bordetella bronchiseptica (BB) is a strictly aerobic Gram-negative coccobacillus that causes diseases in the respiratory tract of animals, such as infectious tracheobronchitis or "canine cough" and pneumonitis in dogs, pneumonia in cats, coryza, pneumonia and otitis media in rabbits and bronchopneumonia in pigs.[1-5]

Infections caused by BB are rare in humans but this bacterium can be isolated as a commensal agent in the human respiratory tract.[1,2,4-6] BB occurs independently from contact with colonized animals, and can persist in the environment for long periods of time.[7,8] In general, humans acquire BB through contact with domestic animals but this can also occur through cross-contamination with hospital patients.[1,2]

In human beings, BB preferentially attacks the respiratory tract and can cause severe pulmonary infections, such as infectious bronchitis, pneumonia and bacteremia. It generally occurs in patients with weak immune systems, such as AIDS patients.[6-9]

Two cases of pneumonia caused by BB in HIV-positive patients at a university hospital are reported below.

CASE REPORTS

Report 1

The patient was a 43-year-old HIV-positive man who had been diagnosed with AIDS in 2006 and was positive for the hepatitis C virus (HCV). He was a homeless drug addict and alcoholic. He had pleural tuberculosis in 2000 and pulmonary tuberculosis in 2012. He was admitted to hospital in 2007 to investigate a cerebral mass lesion suggestive of toxoplasmosis or lymphoma. This diagnosis was not confirmed at the time but the patient showed some improvement with treatment for neurotoxoplasmosis. In November 2012, he was again admitted for treatment of nervous conditions caused by HIV/AIDS. In August 2013, the patient was admitted again showing deep vein thrombosis (DVT) and a subacute respiratory condition that indicated pneumonia, as shown on lung radiography (**Figure 1**). The treatment consisted of anticoagulant therapy and empirical treatment for pneumocystosis. Because the patient showed clinical improvement, he was released.

In May 2014, the patient was admitted again for treatment of disseminated AIDS-related disease, presenting a viral load of 589 copies/ml (reference value, RV: 493-1666 copies/ml) and CD8 212 cell/ml (RV: 224-1112 cell/ml). He complained of chronic pain in the legs, sacral eschar and a dry cough. During his stay in the hospital, antiretroviral treatment was started, with good tolerance to the medicine. Several tests were performed: routine blood tests, C-reactive protein and sputum and blood cultures.

The hemogram showed neutropenia compatible with bacterial infection; elevated C-reactive protein, 0.91 mg/dl (RV: below 0.3 mg/dl), thus suggesting inflammation or infection. BB was isolated from the sputum sample. At this point, antibiotic therapy with trimethoprim/sulfamethoxazole was started to combat BB. However, the antibiogram showed that the bacterium was sensitive to amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem, amikacin, gentamicin, nalidixic acid, levofloxacin, ciprofloxacin and norfloxacin; that it had an intermediate profile in relation to ampicillin, cefalotin and cefepime; and that it was resistant to cefuroxime, cefuroxime axetil, ceftriaxone, nitrofurantoin and trimethoprim/sulfamethoxazole. Thus, the antimicrobial therapy was changed to levofloxacin. The blood culture was negative.

The patient was released at the end of May 2014, with a recommendation to use HIV antiretroviral medication and the antimicrobial agent for an additional seven days.

Report 2

The patient was a 49-year-old male who had been diagnosed positive for HIV, AIDS and HCV in 2012, with no treatment. He had been a smoker for 37 years. He presented hepatitis C with a heterogeneous mass lesion anterior to the 12th thoracic vertebra. In April 2014, the patient reported that he had low-intensity lumbago irradiation pain for one month. Two weeks later, he reported intensified pain irradiating to the abdomen. A biopsy of the mass lesion was performed and showed a benign result. The patient was admitted to hospital at the end of May 2014 with a condition of night sweating, fever peaks (39 °C), abdominal pain, nausea, anorexia and pain in the left thigh for the last 30 days. He presented a viral load of 427 copies/ml, CD4 60 cell/ml

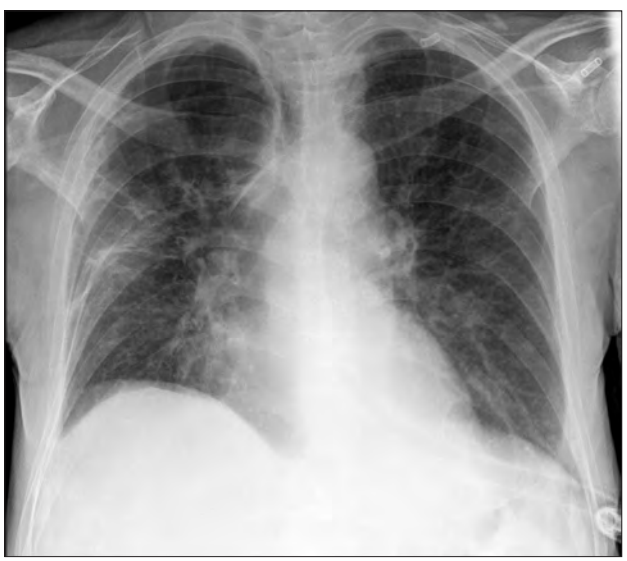

Figure 1. Lung radiograph of the patient in the first report, indicating pneumonia caused by *Bordetella bronchiseptica*.

(RV: 493-1666 cell/ml) and CD8 180 cell/ml (RV: 224-1112 cell/ml). He presented symptoms of peritoneal irritation. He had also been suffering from dyspnea and a dry cough for the last five days. He was suspected of having pneumonia, as shown on lung radiography (**Figure 2**). Therefore, empirical treatment for pneumonia was started.

At the end of May he was taken to the intensive care unit (ICU) because of complications.

At the beginning of June 2014, the patient presented respiratory insufficiency. Thoracic computed tomography (CT) was performed, focusing especially on the right side (**Figure 3**). He remained in the ICU until June 10. Tests comprising sputum culturing, tracheal secretion culturing, bronchoalveolar lavage and blood culturing were then done. The blood cultures showed negative results, but BB was isolated from the samples of the other clinical specimens. The antibiogram of the three materials showed the same sensitivity profile: sensitivity to amikacin, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole and tigecycline; intermediate profile towards ampicillin/sulbactam, ampicillin, cefepime and ceftazidime; and resistance to ceftriaxone and cefuroxime. The patient received treatment for BB initially with trimethoprim/sulfamethoxazole; this was then replaced by treatment with ciprofloxacin (10 days) and meropenem (7 days). At this point, the patient's contact with 25 domestic dogs became known. The patient died at the end of June, and the possible causes included septicemia, pneumonia, AIDS and hypertension.

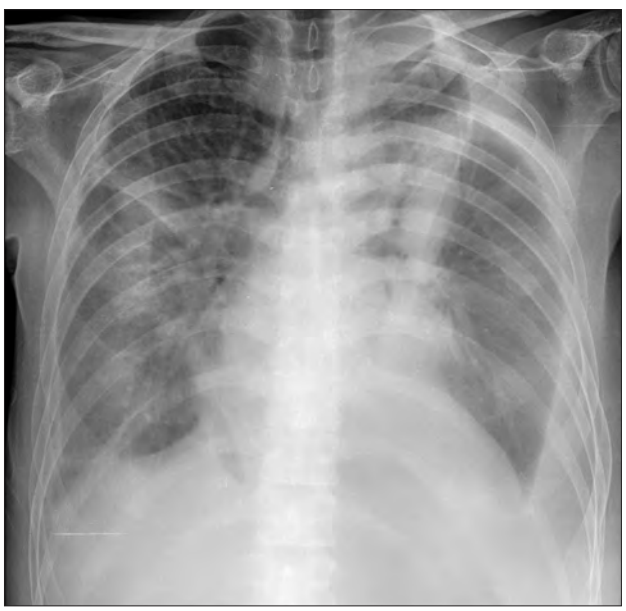

Figure 2. Lung radiograph from the second report, indicating pneumonia caused by *Bordetella bronchiseptica*.

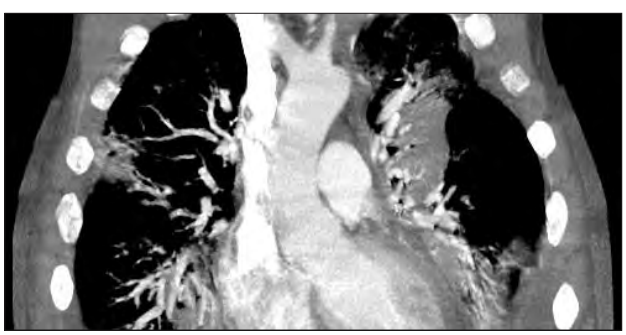

Figure 3. Thoracic computed tomography from the second report.

DISCUSSION

We reported two cases of AIDS patients suffering from pneumonia due to BB. This microorganism is responsible for rare cases of chronic infection of the respiratory tract in humans, especially among individuals with underlying diseases.[10] Formerly, BB used to be an uncommon cause of infection in hosts with a compromised immune system.[11] However, cases among immunocompromised patients have been reported especially among those with HIV. The clinical condition is generally associated with infections in the upper respiratory tract, pneumonia, endocarditis and bacteremia.[6,9,12] In our review of the literature using the Medline (http://www.ncbi.nlm.nih.gov/pubmed/), Embase (http://www.elsevier.com/online-tools/embase), SciELO (http://www.scielo.org/php /index.php) and Lilacs (http://lilacs.bvsalud.org/en/) databases, we did not find any article describing pneumonia in HIV-positive patients caused by *Bordetella bronchiseptica* (**Table 1**).

Table 1. Review of medical databases using the descriptors corresponding to the main features presented by the patient, conducted on December 2, 2015

Database	Search strategy	Results
PubMed	("Bordetella bronchiseptica" [MeshH]) AND "Human Immunodeficiency Virus" [MesH]) AND ("Pneumonia"[MesH]) AND ("Pneumonia"[MesH]) AND ("Case reports"[MesH]) AND ("Humans"[MesH])	0 article
Embase	("Bordetella bronchiseptica" [MeshH]) AND "Human Immunodeficiency Virus" [MesH]) AND ("Pneumonia"[MesH]) AND ("Pneumonia"[MesH]) AND ("Case reports"[MesH]) AND ("Humans"[MesH])	0 article
SciELO	("Bordetella bronchiseptica" [MeshH]) AND "Human Immunodeficiency Virus" [MesH]) AND ("Pneumonia"[MesH]) AND ("Pneumonia"[MesH]) AND ("Case reports"[MesH]) AND ("Humans"[MesH])	0 article
Lilacs	("Bordetella bronchiseptica" [MeshH]) AND "Human Immunodeficiency Virus" [MesH]) AND ("Pneumonia"[MesH]) AND ("Pneumonia"[MesH]) AND ("Case reports"[MesH]) AND ("Humans"[MesH])	0 article

Since 1991, rare cases of BB have been reported in AIDS or immunocompromised patients.[9,13-15] One of these reports was similar to the two reported above: a 42-year-old HIV-positive patient with pulmonary symptoms similar to pneumonia caused by *Pneumocystis jiroveci*. The sputum culture was positive for BB and treatment with levofloxacin, trimethoprim/sulfamethoxazole and azithromycin was successfully used. The patient had been in contact with a domestic dog.[9]

Wernli et al. described eight cases of BB that caused infection or colonization in human beings over a 15-year period.[16] Those researchers showed that seven of their patients had underlying diseases and that only three had been in contact with domestic animals. The patients' symptoms ranged from no symptoms to severe pneumonia. It was not possible to establish a homogeneous pattern regarding clinical disease among the symptomatic patients.[16]

A study by García-de-la-Fuente et al. in 2015 showed that most of the patients from whom BB was isolated presented a compromised immune system as well as an underlying disease, and 82% presented respiratory symptoms.[17] Respiratory tract diseases are the major cause of morbidity and mortality among AIDS patients. The etiology of these respiratory infections varies according to factors such as CD4 levels, location of home, socioeconomic conditions and use of chemoprophylaxis. The CD4 count is the main risk predictor for progression to AIDS and death among HIV-positive patients.[18]

Although *B. bronchiseptica* is only rarely isolated in humans, it should be considered to be potentially pathogenic when found in samples from the respiratory tract in patients with a compromised immune system.[4-6,16] Sputum culturing and investigation of exposure to animals are recommended.[9,16] It became known that the patient in Report 2 had been in contact with 25 domestic dogs. These animals were the potential source of the acquired infection.[1,2]

Empirical antibiotic therapy using trimethoprim/sulfamethoxazole can be justified, given that this drug is the first choice for treating pulmonary pneumocystosis. This is caused by the unicellular fungus *Pneumocystis jiroveci*, a saprophyte of the respiratory tract that causes pneumonia in many HIV-positive patients. Its occurrence decreases substantially through antiretroviral therapy.[18,19]

Given that the antimicrobial treatment for infections caused by BB has not been clearly established, ascertaining the sensitivity profile of the antimicrobials is fundamental.[20] García-de-la-Fuente et al. studied 36 respiratory tracts from which BB was isolated and found that the minimum inhibitory concentration (MIC) of antimicrobial agents was smaller for tigecycline, colistin, tetracycline, minocycline, doxycycline and meropenem; and it was larger for beta-lactams, macrolides and trimethoprim/sulfamethoxazole.[17] According to other studies, this microorganism generally presents strong resistance to macrolides and clindamycin. Thus, treatment can be done using trimethoprim/sulfamethoxazole, fluoroquinolones and penicillins.[11,16]

Other studies have reported 100% sensitivity in vitro to aminoglycosides, penicillins and cephalosporins with anti-*Pseudomonas* activity, and to carbapenems, quinolones and tetracyclines.[6] The Clinical and Laboratory Standards Institute (CLSI) does not recommend inclusion of erythromycin and clindamycin in the antibiogram; it advises that the antibiogram should be produced using quantitative methodology, including gentamicin, tobramycin, piperacillin and ceftazidime as the first line (group A); and piperacillin/tazobactam, ticarcillin/clavulanic acid, cefepime, amikacin, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole (group B) for the more resistant isolates.[21]

B. bronchiseptica has only rarely been isolated from clinical human specimens, in spite of the considerable exposure to this microorganism through colonized animals.[7] Antimicrobial treatment should be started as soon as possible, especially in immunocompromised patients.[6]

CONCLUSION

In spite of the fact that BB is associated with colonization in the respiratory tract and bronchopulmonary disease, it is difficult to clearly establish the pathogenic effect of this microorganism in human beings; its isolation presents challenging results. However, BB should be considered to be a possible agent for pneumonia since it has virulence factors, such as production of toxins. Isolation of BB from biological materials makes it possible to know its resistance profile, which can improve the prognosis considerably. As far as we know, up to the present time, the two cases reported above were the first cases in this university hospital.

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Consent: The evidence of these cases was obtained from a study conducted by our research group that explored the records of patients who were affected by bacteria characterized as *Bordetella bronchiseptica*. Thus, data acquired through analysis on the records led to compilation of this manuscript. The analysis on the records of one patient in this study was conducted subsequent to his death. Therefore, we tried to contact the patient's family through the telephone numbers in the records but were unsuccessful. Although the other patient was still alive at the time of compiling this report, we were unable to contact him

Ethical approval: This study was approved by the Research Ethics Committee of the Federal University of Santa Maria under no. 38850614.4.0000.5346

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Address for correspondence:
Rosmari Hörner
Laboratório de Bacteriologia, Departamento de Análises Clínicas e Toxicológicas (DACT), Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM)
Campus UFSM, Prédio 26, Sala 1.201
Santa Maria (RS) — Brasil
CEP 97015-900
Tel. (+55 55) 3220-8751
Fax: (+55 55) 3220-8751
E-mail: rosmari.ufsm@gmail.com

Antibiotics for mastitis in breastfeeding women

This is the abstract of a Cochrane Review published in the Cochrane Database of Systematic Reviews 2013, issue 2, art. no. CD005458. DOI: 10.1002/14651858.CD005458.pub3. For full text and details about the authors, see reference 1.

Shayesteh Jahanfar, Chirk Jenn Ng, Cheong Lieng Teng

The independent commentary was written by César Eduardo Fernandes

ABSTRACT

BACKGROUND: Mastitis can be caused by ineffective positioning of the baby at the breast or restricted feeding. Infective mastitis is commonly caused by *Staphylococcus aureus*. The prevalence of mastitis in breastfeeding women may reach 33%. Effective milk removal, pain medication and antibiotic therapy have been the mainstays of treatment.

OBJECTIVES: This review aims to examine the effectiveness of antibiotic therapies in relieving symptoms for breastfeeding women with mastitis with or without laboratory investigation.

METHODS:

Search methods: We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (30 September 2012), contacted investigators and other content experts known to us for unpublished trials and scanned the reference lists of retrieved articles.

Selection criteria: We selected randomised controlled trials (RCTs) and quasi-RCTs comparing the effectiveness of various types of antibiotic therapies or antibiotic therapy versus alternative therapies for the treatment of mastitis.

Data collection and analysis: Two review authors independently assessed trial quality and extracted data. When in dispute, we consulted a third author.

MAIN RESULTS: Two trials met the inclusion criteria. One small trial (n = 25) compared amoxicillin with cephradine and found no significant difference between the two antibiotics in terms of symptom relief and abscess formation. Another, older study compared breast emptying alone as 'supportive therapy' versus antibiotic therapy plus supportive therapy, and no therapy. The findings of the latter study suggested faster clearance of symptoms for women using antibiotics, although the study design was problematic.

AUTHORS CONCLUSIONS: There is insufficient evidence to confirm or refute the effectiveness of antibiotic therapy for the treatment of lactational mastitis. There is an urgent need to conduct high-quality, double-blinded RCTs to determine whether antibiotics should be used in this common postpartum condition.

The full text of this review is available free-of-charge from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD005458.pub3/epdf

The abstract is also available in the Portuguese and English languages

COMMENTS

The present review addresses an important issue involving the use of antibiotics to treat mastitis in breastfeeding women, which is common in its infectious form. Considering its infectious nature, the treatment has traditionally consisted of using antibiotics. However, the basis of the present knowledge is empirical and this topic deserves a review.

Only two studies met the inclusion criteria proposed for this review. One, which comprised a small sample, compared two antibiotics, amoxicillin and cephradine, and showed no difference between them in terms of relieving the symptoms and abscess formation. Another study, in 1984, conducted with a larger sample and with no description of blinding methods, monitored breastfeeding women with infectious mastitis divided into three groups: one receiving no treatment; another advised to empty the breasts; and the third treated with antibiotics (penicillin, ampicillin or erythromycin). This study found that the symptoms improved more rapidly among the women who used antibiotics. Based on the studies analyzed, the authors of the review concluded that there was insufficient evidence to confirm or refute the efficacy of antibiotic therapy for treating mastitis and urgently recommended that appropriate studies should be conducted to seek answers. From the conclusion of this Cochrane review and the current knowledge, we believe that the decision to use antibiotics in cases of infectious lactation mastitis remains eminently clinical and the final decision regarding this matter should be made by the physician.

César Eduardo Fernandes. Full Professor of Gynecology, Faculdade de Medicina do ABC (FMABC), Santo André, SP, Brazil.

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 DOI: 10.1590/1516-3180.20161343T2

Vitamin D supplementation for women during pregnancy

This is the abstract of a Cochrane Review published in the Cochrane Database of Systematic Reviews 2016, issue 1, art. no. CD008873. DOI: 10.1002/14651858.CD008873.pub3. For full text and details about the authors, see reference 1.

Luz Maria De-Regil, Cristina Palacios, Lia K Lombardo, Juan Pablo Peña-Rosas

The independent commentary was written by Corintio Mariani Neto

ABSTRACT

BACKGROUND: Vitamin D deficiency or insufficiency is thought to be common among pregnant women. Vitamin D supplementation during pregnancy has been suggested as an intervention to protect against adverse pregnancy outcomes.

OBJECTIVES: To examine whether oral supplements with vitamin D alone or in combination with calcium or other vitamins and minerals given to women during pregnancy can safely improve maternal and neonatal outcomes.

METHODS:

Search methods: We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (23 February 2015), the International Clinical Trials Registry Platform (31 January 2015), the Networked Digital Library of Theses and Dissertations (28 January 2015) and also contacted relevant organisations (31 January 2015).

Selection criteria: Randomized and quasi-randomized trials with randomization at either individual or cluster level, evaluating the effect of supplementation with vitamin D alone or in combination with other micronutrients for women during pregnancy.

Data collection and analysis: Two review authors independently i) assessed the eligibility of studies against the inclusion criteria ii) extracted data from included studies, and iii) assessed the risk of bias of the included studies. Data were checked for accuracy. The quality of the evidence was assessed using the GRADE approach.

MAIN RESULTS: In this updated review we included 15 trials assessing a total of 2833 women, excluded 27 trials, and 23 trials are still ongoing or unpublished. Nine trials compared the effects of vitamin D alone versus no supplementation or a placebo and six trials compared the effects of vitamin D and calcium with no supplementation. Risk of bias in the majority of trials was unclear and many studies were at high risk of bias for blinding and attrition rates.

- Vitamin D alone versus no supplementation or a placebo

Data from seven trials involving 868 women consistently show that women who received vitamin D supplements alone, particularly on a daily basis, had higher 25-hydroxyvitamin D than those receiving no intervention or placebo, but this response was highly heterogeneous. Also, data from two trials involving 219 women suggest that women who received vitamin D supplements may have a lower risk of pre-eclampsia than those receiving no intervention or placebo (8.9% versus 15.5%; risk ratio (RR) 0.52; 95% CI 0.25 to 1.05, low quality). Data from two trials involving 219 women suggest a similar risk of gestational diabetes among those taking vitamin D supplements or no intervention/placebo (RR 0.43; 95% CI 0.05, 3.45, very low quality). There were no clear differences in adverse effects, with only one reported case of nephritic syndrome in the control group in one study (RR 0.17; 95% CI 0.01 to 4.06; one trial, 135 women, low quality). Given the scarcity of data for this outcome, no firm conclusions can be drawn. No other adverse effects were reported in any of the other studies.

With respect to infant outcomes, data from three trials involving 477 women suggest that vitamin D supplementation during pregnancy reduces the risk preterm birth compared to no intervention or placebo (8.9% versus 15.5%; RR 0.36; 95% CI 0.14 to 0.93, moderate quality). Data from three trials involving 493 women also suggest that women who receive vitamin D supplements during pregnancy less frequently had a baby with a birthweight below 2500 g than those receiving no intervention or placebo (RR 0.40; 95% CI 0.24 to 0.67, moderate quality). In terms of other outcomes, there were no clear differences in caesarean section (RR 0.95; 95% CI 0.69 to 1.31; two trials; 312 women); stillbirths (RR 0.35 95% CI 0.06, 1.99; three trials, 540 women); or neonatal deaths (RR 0.27; 95% CI 0.04, 1.67; two trials, 282 women). There was some indication that vitamin D supplementation increases infant length (mean difference (MD) 0.70, 95% CI -0.02 to 1.43; four trials, 638 infants) and head circumference at birth (MD 0.43, 95% CI 0.03 to 0.83; four trials, 638 women).

- Vitamin D and calcium versus no supplementation or a placebo

Women who received vitamin D with calcium had a lower risk of pre-eclampsia than those not receiving any intervention (RR 0.51; 95% CI 0.32 to 0.80; three trials; 1114 women, moderate quality), but also an increased risk of preterm birth (RR 1.57; 95% CI 1.02 to 2.43, three studies, 798 women, moderate quality). Maternal vitamin D concentration at term, gestational diabetes, adverse effects and low birthweight were not reported in any trial or reported only by one study.

AUTHORS CONCLUSIONS: New studies have provided more evidence on the effects of supplementing pregnant women with vitamin D alone or with calcium on pregnancy outcomes. Supplementing pregnant women with vitamin D in a single or continued dose increases serum 25-hydroxyvitamin D at term and may reduce the risk of pre-eclampsia, low birthweight and preterm birth. However, when vitamin D and calcium are combined, the risk of preterm birth is increased. The clinical significance of the increased serum 25-hydroxyvitamin D concentrations is still unclear. In light of this, these results need to be interpreted with caution. Data on adverse effects were lacking in all studies.

The evidence on whether vitamin D supplementation should be given as a part of routine antenatal care to all women to improve maternal and infant outcomes remains unclear. While there is some indication that vitamin D supplementation could reduce the risk of pre-eclampsia and increase length and head circumference at birth, further rigorous randomized trials are required to confirm these effects.

The full text of this review is available free-of-charge from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008873.pub3/epdf

The abstract is also available in the Portuguese, English and Chinese languages

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COMMENTS

This systematic review aimed to ascertain whether supplementation with vitamin D alone or in combination with calcium during prenatal care is beneficial, since vitamin D deficiency or insufficiency is common

among pregnant women. This analysis on fifteen studies, involving nearly 3000 women, showed that the results are still controversial and conflictive. Thus, it seems that supplementation with vitamin D alone reduces the risk of premature birth and low birth weight. However, the lower risk of preeclampsia is more evident only when vitamin D is administered in association with calcium, which, in turn, increases the risk of premature birth. Vitamin D alone also seems to increase the length of the newborn, and also its head circumference. No other results proved conclusive and, moreover, the data on the occurrence of adverse effects were not presented.

Therefore, studies with greater rigor that might confirm the beneficial effects are needed before routine vitamin D supplementation can be recommended for all pregnant women, in order to improve maternal and perinatal outcomes.

Corintio Mariani Neto, MD, MSc, PhD. Professor of Women's Health, Medical School, City of São Paulo University, São Paulo, Brazil.

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Article in journal

- Hurt AC, Hardie K, Wilson NJ, et al. Community transmission of oseltamivir-resistant A(H1N1)pdm09 influenza. N Engl J Med. 2011;365(26):2541-2.

Chapter of book

- Miller WI, Achernabb JC, Fluck CE. The adrenal cortex and its disorder. In: Sperling M. Pediatric endocrinology. 3rd ed. Elsevier Health Sciences; 2008. p. 444-511.

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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 legend or title sentence should be short but comprehensible without depending on reading the article.

All the figures and tables should be cited in the text.

São Paulo Medical Journal/Evidence for Health Care is for now published in black-and-white in its printed version. Photographs, photomicrographs, bar and line graphs and any image to be published must be prepared considering that there will be no color differentiation (any color information will be discarded). Shades of gray and printing patterns (dots, stripes and others) should be used instead, with good contrast.

Original articles

Clinical trials, cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies, and systematic reviews with or without meta-analysis, are considered to be original articles.

The São Paulo Medical Journal/Evidence for Health Care 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, from 2008 onwards, manuscripts on clinical trials have been accepted for publication only if they have received an identification number from one of the clinical trial registers that have been validated in accordance with the criteria established by WHO and ICMJE. Authors of randomized clinical trials must thus register their studies before submitting them for publication in the São Paulo Medical Journal/Evidence for Health Care. The addresses for these registers are available from the ICMJE website (http://www.icmje.org). The identification number should be declared at the end of the abstract.

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;[7,8]
4. Systematic reviews of the literature and meta-analyses: PRISMA[4]

The São Paulo Medical Journal takes the view that these guidelines not only aid in writing and organizing the content of articles in a standardized manner, thereby improving their quality and facilitating reading and assessment, but also these guidelines help to avoid situations in which important information on the methodology of studies remains outside of the manuscript.

As a partner institution of the Cochrane Collaboration and the Brazilian Cochrane Center, the *Associação Paulista de Medicina* considers that production of articles in accordance with these guidelines also aids in future production of systematic reviews of the literature and meta-analyses. Thus, articles submitted for publication that are not in accordance with these norms may be returned to their authors for adjustment before the peer review process begins.

Original articles must be structured so as to contain the following parts: Introduction, Objective, Methods, Results, Discussion and Conclusion. The text must not exceed 5,000 words (excluding tables, figures and references), from the introduction to the end of the conclusion, and must include a structured abstract with a maximum of 250 words.[9] "Structured abstract" means that the abstract must contain the following items: Context and objective, Design and setting, Method, Results and Conclusion.

The structure of the document should follow the format laid out below:

1) *Title and abstract*: the study design and/or the way participants were allocated to interventions, for example "randomized" or "retrospective" study, should be mentioned in the title and in the abstract. The abstract should provide a summary of what was done and what was found.
2) *Introduction:* specify the reasons for carrying out the study, describing the present state of knowledge of the topic. Describe the scientific background and "the state of the art". Do not include here any results or conclusions from the study. Use the last paragraph to specify the principal question of the study, and the principal hypothesis tested, if there is one. Do not include discussions about the literature in the introduction; the introduction section should be short.
3) *Objective:* describe briefly what the main objective or question of the study was. Clearly describe the pre-specified hypotheses.
4) *Methods*

4.1) *Type of study*: describe the design of the study and specify, if appropriate, the type of randomization (the way in which draws were conducted), the blinding (how this was ensured), the diagnostic test standards (gold standard or range of normal values) and the time direction (retrospective or prospective). For example: "randomized clinical trial", "double-blind placebo-controlled clinical trial", "cross-sectional accuracy study", "retrospective cohort study", "cross-sectional prevalence study" or "systematic review of clinical trials".

4.2) *Sample, participants or patients*: describe the eligibility criteria for participants (inclusion and exclusion criteria) and the sources and procedures for selection or recruitment. In case-control studies, describe the rationale for distributing the subjects as cases and controls, and the matching criteria. The numbers of patients at the beginning and end of

the study (after exclusions) must be made clear. A flow diagram showing the initial recruitment, the exclusions and the final sample of patients included should be produced and inserted in the article.

4.3) *Setting:* indicate the place where the study was carried out, including the type of healthcare provided (i.e. whether primary or tertiary; and whether in a private or in a public hospital). Avoid stating the name of the institution where the study was developed (for blinding purposes in the peer review). Only the type of institution should be made clear, for example: "public university hospital" or "private clinic".

4.4) *Procedures (*intervention, diagnostic test or exposure): describe the principal characteristics of any intervention, including the method, the timing and the duration of its administration or of data collection. Describe the differences in interventions administered to each group (if the study is controlled). Detail the procedures in such a way that other researchers will be able to repeat them in other localities.

4.5) *Main measurements, variables and outcome*: state what the primary and secondary outcomes analyzed in the study are. Describe the method of measuring the primary result, in the way in which it was planned before data collection. For each variable of interest, detail the assessment methods. If the hypothesis of the study was formulated during or after data collection (and not before), this needs to be declared. Describe the methods used to enhance the quality of measurements (for example, multiple observers, training, etc.) and to avoid bias. Explain how quantitative variables were handled in the analyses.

4.6) *Sample size and statistical analysis*: describe the sample size calculation method, or the study period in the event that patients were consecutively admitted over a period. Readers need to understand why a given number of patients was used. The planned statistical analysis, the statistical tests used and their significance levels, along with any *post hoc* analyses, should be presented in this section. Describe the methods used to control for confounding factors and variables, and explain how missing data and cases lost from the follow-up were dealt with.

4.7) *Randomization*: describe the method used to implement the random allocation sequence (for example, sealed envelopes containing random sequences of numbers or software for generating random numbers). If appropriate, report that the study used "quasi-randomization".[12] In addition, describe who generated the random sequence, who assigned the participants to each group (in the case of controlled trials) and who recruited the participants.

5) *Results:* describe the main findings. If possible, these should be accompanied by their 95% confidence intervals and the exact level of statistical significance (it is not enough to write "$P < 0.05$": the exact P value should be supplied). For comparative studies, the confidence interval must be stated for the differences between the groups.

5.1) *Participant flow diagram*: describe the flow of participants through each stage of the study (inclusions and exclusions) and the follow-up period, and the number of participants completing the study (or lost from the follow-up). Use a flow diagram to demonstrate the numbers of patients, from the initial recruitment to the end of the study, and the reasons for exclusions. If there was any "intention-to-treat" analysis, describe it.

5.2) *Deviations*: if there was any deviation from the protocol, away from what was initially planned, describe it and the reasons for it.

5.3) *Adverse events*: describe any side effect, adverse event or complication.

6) *Discussion:* provide an interpretation of the results, taking into account the study hypotheses and conclusions. Emphasize the new and important factors encountered in the study, which will form part of the conclusion. Do not repeat data presented in the introduction or results in detail. Mention any limitations of the findings that should be noted and any possible implications for future research. Describe any potential bias. Report any relevant findings from other studies: it is important to review the recent literature to seek new evidence that may have been published, which needs to be discussed. State whether the findings can be generalized to populations (i.e. whether the findings have external validity). It is recommended that the last two paragraphs should contain implications for practice and for further research.

7) *Conclusions:* specify only the conclusions that can be sustained by the results, together with their clinical significance (avoiding excessive generalization). Draw conclusions based on the objectives and hypotheses of the study. The same emphasis should be placed on studies with positive and negative results.

Systematic reviews with or without meta-analyses should comply with the same publication norms established for original articles, and be produced in accordance with PRISMA[4] and the Cochrane Collaboration's systematic review Handbook.[13] The text should not exceed 5,000 words (excluding tables, figures and references)

Short communications, case reports or case series

Short communications and case reports must be limited to 3,000 words (from the introduction to the end of the conclusion). 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 thus: Introduction, Objective, Methods, Results, Discussion and Conclusion, like in original articles.

Individual case reports should contain: 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 key words. The abstracts in short communications should be structured with: Context and objective, Design and setting, Methods, Results and Conclusion, like in original articles. The abstracts in case reports and case series should contain: Context, Case Report (with a description of the case and a pertinent discussion) and Conclusion.

The São Paulo Medical Journal/Evidence for Health Care 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.[14] The results from the systematic search of the main databases — Medline (via PubMed), Embase, Lilacs and Cochrane Library — should be presented in a table with the search strategy for each database and the number of articles obtained.

Narrative reviews

Narrative reviews may be accepted by the São Paulo Medical Journal/Evidence for Health Care and should be structured with: Introduction, Objectives, Methods, Results, Discussion and Conclusions. The abstract must be structured with: Context and objective, Design and setting, Methods, Results and Conclusions, like in original articles. The manuscript must comply with the norms of the Vancouver style[1] and must include a systematic search in the main databases: Medline, Embase, Lilacs and Cochrane Library. The search strategy for each database and the number of articles obtained from each database should be presented 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 must be use for Medline, LILACS and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, search strategy must be performed, at the same time, with English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms. 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).

Letters to the editor

Letters to the editor may address articles published in the São Paulo Medical Journal/Evidence for Health Care 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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