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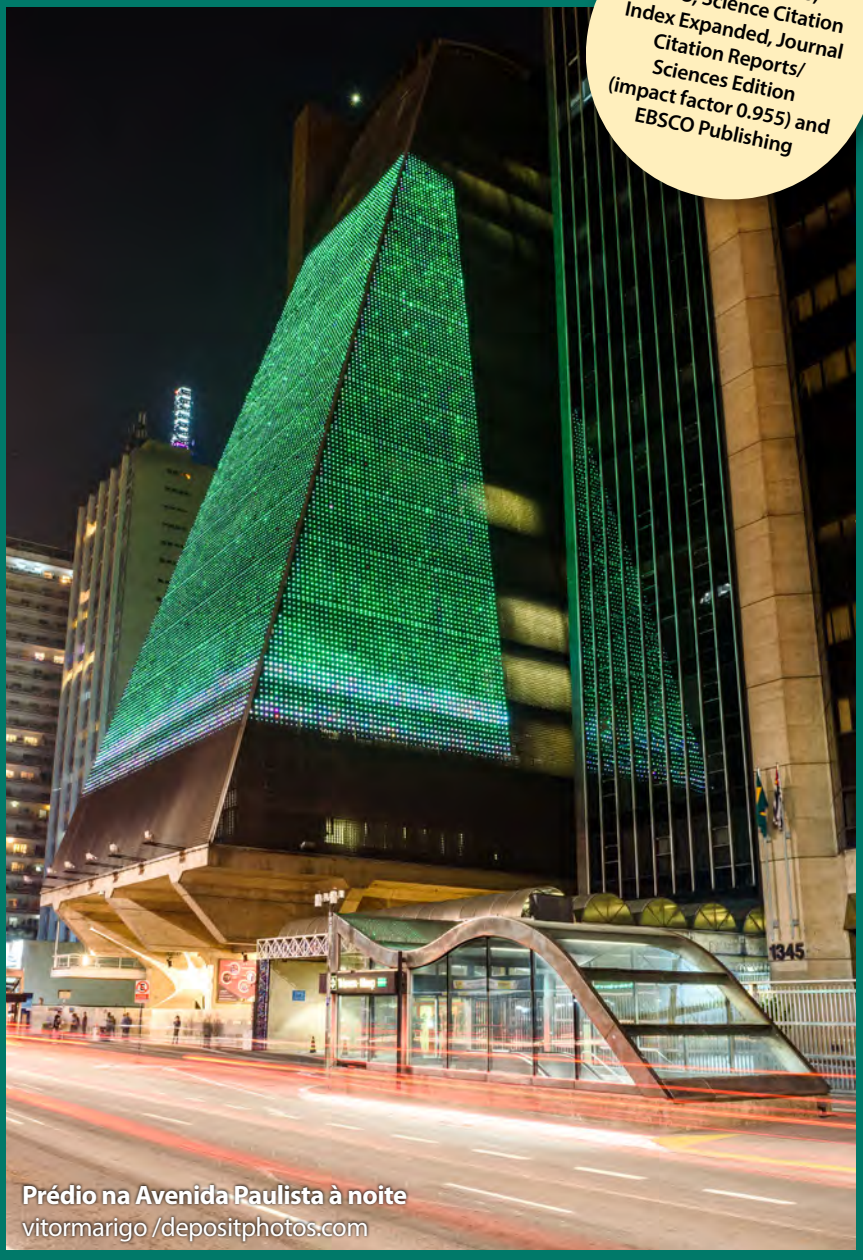

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EVIDENCE FOR HEALTH CARE

INDEX

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How to grade child health in Brazil? Comparison of the results from the Global Burden of Diseases 2015 in Latin America. A call for papers

Como classificar a saúde da criança no Brasil? Comparação dos resultados do Global Burden of Diseases 2015 na América Latina. Convocação de estudos

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Several papers comparing health indicators around the world, derived from the Global Burden of Diseases (GBD) study 2015, were published in October 2016. One of these, authored by the GBD 2015 Child Mortality Collaborators, addressed global, regional, national and selected subnational levels of stillbirths, neonatal, infant and under-five mortality, 1980-2015.[1] This article made it possible to compare mortality rates (per 1000 live births) among Latin American countries in 2015, in terms of neonatal deaths (up to the 27th day of life), postneonatal deaths (from the 28th day to one year of age), deaths at the ages of one to four years and deaths at ages of under five years.[2] Moreover, it was possible to compare the pace of change among those rates over the years, by comparing three periods: 1990-2000, 2000-2015 and 1990-2015; and thus, to ascertain which countries reached Millennium Development Goal 4 (MDG-4), i.e. an annualized rate of decrease in the under-five mortality rate of 4.4%. Particularly, our focus relates to Brazil's performance during the period 1990-2015, among 17 Latin American countries.

Figure 1 shows that Brazil was ranked 14th for neonatal mortality **(A)** with rates 140% higher than Chile, which had the lowest rate. For postneonatal mortality, Brazil ranked 13th with rates 120% higher than Chile, which was again the country with the lowest rate. However, the picture for the age stratum from one to four years **(B)** differed from the traditional infant mortality category of under one year of age, such that Brazil was in fifth place, after Chile, Uruguay, Costa Rica and Argentina.

Table 1 shows the annualized reduction in under-five mortality rates. Peru, El Salvador, Nicaragua, Bolivia, Guatemala and Brazil were the only countries with decreases of 4.4% per year from 1990 to 2015, thus reaching MDG-4. However, the pace of rate reduction was faster over the period 1990-2000 (-5.12%) than in 2000-2015 (-4.08%).

This same article[2] enabled comparisons of prespecified under-five mortality rates among Latin American countries and within Brazil, subdivided according to its states (data not shown). None of these countries were classified in the lowest category of under-five mortality rates (i.e. fewer than five deaths per 1000 live births), as observed in Canada, the United States, Western Europe, Australia, Japan and South Korea. Only Chile and Uruguay were ranked in the category of 5-10 deaths per 1000 live births. Almost all Latin American countries were ranked in the third stratum (10-20 deaths per 1000) including all Brazilian states located in the South, Southeast and Center-West and some in the North and Northeast. The fourth level (20-30 deaths per 1000) was presented in Bolivia, Guatemala, Honduras and the Brazilian states of Acre, Amapá, Maranhão, Piauí, Ceará, Bahia, Alagoas and Pernambuco.

From this summary, readers can grade the quality of child health in Brazil over recent years. The Journal is calling for more articles discussing "Child Health in Brazil".

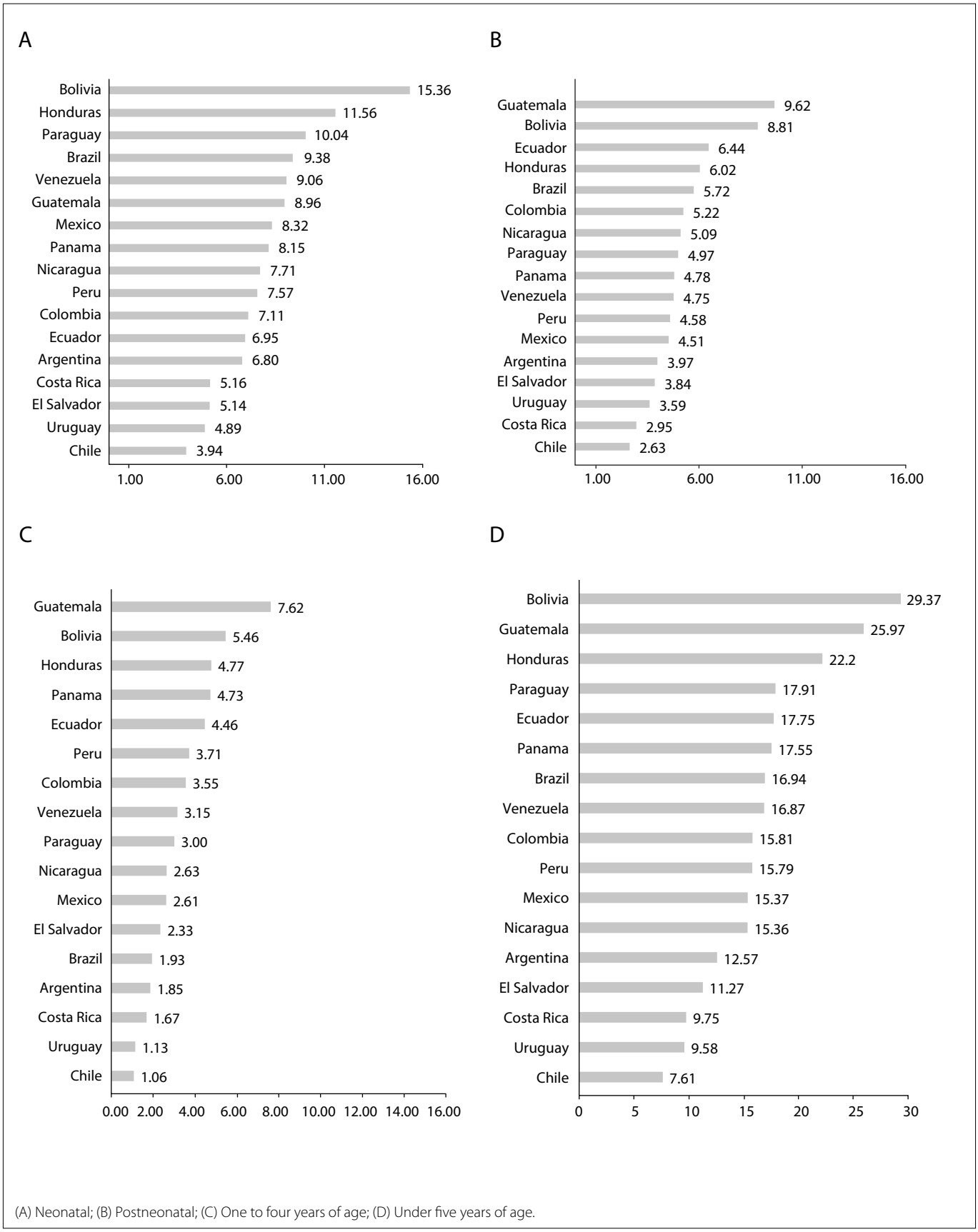

Figure 1. Mortality rates (per 1000 live births) in Latin American countries in 2015.

Table 1. Annual percentage reduction in mortality rates among children under five years of age in Latin American countries

	1990-2000	2000-2015	1990-2015
Peru	-7.99	-5.29	-6.37
El Salvador	-5.96	-6.33	-6.18
Nicaragua	-5.68	-5.60	-5.63
Bolivia	-4.88	-5.62	-5.32
Guatemala	-4.55	-4.54	-4.55
Brazil	-5.12	-4.08	-4.50
Mexico	-4.69	-3.73	-4.11
Ecuador	-3.62	-4.25	-4.00
Colombia	-2.96	-2.75	-3.83
Chile	-5.64	-2.29	-3.63
Honduras	-4.08	-3.27	-3.60
Uruguay	-3.68	-3.39	-3.50
Argentina	-3.56	-2.85	-3.24
Costa Rica	-2.58	-3.15	-2.92
Paraguay	-1.46	-3.00	-2.39
Venezuela	-2.88	-1.49	-2.04
Panama	-1.76	-1.78	-1.77

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Economic evaluation of human albumin use in patients with nephrotic syndrome in four Brazilian public hospitals: pharmacoeconomic study

Avaliação econômica do uso de albumina humana em pacientes com síndrome nefrótica em quatro hospitais públicos brasileiros: estudo de farmacoeconomia

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KEY WORDS:
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ABSTRACT

CONTEXT AND OBJECTIVE: In 2004, the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA) published a resolution establishing guidelines for albumin use. Although the published data do not indicate any definitive conclusions about the benefits of albumin use in patients with nephrotic syndrome (NS), the guidelines recommend this procedure only in cases of edema that is refractory to use of diuretics. The aim here was to analyze albumin use among patients with nephrotic syndrome.

DESIGN AND SETTING: Pharmacoeconomic study conducted in four large public referral hospitals for nephrology services in northeastern Brazil.

METHOD: Cost-effectiveness and cost-utility economic evaluations were performed on a concurrent cohort of patients with nephrotic syndrome, who were divided into two groups according to compliance or noncompliance with the guidelines. Quality-of-life data were obtained from the SF36 and CHQ-PF50 questionnaires.

RESULTS: This study enrolled 109 patients (60% adults and 56% women); 41.3% were using albumin in accordance with the guidelines. The weight, diuresis and fluid balance parameters were more cost-effective for patients who adhered to the guidelines. Regarding days of hospitalization avoided, the incremental ratio showed a daily cost of R$ 55.33, and guideline-compliant patients were hospitalized for five days or fewer. The quality of life improved by 8%, and savings of R$ 3,458.13/QALY (quality-adjusted life year) for the healthcare system were generated through guideline compliance.

CONCLUSION: The economic analyses of this study demonstrated that there were greater cost benefits for patients whose treatment followed the guidelines.

RESUMO

CONTEXTO E OBJETIVO: Em 2004, a Agência Nacional de Vigilância Sanitária (ANVISA) publicou uma resolução que estabelece diretrizes para o uso de albumina. Embora os dados publicados não indiquem conclusões definitivas sobre os benefícios do uso de albumina em pacientes com síndrome nefrótica (SN), a diretriz recomenda o procedimento apenas em casos de edema refratário ao uso de diuréticos. O objetivo aqui foi analisar o uso de albumina em pacientes com síndrome nefrótica.

TIPO DE ESTUDO E LOCAL: Estudo farmacoeconômico realizado em quatro grandes hospitais públicos de referência em serviços de nefrologia no nordeste do Brasil.

MÉTODO: Foram realizadas avaliações econômicas do tipo custo-efetividade e custo-utilidade em uma coorte concorrente de pacientes com síndrome nefrótica, divididos em dois grupos de acordo com o cumprimento ou descumprimento das diretrizes. Dados de qualidade de vida foram obtidos a partir dos questionários SF36 e CHQ-PF50.

RESULTADOS: Este estudo incluiu 109 pacientes (60% adultos e 56% mulheres); 41,3% estavam usando albumina conforme as diretrizes. Os parâmetros de peso, diurese e balanço hídrico foram mais custo-efetivos para pacientes que aderiram às diretrizes. Quanto a dias de internação evitados, a razão incremental mostrou um custo diário de R$ 55,33, sendo que os pacientes que seguiram as diretrizes ficaram cinco dias a menos internados. A qualidade de vida melhorou 8%, gerando economia de R$ 3,458.13/QALY *(quality-adjusted life year)* para o sistema de saúde por meio do cumprimento das diretrizes.

CONCLUSÃO: As análises econômicas deste estudo demonstraram maiores benefícios em termos de custo para os pacientes cujo tratamento seguiu as diretrizes.

INTRODUCTION

Healthcare needs have expanded exponentially over recent years, thus increasing the demand for more effective results. A number of analysis tools are available to public policy managers, to enable justification of decisions that are made. One of these tools is economic evaluation, which is characterized by comparative analysis of different interventions in terms of costs and their consequences. Economic evaluation has been regarded as an excellent decision-making support tool.[1-5] Production of clinical protocols and therapeutic guidelines rationalizes the use and safety of technologies, since they organize logical thinking towards positive results in terms of economy, effectiveness, safety and efficiency.[6]

Albumin is an endogenous liver-synthesized protein. It is present at high concentrations in human plasma and is primarily responsible for maintaining intravascular oncotic pressure.[7-10] As a pharmaceutical product, human albumin is an injectable blood product from human plasma that is found at hyperosmotic concentrations in plasma (4% to 25%). Its primary therapeutic indications are for restoration of oncotic and iso-osmotic pressure (4%), and also, although less indicated, for plasma volume restoration.[7,10]

Because of variability in the way in which human albumin is used in Brazil, as well as its high cost, the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA) published guidelines for therapeutic use of this drug in 2004.[7] Brazil's expenditure on imported blood products in 2010 reached US$ 330 million and, within this amount, 12 tons of human albumin were imported, at a cost of approximately US$ 50 million.[11]

One of the formal indications for the use of human albumin stated within the scope of ANVISA's guidelines is nephrotic syndrome (NS). This involves the presence of large-scale edema that is refractory to diuretics, thereby endangering these patients' lives (due to pleural effusion, pericardial effusion or bulky ascites). In these cases, treatment with albumin would be short-term and would aim to resolve the patient's acute decompensation. Presence of hypoalbuminemia alone in patients with NS (a condition that is caused by the disease) does not justify albumin use.[7]

NS is a clinical condition characterized by the presence of massive proteinuria, edema, hypoproteinemia and dyslipidemia. Massive proteinuria is defined as excretion of more than 3.5 g of protein per 1.73 m^2 of body surface area within 24 hours or more than 50 mg/kg of bodyweight within 24 hours through the urinary tract. NS affects both adults and children and is primarily caused by kidney disease (idiopathic or primary NS) or by various pathological conditions (secondary NS)[12-15] Generalized edema is the main complication of NS, since it can lead to serious conditions such as pulmonary edema, heart failure and hypertension.[13] Formation of this edema can be explained through pathophysiological mechanisms that are activated due to decreased glomerular filtration rates resulting from prior renal disease, inadequate sodium excretion in the distal tubules and hypoalbuminemia.[13,15,16] The treatments for nephrotic syndrome depend on two factors, namely, the patient's general condition and the type of primary renal disease. In cases of generalized edema, the first procedure should be grounded in removing the patient from the critical state.

The use of human albumin in association with diuretics as the first choice for patients with NS has been extensively discussed in the literature.[17] Some studies have shown that human albumin enhances the effect of diuretics,[13,18-21] while other studies have failed to show any difference in the results from comparisons between ways of using diuretics: on a stand-alone basis or in association with albumin.[17,22-25] Some other studies have contraindicated this combination of human albumin and diuretics for treating edema in NS,[26] while yet others have shown benefit from stand-alone use of albumin.[27]

Knowledge of patients' profiles and economic evaluation of human albumin use among patients with NS can be valuable tools in decision-making for the healthcare system. This information can assist managers in spreading the use of these guidelines to other healthcare units, thereby optimizing disease management and use of public resources, or it may spur a review of standards.

OBJECTIVE

The aim of this study was to analyze albumin use among patients with nephrotic syndrome, from the perspective of the Brazilian National Health System (Sistema Único de Saúde, SUS), through a pharmacoeconomic study.

METHODS

Method, population and data source

Cost-effectiveness and cost-utility economic evaluations were performed on a concurrent cohort of patients with nephrotic syndrome that was observed between December 2010 and July 2012.

This study was conducted in four public referral hospitals for nephrology services that are located in Salvador, Bahia. The criteria for selecting these institutions comprised their care profile (referral centers for nephrology within the state's public health network), level of human albumin consumption and formal acceptance of participation.

The study population consisted of consecutive adult and pediatric patients with nephrotic syndrome, who were monitored from the start to the end of their treatment and were divided into two groups:

- Group 1: patients whose treatment followed the instructions for human albumin use, as recommended by ANVISA, i.e. human albumin was only used in cases of large-scale edema that was refractory to diuretics;
- Group 2: patients whose treatments did not follow policy guidelines.

Patients were required to agree to and sign a free and informed consent statement in order to join the study.

The research team was independent from the clinical team, and only allocated the case to one or other of the groups shortly after the beginning of the treatment prescribed. Thus, if after the case had been diagnosed as nephrotic syndrome and the use of albumin alone or albumin in association with diuretics was prescribed as the first-choice treatment, this patient would belong to group 2 (treatment not following the guidelines). If the patient was using diuretics alone and an inadequate therapeutic response was obtained (i.e. the patient was refractory to diuretics), and then albumin use was added, this patient would be allocated to group 1, since the guidelines indicate that albumin should only be used for patients with nephrotic syndrome in cases of large-scale edema that was refractory to diuretics.

In order to check the allocations of patients to groups 1 and 2 that the researchers made, all cases were reviewed and validated weekly by a nephrologist.

Data-gathering

Clinical, laboratory, socioeconomic and demographic data were gathered upon confirmation of the case of nephrotic syndrome during hospitalization. The main sources of information used were the human albumin stock movement records in the pharmacy service, daily medical prescriptions, hospitalization censuses and medical records, along with interviews with patients and/or guardians. The data-gathering was conducted by trained field workers who had been instructed about all the study methodology and research instruments.

The variables were grouped into general data, socioeconomic and demographic data, clinical data, indications for human albumin, hospital length of stay, reason for discharge, deaths and quality-of-life data.

The quality-of-life data were obtained by applying two generic health-related quality-of-life questionnaires, which have both been adapted and validated for the Brazilian population. The Medical Outcomes Survey Short Form 36 (SF36),[28,29] which consists of 36 questions addressing eight quality-of-life domains, was used for adults. Scale scores are converted into values from 0 (worst quality of life) to 100 (best quality of life). Adolescents aged 12 and over could also answer this questionnaire.

The "50-item, parent complete short form, Child Health Questionnaire" (CHQ-PF50)[30] was used for pediatric patients up to the age of 12 years. This can be applied to these patients either directly or indirectly through their parents or guardians.[31,32] It consists of ten patient-related health concepts and four family-related concepts, in order to measure the emotional impact of child health on the adult responsible for that child. The results range from 0 to 100, with higher scores indicating better quality of life.

The patients included in this study gave responses to the quality-of-life questionnaires at two stages: before the onset of treatment and four weeks after the first application.

The Short Form 6D (SF-6D) algorithm was applied to data gathered through the SF36. This enabled direct measurement of utility, through calculation of quality-adjusted life years (QALYs).[33]

Cost data were obtained by calculating the patients' direct treatment costs during the hospitalization period through the absorption costing technique, since the participating hospitals did not have systematic computerized overhead cost information to enable their estimation. The direct costs used in this study were drawn from the medical billing sectors of the institutions and were gathered from the patients' billing sheets (patient/days of hospitalization), in accordance with the SUS procedures table. Costs were adjusted for inflation to June 2013, based on the Market General Price Index (IGP-M) of the Getúlio Vargas Foundation (Fundação Getúlio Vargas, FGV). The study was conducted from the economic perspective of SUS.

All the cases were presented and discussed before officially defining their inclusion in the study, at meetings in which both the data-gatherers and the advisory members of the project (two pharmacists, one economist and one nephrologist) participated. All data collected were checked by a supervisor before database entry. Where divergences and/or lack of data were noted, the questionnaires were reevaluated by the researchers using complementary information and were analyzed again by the supervisor.

Data analysis

The data were entered into the Microsoft Excel 2007 software and were analyzed using SPSS version 20.0. Continuous variables were expressed as means and standard deviations. The Kolmogorov-Smirnov test was used to evaluate the normality pattern of the distribution of continuous variables. P-values of less than 0.05 with 95% confidence interval were considered to be statistically significant. Categorical variables were reported as proportions and were tested using the chi-square test.

The cost-effectiveness economic analysis was performed starting from cost-effectiveness mean ratios and, when necessary, incremental cost-effectiveness ratio (ICER) analysis was applied, in which costs and health outcomes were calculated by dividing the difference in strategy cost by the difference in health results for each group. For the cost-effectiveness economic analysis, the effectiveness indicators used to compare the groups were: weight, diuresis, water balance and days of hospitalization avoided.

The mean cost-utility ratio was also used for cost-utility analysis and, when required, the incremental cost-utility ratio (ICUR) was applied. In this case, the health outcome measurements were scores from the results of the quality-of-life questionnaires, transformed into quality-adjusted life years (QALYs).

Univariate sensitivity analysis was performed. Each parameter was assessed separately within its range of variation, while the other remained constant. The objective was to ascertain the influence of the parameter analyzed on the final result, so as to determine whether or not this was sensitive to change.

The study was approved by the Research Ethics Committee of the Prof. Edgard Santos University Hospital Complex, Federal University of Bahia, under report number 063/2007.

RESULTS

One hundred and nine patients were enrolled by the end of the study period, and the treatments for 45 (41.3%) of them followed the human albumin use guidelines. Two patients were excluded because of lack of information for the economic analysis that had been planned.

In Group 1, 64% (29/45) were pediatric patients with a mean age of 16 years, and the CHQ-PF50 questionnaire was applied in most cases. In group 2, 77% (49/64) were adult patients with a mean age of 28 years, and the SF36 was used in most cases. Comparison between the average ages of the groups showed differences ($P < 0.0001$). **Table 1** shows the comparison groups according to the four hospitals surveyed.

The demographic and socioeconomic data are shown in **Table 2**. Overall, an average of four people were living with each patient, and this average was also maintained in the group analysis.

In Group 1, 77.8% (35/45) of the patients were not in their first treatment, whereas a balance was noted in Group 2 between first and non-first treatment, with 50% (32/64) of the cases in each treatment situation.

The overall average hospitalization cost for SUS (Sistema Único de Saúde, the Brazilian public health system) in this study was R$ 2,360.00, with an average of 22 days of hospitalization. The average cost for Group 1 was R$ 2,221.68, with an average of 20 days of hospitalization and it was R$ 2,498.33 for Group 2, with an average of 25 days of hospitalization. The incremental cost-effectiveness ratios for days of hospitalizations and urine output parameters are shown in **Table 3**. The weight loss and fluid balance parameters were better in Group 1.

Data from the quality-of-life analyses are shown in **Figure 1**, through QALY indices before and after the procedure for each group. **Table 4** displays the ICUR.

Univariate sensitivity analyses were performed based on the ICER and ICUR results. The ICER sensitivity data showed that

Table 2. General characteristics of patients with nephrotic syndrome treated at four hospitals in Salvador, Bahia, in 2012, according to group

Parameter	Group 1 n (%)	Group 2 n (%)	Total n (%)
Gender			
Female	25 (55.6)	36 (56.2)	61 (56)
Male	20 (44.4)	28 (43.8)	48 (44)
Age			
Up to 12 years	29 (64.4)	15 (23.4)	44 (40.4)
12-59 years	15 (33.4)	46 (71.9)	61 (56)
60 years and over	1 (2.2)	3 (4.7)	4 (3.6)
Ethnic group			
White	7 (15.6)	10 (15.6)	17 (15.6)
Black	19 (42.2)	21 (32.8)	40 (36.7)
Brown	19 (42.2)	33 (51.6)	52 (47.7)
Status			
Single	36 (80)	45 (70.3)	81 (74.3)
Married	8 (17.8)	17 (26.6)	25 (22.9)
Divorced	1 (2.2)	2 (3.1)	3 (2.8)
Schooling			
Illiterate	14 (31.1)	5 (7.8)	19 (17.4)
Incomplete elementary school	20 (44.4)	25 (39.1)	45 (41.3)
Complete elementary school	1 (2.2)	2 (3.1)	3 (2.8)
Incomplete high school	6 (13.4)	11 (17.2)	17 (15.7)
Complete high school	4 (8.9)	16 (25)	20 (18.3)
Incomplete university education	–	3 (4.7)	3 (2.7)
Complete university education	–	2 (3.1)	2 (1.8)
Employment situation			
Formally employed	5 (11.1)	8 (12.5)	13 (11.9)
Informally employed	2 (4.4)	7 (10.9)	9 (8.3)
Unemployed	4 (8.9)	11 (17.2)	15 (13.8)
Housewife	1 (2.2)	7 (10.9)	8 (7.3)
Retired/pensioner	3 (6.7)	5 (7.8)	8 (7.3)
Student	6 (13.4)	21 (32.9)	27 (24.7)
Less than 7 years of age	24 (53.3)	5 (7.8)	29 (26.7)
Patient's monthly income			
Up to R$ 622.00	40 (88.9)	49 (76.6)	89 (81.6)
R$ 622.00 and over	5 (11.1)	15 (23.4)	20 (18.4)
Household's monthly income			
Up to R$ 622.00	25 (55.6)	29 (45.3)	54 (49.5)
R$ 622.00 and over	20 (44.4)	35 (54.7)	55 (50.5)
Origin of patient			
State capital	19 (42.2)	28 (43.7)	47 (43.2)
Rural area	26 (57.8)	36 (56.3)	62 (56.8)
Family rank			
Head	7 (15.6)	15 (23.4)	22 (20.2)
Spouse	2 (4.4)	12 (18.8)	14 (12.8)
Son/daughter	35 (77.8)	35 (54.7)	70 (64)
Other relatives	1 (2.2)	2 (3.1)	3 (3)

Table 1. Total number of patients with nephrotic syndrome in each study group, according to hospital of origin, in Salvador, Bahia, in 2012

Adherence to guidelines	Institution				Total
	Hospital A	Hospital B	Hospital C	Hospital D	
Treatment followed the guidelines – Group 1	26	3	13	3	**45**
Treatment did not follow the guidelines – Group 2	19	28	4	13	**64**
Total	**45**	**31**	**17**	**16**	**109**

there was an increase of 60% in the value of avoided hospitalization days, with a difference of 2 days of hospitalization between the groups; the amount of expenditure avoided per day was only R$ 138.33. The ICUR sensitivity analysis showed that there was a 30% improvement in the quality of life, which generated savings of R$ 1,024.63 for SUS.

DISCUSSION

This was the first pharmacoeconomic study in Brazil examining the use of human albumin among patients with nephrotic syndrome. The general economic analyses showed that the treatments that followed ANVISA's guidelines were more cost-effective. These data suggest that these guidelines should be implemented in SUS services where they are currently not being used. This can be justified since they produce clinical and economic results for the system.

These data show that treatments have better cost-effectiveness and usefulness for patients when the guidelines are followed, even though this issue remains undefined in the literature. This seems to lead to a broad discussion on the appropriateness of using an initial association of human albumin and diuretics as the first choice in treatments for NS. It has been suggested in the literature that the clinical relevance of the effects of furosemide and albumin is still obscure and that there is no accumulated scientific evidence justifying use of this combination in the initial intervention, or as a routine treatment.[25] The review by Elwell et al.[25] confirms that the results from different studies are conflicting with regard to using human albumin coupled with furosemide in cases of NS. Therefore, this combination should be reserved for specific groups of patients whose doses of diuretics have been maximized or for cases of severe hypoalbuminemia.[17]

The findings from the review by Caraceni et al.[34] show that use of human albumin alone for correcting hypoalbuminemia is not backed by scientific evidence. This confirms the stance taken in the guidelines, i.e. that thorough analysis is required before using human albumin in a direct association and as the first-choice treatment.[7,24,34]

The possible positive impact within clinical practice from using a combination of human albumin and furosemide among patients with edema is not associated with correction of hypovolemia. There is agreement that one possible explanation for the

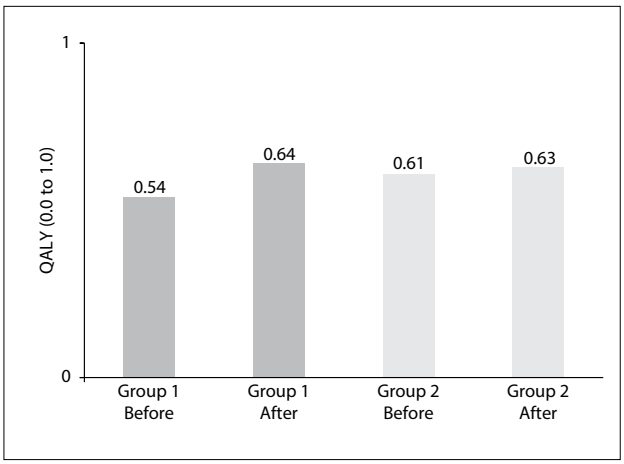

Figure 1. Comparison of QALY (quality-adjusted life year) indices before and after treatment with human albumin, according to groups of patients with nephrotic syndrome treated at four hospitals in Salvador, Bahia, in 2012.

Table 3. Incremental cost-effectiveness ratio according to "days of hospitalization" and "diuresis" among patients with nephrotic syndrome treated at four hospitals in Salvador, Bahia, in 2012

Parameter	Average cost to SUS per hospitalization (R$)	Average parameter value	Difference in health outcomes	Difference in costs	Incremental cost-effectiveness (R$)
Days of hospitalization					
Group 2	2498.33	25	-5	-276.65	55.33/day
Group 1	2221.68	20			
Diuresis (ml)					
Group 2	2498.33	1045.16	-54.75	-276.65	5.05/ml
Group 1	2221.68	990.41			

SUS = Sistema Único de Saúde, the Brazilian National Health System.

Table 4. Incremental cost-effectiveness ratio per QALY of patients with nephrotic syndrome treated at four hospitals in Salvador, Bahia, in 2012

Parameter	Interventions	Average cost to SUS per hospitalization (R$)	Average QALY value gained	Difference in health outcomes	Difference in costs	Incremental cost-effectiveness (R$)
QALY	Group 2	2498.33	0.02	0.08	-276.65	-3458.13
QALY	Group 1	2221.68	0.1			

QALY = quality-adjusted life year; SUS = Sistema Único de Saúde, the Brazilian National Health System.

boosting of the effect of furosemide is that greater availability of the drug at its site of action is achieved.[13,35]

The present study based on economic analyses had the aim of assessing ANVISA's guidelines regarding the use of human albumin among patients with NS. It also poses the question of whether the use of protocols and guidelines in clinical practice is relevant. It has been suggested in the literature that institutionalization and dissemination of guidelines or protocols in itself does not necessarily translate into changes in practices or into lasting clinical results.[21] Data from one study have shown that it takes five to ten years to achieve significant results from changes in practice after implementation of these instruments.[36]

The process of establishing therapeutic guidelines should also be followed by continuing education, review of processes and monitoring of practices. These actions can definitely bring about real and positive results.[10,36,37]

Lastly, the benefits of guidelines and clinical protocols and their negative aspects and the obstacles to implementing them have been widely assessed in the literature. The information from the present study, in which the treatments for most of the cases did not follow the policy guidelines, reinforces the previously published data, thus reaffirming that merely disseminating a guideline or protocol does not warrant safer, more effective and more efficient practices for patients, government and society itself.

The assessment of the numbers of cases distributed per hospital showed the situation of bed availability within these SUS hospitals in Bahia. One of them is the largest state hospital in the public network, with approximately 1,792 beds and emergency care, whereas the others are traditional referral centers for nephrology.

Ethnic group analysis showed that brown and black individuals together accounted for more than 90% of the cases, which reflects the state's population composition.[38]

An analysis on the patients' origins revealed that most of them were from municipalities in rural areas of the state. This may demonstrate the fragility or lack of hospitals in rural macroregions to serve the needs of this profile of patients.

Regarding the economic analysis, the cost-effectiveness analysis results calculated from the average ratios for weight loss and fluid balance outcomes showed better relationships for Group 1. An incremental ratio method was used for the parameter of days of hospitalization, since treatments that followed the guidelines were more effective but also more costly. This allowed the hospitalization to be five days shorter but with an increased daily cost (R$ 111.08/day versus R$ 99.93/day). Thus, the ICER result for the number of days of hospitalization showed that an additional cost of R$ 55.33 will be required per day avoided. This data needs to be analyzed in the light of the reality of the lack of public hospital beds in Brazil and the consequent long waiting lists for care and attempts to make overall savings for SUS through deinstitutionalization of patients.

In fact, the additional cost should be analyzed as an investment in the system, since having a bed available five days earlier brings more benefits. Data gathered by the Organization for Economic Cooperation and Development (OECD) show that the number of beds per capita varies greatly between countries and over time.[39] Brazil has one of the lowest levels in the Americas (1.7 beds per 1,000 population).[39]

Several other risks are associated with longer hospital stays. Hospital infection and various adverse events can be highlighted among these risks, and these give rise to additional diagnostic investigations and tests, along with greater routine stay expenses (accommodation, meals, general nursing, etc.), which all contribute towards increased hospitalization costs. These explanations ultimately indicate that investments in deinstitutionalization need to be made. Univariate sensitivity analysis showed that a difference of only two days of hospitalization between the groups would generate a 60% increase in the amount saved through avoidance of hospital stay. Thus, investment of these amounts can be justified, given the other costs of keeping patients hospitalized.

The average cost-effectiveness rate for the diuresis parameter in Group 1 was less effective and lower, in relation to the possible cost-effectiveness responses. The ICER analysis for diuresis highlighted the need to invest resources, so as to achieve clinical results better than those that have been found so far. However, in clinical practice, these shortfalls probably would not have an impact on patients great enough to justify investment.

The results from the cost-utility analysis showed that Group 1 achieved greater improvement of quality of life than Group 2. This benefit can be put back into society and into SUS.

There was an increase in quality of life of 10% for Group 1 and 2% for Group 2. For treatments that followed the guidelines, ICUR showed that there were savings of R$ 3,458.13 per QALY for SUS.

Univariate sensitivity analysis on ICUR, setting the QALY gained in Group 2 at 2%, showed that the savings for the system were maintained even when the difference in QALY gained by the groups was more than 30%. In other words, savings for SUS were generated through treatments that followed the guidelines, even with better quality of life in Group 1.

It is important to highlight that there is no high-quality evidence supporting use of albumin for treating nephrotic syndrome. Caution is still needed in using albumin, since this is not considered at all for other clinical situations. For example, a non-concurrent historical cohort study that was carried out in Brazil based on DATASUS records found that use of albumin among patients with major burns was associated with considerably increased mortality (seven times higher than when crystalloid solutions were used).[40]

The clinical evaluation on the effectiveness of intermediate outcomes for weight, diuresis and fluid balance parameters more

directly related to the care process showed differences between the groups in this study. However, within clinical practice, these differences would probably not demonstrate observable relevance. Nonetheless, what can be highlighted is the difference in the number of days of hospitalization, i.e. the number of days avoided and the increased quality of life for patients whose treatments followed the guidelines. These analyses showed both clinical benefits for patients and savings for the system.

CONCLUSION

The cost-effectiveness results, and especially the cost-utility results of this study provided information on the use of human albumin among patients with nephrotic syndrome. This information, along with other data, can guide healthcare practices within SUS from both the clinical and the economic and financial viewpoints.

Economic analyses suggest that the use of guidelines for using human albumin among patients with NS has been beneficial, since there was a gain in effectiveness and quality of life for patients and economic gains for SUS.

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Association of risk factors with smoking during pregnancy among women of childbearing age: an epidemiological field study in Turkey

Associação de fatores de risco com tabagismo durante a gravidez em mulheres em idade fértil: estudo epidemiológico de campo na Turquia

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KEY WORDS:
Smoking.
Prevalence.
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Pregnancy.
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PALAVRAS-CHAVE:
Hábito de fumar.
Prevalência.
Mulheres.
Gravidez.
Idade gestacional.

ABSTRACT

CONTEXT AND OBJECTIVE: Smoking during pregnancy is an important risk factor for maternal and infant health that is preventable. This study aimed to investigate the risk factors associated with smoking behavior during pregnancy.

DESIGN AND SETTING: A household-based probability sample survey of 1,510 women was conducted in the center of the city of Sivas, Turkey, between September 2013 and May 2014.

METHODS: The prevalence of smoking during pregnancy was estimated according to independent variables by means of regression analysis.

RESULTS: The prevalence of smoking during pregnancy was 16.5%. Logistic regression showed that being at a relatively young age (odds ratio, OR = 1.92, P = 0.025 for 15-24 age group; and OR = 2.45, P = 0.001 for 25-34 age group), having a low educational level (OR = 1.76, P = 0.032), being unmarried (OR = 1.48, P = 0.002) and living in an extended family (OR = 1.98, P = 0.009) were the factors associated with the risk of smoking during pregnancy.

CONCLUSIONS: Systematic attention should be paid to socioeconomic inequalities, to support women towards quitting smoking before or at an early stage of their pregnancies. Younger women and particularly those in lower socioeconomic groups should be targeted. This will lead to better pregnancy status, especially among young women.

RESUMO

CONTEXTO: Fumar durante a gravidez é um fator de risco importante para a saúde materna e infantil e que pode ser evitado. O estudo teve como objetivo investigar os fatores de risco associados com o comportamento de fumar durante a gravidez.

TIPO DE ESTUDO E LOCAL: Inquérito por amostragem probabilística de base domiciliar de 1.510 mulheres foi realizado no centro da cidade de Sivas, Turquia, entre setembro de 2013 e maio de 2014.

MÉTODOS: A prevalência do hábito de fumar durante a gravidez foi estimada de acordo com as variáveis independentes por meio da análise de regressão.

RESULTADOS: A prevalência do hábito de fumar durante a gravidez foi de 16,5%. A regressão logística mostrou que estar em idade relativamente jovem *(odds ratio*, OR = 1,92, P = 0,025 por grupo de idade 15-24e OR = 2,45, P = 0,001 para a faixa etária 25-34), com alto nível de escolaridade (OR = 1,76, P = 0,032), sendo solteira (OR = 1,48, P = 0,002) e vivendo em família grande (OR = 1,98, P = 0,009) foram os fatores associados ao risco de fumar durante a gravidez. Parto prematuro e baixo peso ao nascer foram mais frequentes entre as fumantes.

CONCLUSÕES: Atenção sistemática deve ser dada às desigualdades socioeconômicas para apoiar mulheres a parar de fumar antes ou na fase inicial da gravidez, tendo como alvo mulheres mais jovens, e particularmente aquelas de grupos socioeconômicos mais desfavorecidos. Isso conduzirá a um melhor estado de gravidez em mulheres, especialmente às jovens.

INTRODUCTION

Maternal smoking during pregnancy has been identified as the most preventable source of newborn morbidity and mortality throughout the world. The health problems, economic burden and dependence caused by smoking are predictably greater in vulnerable populations such as pregnant women. Smoking during pregnancy increases the risk of adverse pregnancy outcomes such as perinatal mortality or miscarriage, premature births, low birthweight and small fetuses.[1-4]

According to a recent study on trends within the prevalence of smoking in developing and developed countries, the prevalence of smoking has decreased significantly worldwide.[5] However, as suggested by some studies, because the rates of smoking cessation are lower in socioeconomically vulnerable populations, the differences in smoking rates between socioeconomic levels may have increased.[6,7]

Several variables relating to sociodemographic conditions are closely connected with the likelihood of smoking behavior. Furthermore, smoking behavior is inversely associated with socioeconomic position, such that vulnerable people in the community are more likely to continue their smoking behavior.[8,9] Despite considerable public understanding of the dangers of smoking during pregnancy, more than a third of women who smoke continue their smoking behavior during pregnancy.[10-12]

Research has documented factors relating to smoking behavior during pregnancy and their effect on adverse pregnancy outcomes. The social structure of smoking behavior during pregnancy is largely determined by social factors.[13-15] The consequences of pregnancy in situations of low socioeconomic level are determined through internal factors and the habit of cigarette smoking is among these factors.[16] Up to half of the adverse consequences of pregnancy are caused by smoking behavior among women living under low socioeconomic conditions.[17] It is even more important to take into account the effect of smoking during pregnancy among women living in different socioeconomic levels in developing countries like Turkey, which have high inequality in maternal and infant mortality rates.

Because of the direct risks to infant health, much attention has been paid to maternal smoking during pregnancy over recent years. However, few data on the characteristics of high-risk populations regarding smoking behavior are available. Previous studies have been limited by several factors. Most of these studies have been conducted in the hospital setting rather than community setting or have had small sample sizes.[11,12]

OBJECTIVE

The aim of the present study was to investigate the risk factors associated with smoking during pregnancy.

METHODS

Setting and ethics

This cross-sectional population-based study was conducted in the center of the city of Sivas, Turkey, between September 2013 and May 2014. Sivas is a Middle Anatolian city with approximately 625,000 inhabitants. The number of women of reproductive age (15-49 years) in the urban area of this city is about 85,000. Compared with other cities in Turkey, it has an average structure with regard to socioeconomic and demographic conditions.

Women were interviewed in person at their homes. All participants were informed that all information obtained through the interview would be kept confidential. The study was approved by the Ethics Committee of Cumhuriyet University.

Sampling design

A large sample consisting of 1,510 individuals was used. This sample size was enough to estimate an expected prevalence of smoking during pregnancy of 20% from a large population with a margin of error for a 95% confidence interval of ± 2%.[11,12] The target population of the study comprised around 38,000 households in 63 districts. A multistage cluster sampling scheme was used in this study. The sampling scheme was prepared by listing the number of households in each district. The electricity company's records were used to find the number of households. Firstly, a total of 11 districts were randomly selected. Secondly, the street and street number of the dwelling on the street were selected randomly in each of the districts. Households were sampled with probability proportional to size. Buildings primarily providing short-term or temporary accommodation such as hotels, rental homes, etc., were excluded. Over the study period, to increase the chance of obtaining an interview, two revisits to each household were made on different days of the week. If a household contained more than one eligible woman, one of them was randomly chosen by drawing lots for the interview. The eligible women enrolled into the study were those who had been pregnant at some time during the previous three years (before the time of the interview), who did not present any communication difficulties and who gave their informed consent to participate.

Before starting the face-to-face interview, written and verbal consent was sought from every participant. All interviews were conducted in a quiet room in each respondent's home, by trained final-year medical students.

Survey instrument

The survey questionnaire for this study was developed based on the existing literature and was reviewed by two research experts. In order to judge the time needed to administer the

questionnaire and to test it for clarity and logical flow, it was piloted with 20 women.

The questionnaire consisted of two sections. The first section of the questionnaire requested demographic information on the participants, including maternal age, current marital status, health insurance, family type (nuclear or extended, i.e. a family that included not only parents and children but also other relatives such as grandparents, aunts or uncles), number of previous deliveries, education level and employment status. Employment status was categorized as an office job, manual work, or unemployed. In this study, subjects who were students or housewives were registered as unemployed. Also in this section, the subjects were asked about the place where they had spent the majority of their lives, whether they had any type of health insurance and about their perceived health status and income level. Based on self-reported data, the annual household income was categorized into two groups: 1 (≤ US$ 7,000) and 2 (> US$ 7,000). Likewise, health-related problems were coded as 1 = present or 0 = absent; the women were asked to self-report whether during their last pregnancy they had had one or more of the following chronic medical conditions: diabetes, hypertension, arthritis, thyroid disorders, migraines, asthma, gastrointestinal disorders, cancer or physical disability.

In the second section of the questionnaire, the women were asked whether they had smoked during their last pregnancy. Smoking was defined as cigarette smoking at least once a week. Furthermore, alcohol consumption was determined through the frequency of drinking, defined as: often (least once a week), occasionally (rarely, less than one beverage per month) or never.

Independent and dependent variables

Associations of the following independent contextual variables were considered to be relevant to smoking during pregnancy: age, maternal education, marital status, employment status, family type and annual household income.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) for Windows, version 16.0, was used for the data analysis. Categorical data were expressed as percentages. Quantitative data were presented as mean ± standard deviation, SD. To evaluate associations between dependent and independent variables, bivariate analysis using the chi-square test was performed. Also, multiple logistic regression analyses were performed to assess which variables were significantly associated with smoking behavior as dependent variables. Thus, age, maternal education, marital status, employment status, family type and annual household income were included in the model for smoking behavior as independent variables. Purposeful selection of candidate variables was done based on a bivariate P-value < 0.15. The fit of the multiple logistic models was assessed using the Akaike information criterion (AIC). The model with the lowest AIC was accepted as the best-fitting model. To determine multicollinearity among the variables, collinearity diagnostic tests were conducted. In none of the cases were the "tolerance values" less than 0.2, and no variance inflation factor was greater than 10; P-values less than 5% were considered to be statistically significant.

RESULTS

Out of the total of 1,510 women initially in the sample, 24 did not meet the inclusion criteria. A further 187 excluded themselves due to domestic commitments, while 26 could not be contacted at their home. Consequently, the survey involved 1,273 eligible women who agreed to participate in a face-to-face interview, thus yielding a survey response rate of 84.3%. In the present survey, the retrospective evaluation took place approximately 1.3 years (1.3 ± 1.1) after pregnancy.

Characteristics of the sample

All the study participants were from urban areas. Their mean age was 36.4 ± 7.9 years (range: 15-49 years). Most of the participants (62%) were over 34 years old. As presented in **Table 1**, with regard to the demographic characteristics of the sample over the year prior to the survey, more than half of the participants (57.2%) were living in nuclear families, and approximately 80% of them did not have a university degree. Most of the participants (85.5%) had simple health insurance, 91% were married, more than 25% were employed and 81% had an annual household income of ≤ US$ 7,000 (**Table 1**).

Prevalence and factors relating to smoking during pregnancy

The prevalence of self-reported smoking during pregnancy was found to be 16.5% in this study sample (**Table 1**). Bivariate analysis results comparing smokers and nonsmokers are shown in **Table 1**. Regarding smoking behavior, there were differences among the women in terms of age, education, marital status and type of family. However, there were no significant differences regarding their health insurance, employment status, alcohol consumption or annual household income.

Bivariate analysis showed that, compared with nonsmokers, smokers were more likely to be under 25 years of age (14.8% versus 8.3%; P = 0.010) and had a higher level of education (26.6% versus 18.9%;P = 0.014). More smokers were unmarried (16.2% versus 7.2%; P = 0.003) and were living in extended families (51.9% versus 41.0%; P = 0.003), compared with nonsmokers. **Table 2** presents variables associated with being a smoker during pregnancy, based on multivariate logistic regression analysis. Being at a relatively young age (odds ratio, OR = 1.92, r = 0.65, P = 0.025 for the

Table 1. Characteristics of the participants: total sample and sample according to smoking status during pregnancy

Characteristics	Total sample (n %)	Smoking status during pregnancy		P-value (chi-square test)
		(+) (n %)	(-) (n %)	
Total	1,273 (100.0)	210 (16.5)	1,063 (85.5)	
Age groups (years)				
15-24	119 (9.3)	31 (14.8)	88 (8.3)	0.010
25-34	366 (28.8)	62 (29.6)	304 (28.6)	
≥ 35	788 (61.9)	107 (55.6)	671 (63.1)	
Education level*				
Primary school	388 (30.5)	51 (24.3)	337 (31.7)	0.015
Secondary school	628 (49.3)	103 (49.1)	525 (49.4)	
High school and beyond	257 (20.2)	56 (26.6)	201 (18.9)	
Marital status				
Married	1,161 (91.2)	176 (83.8)	985 (92.8)	< 0.001
Unmarried†	112 (8.8)	34 (16.2)	78 (7.2)	
Family type				
Nuclear	728 (25.6)	101 (48.1)	627 (59.0)	0.003
Extended	545 (74.4)	109 (51.9)	436 (41.0)	
Employment status				
Employed	326 (25.6)	58 (27.6)	268 (25.2)	0.144
Unemployed	947 (74.4)	152 (72.4)	795 (74.8)	
Alcohol consumption during pregnancy				
Yes	21 (1.7)	5 (2.4)	16 (1.5)	0.329
No	1,252 (98.3)	152 (97.6)	1,047 (98.5)	
Annual household income (self-reported)				
≤ US$ 7,000	1,031 (81.0)	174 (82.9)	855 (80.4)	0.117
> US$ 7,000	242 (19.0)	36 (17.1)	208 (19.6)	
Health insurance				
Yes‡	1,088 (85.5)	172 (82.9)	914 (86.1)	0.490
No	185 (14.5)	36 (17.1)	149 (13.9)	
Having preterm birth				
Yes	207 (16.3)	145 (21.4)	163 (15.3)	0.034
No	1,064 (83.7)	165 (78.6)	900 (84.7)	
Having low birthweight baby				
Yes	225 (17.7)	49 (23.3)	176 (16.6)	0.024
No	1,048 (82.3)	161 (76.7)	887 (83.4)	

*The normal age ranges for starting to attend the different school levels in Turkey are 6-7 years old for primary school (mandatory), 10-11 years old for secondary school and 13-14 years old for high school. †Including single, separated, divorced and widowed women. ‡Including the Greencard that is given to poor individuals by the Turkish Health Ministry for free healthcare but which is limited to emergency care.

15-24 age group; and OR = 2.45, r = 0.90, P = 0.001 for the 25-34 age group), having primary educational level (OR = 1.76, r = 0.56, P = 0.032), being unmarried (OR = 1.48, r = 0.48, P = 0.002) and living in an extended family (OR = 1.98, r = 0.69, P = 0.009) were significantly associated with a risk of smoking during pregnancy (**Table 2**).

DISCUSSION

This survey highlights the social factors involved in smoking during pregnancy among women living in a Middle Anatolian city. It has been estimated that 14%-30% of pregnant women continue smoking during pregnancy, including 17% in England and Wales,[18] 15% in Romania and in Australia,[19,20] 14% in the United States,[21] 13% in Israel[22] and 30% in Poland.[23] In line with these recent studies, the prevalence of smoking in the present study was 16.5%. However, the prevalence of smoking found in the present study was lower than in other studies on Turkish women, including 18% among 499 pregnant women[11] or 23% among 256 women.[12] These disparities in prevalence levels may be due to differences between geographic locations or due to hospital setting designs.[19] Furthermore, it should be noted here that the true prevalence of smoking during pregnancy may be difficult to discern, because of possible underreporting in studies that depend on self-reporting.[24] Also, sociocultural norms discouraging smoking may lead women to fail to disclose their true smoking status during pregnancy.

Table 2. Relationship between sociodemographic variables and smoking during pregnancy, based on multivariate logistic regression analysis, with odds ratios and 95% confidence intervals (n = 1,273)

Independent variables	Smoking during pregnancy	
	β	OR (95% CI)
Age groups (years)*		
≥ 35		1.00
25-34	0.90	**2.45 (1.52-3.95)**
15-24	0.65	**1.92 (1.15-3.22)**
Education level		
High school and beyond		1.00
Secondary school	0.35	1.42 (0.86-2.34)
Primary school	0.56	**1.76 (1.07-2.90)**
Marital status		
Married		1.00
Unmarried†	0.48	**1.48 (0.46-1.07)**
Employment status		
Employed		1.00
Unemployed	0.35	0.70 (0.46-1.07)
Family type		
Nuclear		1.00
Extended	0.69	**1.98 (1.42-2.77)**
Annual household income (self-reported)		
> US$ 7,000		1.00
≤ US$ 7,000	0.29	1.34 (0.89-2.02)

All statistically significant P-values are in bold type; *during pregnancy; †including single, separated, divorced and widowed women; OR = odds ratio; CI = confidence interval.

The harmful behavior of smoking contributes substantially towards deterioration of maternal and child health, especially among those living with socioeconomic disadvantages. Previous studies have reported that women who continued to smoke throughout the pregnancy had about twice the risk of adverse pregnancy outcomes, compared with those who did not smoke or reduced their smoking habit.[11,18]

The smoking pattern demonstrated in this study confirms previous research findings that smoking is more prevalent among relatively young women and among women who live in poor socioeconomic circumstances.[11,12,15,22] This may be explained by the marketing campaigns that are designed specifically to target young women.[25]

In this study, it was found that women for whom primary school was their highest education level had around twice as much risk of smoking during pregnancy. This was in line with previous research that showed that there were prominent socioeconomic differences between women who continued smoking during pregnancy and those who did not.[26,27]

An association between marital status and smoking during pregnancy was found in the present study. There was around a 1.5 times greater risk of continuing smoking during pregnancy among unmarried women, and this finding was consistent with previous research indicating that unmarried women had the highest prevalence of smoking.[26,27] A similar association was also found in the present study between women who were living in an extended family and continuing to smoke during pregnancy. This may be explained by the fact that in addition to women's roles as mothers and homemakers, their participation in the workforce has increased in recent years. From this perspective, smoking not only is a coping mechanism for escaping from or avoiding negative emotions, but also is important in relation to strong feelings of autonomy for finding one's own place in society.[28,29]

In this survey, the retrospective evaluation took place around 1.3 years after pregnancy. Previous research has been called into question with regard to recall bias, because bias in recollecting data and behavior is a severe risk in using retrospective methods. Nonetheless, concerning smoking during pregnancy, a previous study demonstrated that recollections relating to pregnancy were still accurate five or six years afterwards.[30] Pregnancy is an important event within life and the social stigma relating to smoking during pregnancy is assumed to provide a model for this accuracy. Hence, in the present survey, the time of 1.3 years after pregnancy that elapsed should not be considered problematic with regard to validity. Because of the characteristics of cross-sectional designs, self-reporting may be an important limitation in this survey. However, evidence from population-based studies has demonstrated that self-reporting of smoking status has a high level of validity.[31,32] In the present study, a face-to-face design was used rather than a self-administered design, and the fact that the information was obtained from the women in their homes means that the data regarding sociodemographic and socioeconomic determinants is likely to have been more reliable and more accurate.

Limitations

There were some limitations to this study. Firstly, the study was undertaken on a community-based sample in a single city. Because of the nature of cross-sectional designs, the findings from the study sample can possibly be generalized to the population of women of childbearing age, but no assessment of temporal relationships and thus potentially causal relationships between the variables was possible. Secondly, all the data were dependent on the women's perceptions and their accuracy of recall. No objective measurements were used to validate the participants' responses regarding smoking behavior. Furthermore, underreporting bias is common in relation to socially undesirable behavior like smoking. Finally, because smoking during pregnancy is considered to be an unacceptable and avoidable exposure, there was a possibility of reporting bias.

CONCLUSION

Smoking rates during pregnancy and adverse outcomes from pregnancy were found to vary according to social circumstances. Systematic attention should be paid to socioeconomic inequalities, to support women towards quitting smoking before or at an early stage of their pregnancies. Younger women and particularly those in lower socioeconomic groups should be targeted. This will lead to better pregnancy status, especially among young women.

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Translation and validation of the Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire for the Brazilian Portuguese language

Tradução e validação do questionário Uterine Fibroid Symptom and Quality of Life (UFS-QOL) para a língua portuguesa brasileira

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PALAVRAS-CHAVE:
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ABSTRACT

CONTEXT AND OBJECTIVE: Uterine fibroids (UF), also known as leiomyomas, are the most prevalent gynecological tumors. The Uterine Fibroid Symptoms and Quality of Life (UFS-QOL) is the only specific questionnaire that assesses symptom intensity and quality-of-life issues for women with symptomatic UF; however, it only exists in the English language. Thus, we aimed to translate and culturally validate the UFS-QOL questionnaire for the Brazilian Portuguese language.

DESIGN AND SETTING: Cross-sectional study, Department of Gynecology and Obstetrics, FMRP-USP.

METHODS: 113 patients with UF (case group) and 55 patients without UF (control group) were interviewed using the UFS-QOL questionnaire after translation and cultural adaptation. The Short Form-36 questionnaire was used as a control. Demographic and psychometric variables were analyzed.

RESULTS: Women with UF presented higher mean age, body mass index, weight, parity and comorbidities than the control group ($P < 0.05$). The most prevalent complaints were abnormal uterine bleeding (93.8%), pelvic pain (36.3%) and extrinsic compression (10.6%) and these presented adequate construct validity regarding UFS-QOL severity ($P < 0.05$). The UFS-QOL questionnaire presented good internal consistency regarding symptom severity and quality-of-life-related domains (intraclass correlation coefficient, ICC = 0.82/0.88). Structural validity presented correlation coefficients ranging from 0.59 to 0.91. Test-retest comparison did not show differences among the UFS-QOL subscales. After treatment, women with UF presented improvements on all subscales.

CONCLUSION: The UFS-QOL questionnaire presented adequate translation to the Brazilian Portuguese language, with good internal consistency, discriminant validity, construct validity, structural validity and responsiveness, along with adequate test-retest results.

RESUMO

CONTEXTO E OBJETIVO: O leiomioma uterino (LU) é o tumor ginecológico mais comum. Existe apenas um questionário específico que avalia a intensidade de sintomas e qualidade de vida de mulheres com LU sintomático, o Uterine Fibroid Symptom and Quality of Life (UFS-QOL), porém somente na língua inglesa. Dessa forma, objetivamos traduzir e validar culturalmente o questionário UFS-QOL para a língua portuguesa brasileira.

TIPO DE ESTUDO E LOCAL: Estudo transversal, Departamento de Ginecologia e Obstetrícia da FMRP-USP.

MÉTODOS: 113 pacientes portadoras de LU (grupo caso) e 55 pacientes-controle foram entrevistadas com o questionário UFS-QOL após tradução e adaptação cultural. O questionário Short Form-36 foi utilizado para controle. Variáveis demográficas e psicométricas foram analisadas.

RESULTADOS: As mulheres com LU apresentaram maior média de idade, índice de massa corporal, peso, paridade e comorbidades do que o grupo controle ($P < 0,05$). As queixas mais prevalentes foram sangramento uterino anormal (93,8%), dor pélvica (36,3%) e compressão extrínseca (10,6%) e estas apresentaram adequada validade de constructo com a gravidade indicada pelo UFS-QOL ($P < 0,05$). O questionário UFS-QOL apresentou boa consistência interna com a gravidade dos sintomas e com os domínios relacionados a qualidade de vida (coeficiente de correlação intraclasse, CCI = 0,82/0,88). A validade estrutural mostrou coeficientes de correlação variando de 0,59 até 0,91. A comparação teste-reteste não mostrou diferença entre as subscalas do UFS-QOL. Depois do tratamento, as mulheres com LU apresentaram melhora em todas as subscalas.

CONCLUSÃO: O questionário UFS-QOL apresentou adequada tradução para a língua portuguesa brasileira, com boa consistência interna, validade de constructo/discriminatória, estrutural e responsividade, assim como adequados resultados teste-reteste.

INTRODUCTION

Uterine fibroids (UF), also known as leiomyomas, are the most common gynecological tumors and they originate from the smooth muscle cells of the uterine wall. Although 60 to 80% of women present these tumors,[1] only 20 to 30% present symptoms, such as abnormal uterine bleeding and pelvic pain.[2] In Brazil, there are no accurate epidemiological data for this disease; however, the number of hysterectomies performed in this country has stabilized,[3] even though clinical management of UF is still a challenge.[4]

An observational study showed that UF had a negative impact on the patients' quality of life.[5] They presented social and professional limitations, with fear, disbelief and despondency regarding their symptoms, and these factors strengthened their will to undergo hysterectomy.[6] Thus, the impact of UF symptoms on women's health-related quality of life (HRQL) is a major indicator for treatment.

Questionnaires may be useful for measuring the impact of symptoms and the results from clinical and/or surgical interventions. Fourteen years ago, the only questionnaire directed towards UF, the Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire, was published.[7] The responsiveness of this questionnaire was subsequently assessed[8] and it was validated in a non-randomized prospective study.[9] Thus, many studies that have investigated UF have been using this questionnaire.

To our knowledge, from searching in the literature, there is no cultural translation/validation of the UFS-QOL questionnaire in the Brazilian Portuguese language. A simple translation is not valid for use, given that it is known that adapting questionnaires to the local language is a difficult task.[10] Moreover, regional expressions should be incorporated and therefore there is a need to conduct a study to confirm the cultural validity of the questionnaire.

OBJECTIVE

The authors aimed to translate and culturally validate the UFS-QOL questionnaire for the Brazilian Portuguese language.

METHODS

Type of study and patients

A cross-sectional study was conducted at the Gynecological Surgery Clinic of Hospital das Clínicas, Faculdade de Medicina de Ribeirão, Universidade de São Paulo (HCFMRP-USP), between April and September 2015. Women were recruited through the consultations that are provided in this setting and were treated by means of clinical or surgical management. The control group was constituted by women from other outpatient gynecological clinics at HCFMRP-USP who did not present abnormal uterine bleeding or pelvic pain as their main complaints.

The inclusion criteria were that the subjects should be women of fertile age presenting UF with complaints of abnormal uterine bleeding or pelvic pain or a sensation of external compression. We excluded pregnant women, those who were using anticoagulants or presented abnormal uterine bleeding of other secondary causes (endometrial or ovulatory), and those with cognitive impairments or who were illiterate.

All participants signed an informed consent statement and this study was approved by our Institutional Review Board (procedural number 531.584). This study was drawn up in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

UFS-QOL questionnaire: translation and validation

The UFS-QOL questionnaire specifically assesses severity of symptoms (8 questions) and HRQL (29 questions) among women with UF. The HRQL scale comprises the following subscales: concern, activities, energy/mood, control, self-consciousness and sexual function.[7] All answers have five options on a Likert scale. The higher the score on the severity subscale of the questionnaire is, the greater the severity of symptoms is; while the lower the scores on the HRQL subscales are, the poorer the quality of life is.

Written authorization was firstly obtained from the Society for Interventional Radiology and from Prof. James Spies to translate and validate the UFS-QOL questionnaire. Following this, the initial translation was performed by two notarized bilingual professors. This translation was evaluated by a committee composed of three gynecologists (LGOB, DAML and MAFA) and was pilot-tested on ten women who were attended in the outpatient setting. These patients were asked about any difficulties in comprehending the questionnaire. Some words were adapted by the interviewer to ease their understanding. The changes thus made were reviewed by the same medical committee and the final version was released for application. A back translation was performed by a native speaker of English who had knowledge of the Portuguese language. The final questionnaire was submitted to and received consent for publication from the Society for Interventional Radiology.[11]

There was no standardization regarding the mode of application of the questionnaire (oral interview or self-application). However, most of the questionnaires were self-administered by the patients, with the option of asking the interviewer for help if any queries arose while answering the questionnaire.

In addition, the Short Form-36 (SF-36) questionnaire was used as a control, to make comparisons with the UFS-QOL questionnaire and thus calculate its construct validity. The SF-36 questionnaire measures HRQL using eight domains: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning and mental health.[12] The individual items from each subscale are

combined to form an overall score, which is then transformed into a score from 0 to 100. Higher scores indicate better HRQL.[13]

Variables

The following demographic data were obtained: age, weight, height, body mass index (BMI), gravidity, parity and comorbidities. The following clinical symptoms were investigated: presence of abnormal uterine bleeding, pelvic pain and a sensation of external compression.

With regard to psychometric properties for measuring questionnaires, the variables analyzed were: internal consistency, construct validity, test-retest similarity and responsiveness. Internal consistency assesses the correlation between the items and is determined from the subscale and total scores. Higher values indicate higher correlation among several items on the scales, i.e. one or several items may be measuring the same concept. Values over 0.70 were considered adequate or acceptable.[14] Construct validity was calculated by comparing the symptoms with the UFS-QOL score response and was considered adequate when different responses were perceived between the groups with and without symptoms. Structural validity was explored by means of principal-component analysis, with standardized coefficients comparing all questions from each quality-of-life subscale with the severity subscale, in order to identify the highest correlation coefficient. It is important to note that this psychometric analysis was previously done by Spies et al.,[7] to individualize the subscales. A retest (n = 20) was performed 1-2 weeks after first application of the questionnaire. Responsiveness (n = 44) was assessed after three months of clinical and/or surgical treatment, to ascertain whether the UFS-QOL scores had changed after the first application of the questionnaire.

Statistical analysis

The data were tabulated in Microsoft Excel (Microsoft Co., Redmond, WA, USA) and statistical analyses were performed by means of Intercooled Stata 13.0 (College Station, TX, USA). A significance level of 5% was stipulated in all two-sided tests. Normality analysis was performed using the Shapiro-Wilk test, and the continuous variables (age, body mass index and weight) were found to have parametric distribution. The chi-square test was used for binomial variables and Student's t test for continuous variables.

No power calculation was performed, because of the extensive variability of formulae that have been suggested for generating the minimum number of subjects for a given study that involves translating and validating a standardized questionnaire.[10]

The internal consistency was calculated by means of Cronbach's alpha (over 0.9, excellent; 0.7-0.9, acceptable to good; 0.6-0.7, questionable; 0.5-0.6, poor; and below 0.5, unacceptable) and by means of item correlation. The structural validity was calculated by means of structural equation modeling between severity and other quality-of-life subscales. Test-retest similarity and responsiveness were calculated using a paired t test, with extraction of the mean and standard deviation and the mean difference between variables.

Missing data were not treated using imputation methods. All patients answered at least 50% of the questions on both questionnaires.

RESULTS

The study group was formed by 113 women and the control group by 55 women, thus totaling 168 patients recruited over the study period. We had 5 refusals to participate, and 12 women were excluded from the study (due to cognitive incapacity to understand the questionnaire) during the enrollment period.

The demographic data are described in **Table 1**. The women with UF presented higher mean age (42.6 ± 6.5 years; P < 0.005), weight (78.9 ± 16.7 kg), BMI (30.3 ± 6 kg/m²; P = 0.01) and parity (P < 0.005). The presence of comorbidities was higher among women with UF than in the control group, without any statistically significant difference (53.1% versus 38.2%; P = 0.069). The most common diseases in the study group were arterial hypertension and anxiety/depressive disorders. Among the clinical symptoms, abnormal uterine bleeding was the most prevalent (93.8%), followed by pelvic pain (36.3%) and external compression (10.6%).

Table 2 shows the analysis of the UFS-QOL and SF-36 scores in both groups. All UFS-QOL subscales showed statistically significant differences between women with UF and controls (P < 0.05). The women with UF presented higher severity scores and lower scores on the subscales that measured quality of life, compared with the controls. The self-consciousness and sexual function subscales presented greater reductions in mean difference scores between

Table 1. Demographic and clinical baseline characteristics

Variables	Control group (n = 55)	Study group (n = 113)	P-value
Age (mean ± standard deviation)	37.9 (8.2)	42.6 (6.5)	< 0.005
Gravidity (median/range)	1 (0-6)	2 (0-6)	< 0.005
Parity (median/range)	1(0-5)	2(0-6)	< 0.005
Educational level (n, %)			
0-6 years	15 (27.3)	44 (38.9)	< 0.005
7-12 years	21 (38.2)	63 (55.9)	
> 12 years	18 (32.7)	3 (2.6)	
Not informed	1 (1.8)	3 (2.6)	
Body mass index (mean ± standard deviation)	27.7 (5.8) (n = 54)	30.3 (6.0) (n = 110)	0.010
Comorbidities (n, %)	21 (38.18)	60 (53.1)	0.069
Symptoms			
Abnormal uterine bleeding	1 (1.8)	106 (93.8)	< 0.005
Pelvic pain	3 (5.5)	41 (36.3)	< 0.005
External compression	1 (1.8)	12 (10.6)	< 0.005

the women with UF and the others. Regarding the SF-36 domains, women with UF presented lower quality of life ($P < 0.05$) except for the vitality domain ($P = 0.07$). Thus, discriminant validity could be demonstrated.

The internal consistency calculation on the UFS-QOL questionnaire is shown in **Table 3**. Values over 0.75 were found for all its subscales and for the HRQL score in the control and study groups, thus showing that the concordance was adequate. Item correlation presented moderate concordance regarding symptom severity. Similarly, in the study group, moderate concordance was seen regarding symptom severity, self-consciousness and sexual function. The construct validity showed statistical significance between the presence of symptoms and higher mean UFS-QOL severity score (**Table 4**). With regard to responsiveness, all UFS-QOL subscales presented improvement in quality of life and reduction in the severity of symptoms after treatment (**Table 5**). The analysis on standardized coefficients for quality-of-life subscales, with severity as covariance, showed values ranging from 0.59 to 0.91, as seen in **Table 6**. The covariance analysis with severity showed highest correlations with the following subscales: concern (0.91), control (0.80) and self-consciousness (0.81). Finally, no differences were noted in the test-retest comparison of the UFS-QOL version for the Brazilian Portuguese language (**Table 7**). The final version is presented with this paper (**Annex**).

Table 2. Discriminant validity of the Uterine Fibroid Symptom and Quality of Life (UFS-QOL) and Short-Form-36 (SF-36) questionnaires among women with uterine fibroids and control patients

Questionnaires	Mean (SD)		P-value
	Control group (n = 55)	Study group (n = 113)	
UFS-QOL			
Symptom severity	15.7 (17.6)	59.4 (19.1)	< 0.001
Concern	95.2 (10.9)	33.6 (28.7)	< 0.001
Activities	96.5 (10.6)	53.8 (26.0)	< 0.001
Energy/Mood	92.9 (18.6)	47.3 (28.5)	< 0.001
Control	94.5 (16.2)	51.3 (27.3)	< 0.001
Self-consciousness	94.7 (16.2)	49.3 (25.7)	< 0.001
Sexual function	90.7 (23.8)	43.9 (36.6)	< 0.001
Total Score	361.7 (73.7)	115.7 (116.9)	< 0.001
SF-36			
Physical functioning	74.9 (32.3)	59.2 (29.6)	0.0021
Physical role functioning	73.2 (39.0)	38.3 (44.9)	< 0.001
Bodily pain	62.5 (26.2)	52.0 (28.4)	0.023
General health perceptions	58.6 (17.9)	50.5 (20.2)	0.013
Vitality	56.9 (21.7)	49.6 (25.8)	0.072
Social role functioning	74.8 (30.7)	59.9 (32.0)	0.003
Emotional role functioning	72.7 (40.1)	44.8 (44.5)	< 0.001
Mental health	60.7 (21.5)	50.8 (26.5)	0.017

Table 3. Internal consistency (ICC) and item correlation among women with uterine fibroids and control group

UFS-QOL	Control group (n = 55)		Study group (n = 113)	
	Item correlation	Cronbach's alpha	Item correlation	Cronbach's alpha
Symptom severity	0.59	0.84	0.65	0.82
Concern	0.84	0.84	0.80	0.80
Activities	0.89	0.83	0.81	0.81
Energy/mood	0.96	0.81	0.86	0.80
Control	0.92	0.82	0.86	0.80
Self-consciousness	0.90	0.82	0.66	0.82
Sexual function	0.90	0.80	0.72	0.81
HRQL score	1.00	0.91	1.00	0.88

UFS-QOL = Uterine Fibroid Symptom and Quality of Life; HRQL = health-related quality of life.

Table 4. Construct validity between uterine fibroid symptoms and the Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire

	Study group	Control group	Mean difference	P-value
	Mean ± standard deviation			
Abnormal uterine bleeding	59.57 ± 19.92	19.67 ± 20.10	-39.9	< 0.001
Pelvic pain	60.51 ± 20.15	39.61 ± 28.02	-20.89	< 0.001
External compression	56.00 ± 23.74	44.17 ± 27.88	-11.83	0.014

Table 5. Responsiveness of women with uterine fibroids after treatment (n = 44)

UFS-QOL	Before treatment	After treatment	Mean difference	P-value
	Mean/standard deviation			
Symptom severity	54.97 (14.57)	15.05 (17.91)	-39.91	< 0.005
Concern	32.84 (24.97)	86.59 (24.97)	+53.75	< 0.005
Activities	57.95 (24.96)	91.72 (13.81)	+33.76	< 0.005
Energy/mood	50.08 (28.37)	83.76 (22.69)	+33.69	< 0.005
Control	54.32 (26.86)	87.61 (20.50)	+33.29	< 0.005
Self-consciousness	50.95 (22.68)	75.19 (27.70)	+24.24	< 0.005
Sexual function	43.18 (36.21)	65.91 (39.19)	+22.72	< 0.005
HRQL score	124.42 (108.26)	298.09 (110.96)	+173.68	< 0.005

UFS-QOL = Uterine Fibroid Symptom and Quality of Life; HRQL = health-related quality of life.

DISCUSSION

This study showed that in the UFS-QOL questionnaire, the symptoms of women with UF strongly correlated with lower HRQL subscales and higher severity scores, and thus that the women's symptoms had an impact on their quality of life. No differences with regard to the ICC were seen on the UFS-QOL subscales among the women with UF, although item correlation was moderate among the symptoms of severity, self-consciousness and sexual function. These data are similar to what was found in the validation studies conducted by Spies et al. and Coyen et al.[7,9] Moreover, women with UF presented worse quality of life when analyzed by means of the SF-36 questionnaire, except for the vitality domain. This may indicate that the UFS-QOL questionnaire is fulfilling its task of targeting women with UF.

Table 6. Structural validity of all quality-of-life subscales compared with the severity scale of the UFS-QOL questionnaire by means of structural equation modeling

UFS-QOL questionnaire (model showing interaction of quality-of-life subscales with the severity subscale)	Standardized coefficient (standard error)	Variance
Concerns (last 3 months)		
Anxiety about unpredictable onset/duration of periods (Q9)	0.78 (0.031)	0.38
Soiling underclothes (Q15)	0.91 (0.015)	0.16
Soiling bed linen (Q22)	0.88 (0.018)	0.21
Feeling inconvenienced about carrying extra pads (Q28)	0.67 (0.044)	0.54
Soiling outer clothes (Q32)	0.91 (0.015)	0.15
Activities (last 3 months)		
Anxiety about traveling (Q10)	0.62 (0.050)	0.60
Interfered with physical activities (Q11)	0.77 (0.036)	0.39
Decreased amount of time on exercise (Q13)	0.73 (0.041)	0.45
Felt that activity was difficult to carry out (Q19)	0.78 (0.034)	0.38
Interfered with social activities (Q20)	0.78 (0.034)	0.38
Plan activities more carefully (Q27)	0.82 (0.028)	0.31
Caused embarrassment (Q29)	0.78 (0.034)	0.38
Energy (last 3 months)		
Felt tired (Q12)	0.81 (0.029)	0.34
Felt drowsy or sleepy during day (Q17)	0.72 (0.039)	0.47
Felt sad, discouraged or hopeless (Q23)	0.88 (0.020)	0.21
Felt downhearted and blue (Q24)	0.88 (0.019)	0.21
Felt wiped out (Q25)	0.86 (0.022)	0.25
Felt irritable (Q31)	0.83 (0.025)	0.29
Felt weak as energy drained from her body (Q35)	0.87 (0.020)	0.24
Control (last 3 months)		
Felt that she was not in control of her life (Q14)	0.79 (0.033)	0.37
Felt less productive (Q16)	0.78 (0.034)	0.38
Concerned about her health (Q26)	0.84 (0.027)	0.29
Felt uncertain about her future (Q30)	0.77 (0.035)	0.39
Felt that she was not in control of her health (Q34)	0.84 (0.028)	0.29
Self-consciousness (last 3 months)		
Felt self-conscious of weight gain (Q18)	0.71 (0.048)	0.49
Felt conscious about the size and appearance of her stomach (Q21)	0.80 (0.041)	0.35
Affected the size of clothing she wore during her periods (Q33)	0.59 (0.058)	0.64
Sexual function (last 3 months)		
Diminished sexual desire (Q36)	0.90 (0.031)	0.17
Caused patient to avoid sexual intercourse (Q37)	0.90 (0.032)	0.18
Covariance severity		
Concern	0.91 (0.018)	----------
Activity	0.79 (0.036)	----------
Energy	0.78 (0.034)	----------
Control	0.80 (0.035)	----------
Self-consciousness	0.81 (0.043)	
Sexual function	0.65 (0.050)	----------

With regard to the structural validity of the UFS-QOL questionnaire, we prepared structural equation modeling to compare the severity subscale with the other subscales, with the aim of determining which of them presented the highest correlation coefficient. We did not analyze all possibilities because this had already been done by Spies et al.[7] when they published the UFS-QOL questionnaire in the English language. Structural validity is usually determined when a questionnaire is initially launched, to prove that the questions are correctly divided between the best-fit subscales.

Our test-retest reliability determination showed through the paired t test that there was no systematic difference at the second visit, after an interval ranging from 1 to 2 weeks. This is similar to what Spies et al.[7] found with 27 patients and they did not consider the sample size to be underpowered for detecting differences in baseline characteristics. Our total sample size was larger than that of the first publication of this questionnaire.[7]

Furthermore, the UFS-QOL questionnaire for the Brazilian Portuguese language also demonstrated responsiveness to change among women treated with medications or surgery, with mean change scores greater than 20 points for all subscales. Although only 40 patients were included, these data are supported by those of Harding et al.,[8] who analyzed 102 patients with UF who were treated with thermal ablation by means of MRI-guided focused ultrasound (MRgFUS); and by data from larger studies such as the Fibroid Registry for Outcomes Data (FIBROID), a database in which more than 2,000 women undergoing uterine embolization for leiomyomata were studied.[15] Recently, the questionnaire was slightly modified for women who have undergone hysterectomy.[16] Some non-English-speaking countries have used this questionnaire for studies on women with UF without validating it, such as a study conducted in Korea.[17]

This study presented some limitations: we did not calculate the criterion validity, which would consist of assessment of the UFS-QOL questionnaire using a clinical tool that is considered to be the gold standard. In this case, we would have had to compare it with the alkaline hematin technique for quantifying blood loss, or with a pictorial blood assessment chart (PBAC). On the other hand, although the control and study groups were different with regard to age and comorbidities, we do not believe that these variables caused a great impact on our results, just as stated by Spies et al., probably because women with UF present epidemiological features that would be difficult to create in a similar control group.[7]

We believe that we met our goal of confirming that the UFS-QOL questionnaire could be translated without language impairment. Thus, we confirmed that the Brazilian Portuguese version is adequate for identifying women with UF, in the same way that it does this in English-speaking countries. Our expectation is that the translation and validation of this questionnaire will be very useful as a tool for measuring the impact of symptoms and the results from interventions relating to UF in Brazil and will strengthen future data produced in our setting.

Table 7. Test-retest similarity of the UFS-QOL questionnaire in the study group (n = 20)

UFS-QOL	Test	Retest	Mean difference	P-value
	Mean (standard deviation)			
Symptom severity	57.19 (19.35)	52.03 (32.81)	-5.16	0.453
Concern	39.5 (33.34)	48.25 (35.37)	+8.75	0.088
Activities	54.8 (27.79)	61.79 (30.35)	+6.96	0.270
Energy/mood	54.64 (32.36)	54.11 (33.83)	-0.53	0.929
Control	53.5 (27.29)	60 (32.16)	+6.5	0.223
Self-consciousness	50.83 (31.75)	53.33 (32.82)	+2.5	0.746
Sexual function	46.25 (35.14)	45 (39.82)	-1.25	0.892
HRQL score	133.23 (133.27)	152.99 (158.69)	+19.77	0.452

UFS-QOL = Uterine Fibroid Symptom and Quality of Life; HRQL = health-related quality of life.

CONCLUSION

The Brazilian Portuguese version of the UFS-QOL questionnaire is a valid and reliable instrument for assessing the HRQL of women with UF. It demonstrated good internal consistency, discriminant validity, construct validity, structural validity, test-retest similarity and responsiveness.

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Annex. Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire after translation and cultural validation for the Brazilian Portuguese language

RUBRICA DA PACIENTE: _____

IDENTIDADE ou REGISTRO DA PACIENTE: _____

DATA: _____

QUESTIONÁRIO SOBRE SINTOMAS DO LEIOMIOMA/MIOMA UTERINO E QUALIDADE DE VIDA (UFS-QOL)

Encontram-se listados, abaixo, sintomas experimentados por mulheres portadoras de mioma uterino. Avalie, por favor, a maneira como cada sintoma se relaciona com o mioma uterino ou seu ciclo menstrual. O objetivo de cada pergunta é saber o quanto cada um desses sintomas tem incomodado você nos últimos três meses.

Não há respostas certas ou erradas. Certifique-se, por favor, de responder cada pergunta assinalando (X) no quadrado mais apropriado. Se a pergunta não se aplicar a você, marque a resposta "nada"

Durante os últimos três meses, diga o quanto você ficou incomodada com ...	**Nada**	**Muito pouco**	**Um pouco**	**Muito**	**Muitissímo**
1. Sangramento intenso durante sua menstruação	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Eliminação de coágulos durante a menstruação	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Variação na duração do seu ciclo menstrual quando comparada com seus ciclos anteriores	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Variação no intervalo do seu ciclo menstrual quando comparada com seus ciclos anteriores	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5. Sensação de aperto ou pressão na região do baixo ventre ("pé da barriga")	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
6. Aumento da vontade de urinar durante o dia	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
7. Aumento da vontade de urinar durante a noite	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
8. Sensação de cansaço	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

As próximas questões procuram saber sobre seus sentimentos e experiências em relação ao impacto do leiomioma uterino em sua vida. Considere, por favor, cada pergunta na medida em que ela se relaciona com suas experiências sobre esse assunto nos últimos três meses.

Não há respostas certas ou erradas. Certifique-se, por favor, de responder cada pergunta assinalando (X) no quadrado mais apropriado. Se a pergunta não se aplicar a você, marque a resposta "nunca"

Durante os últimos três meses, com que frequência seus sintomas relacionados ao mioma uterino ...	**Nunca**	**Poucas vezes**	**Algumas vezes**	**A maior parte do tempo**	**O tempo todo**
9. Fizeram com que você se sentisse ansiosa sobre a falta de previsão da data ou duração da menstruação?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
10. Fizeram com que você se sentisse ansiosa em relação a viajar?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
11. Interferiram em suas atividades físicas?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
12. Fizeram com que você se sentisse cansada ou exausta?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
13. Fizeram com que você diminuísse a quantidade de tempo que você usa em exercícios ou outras atividades físicas?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
14. Fizeram com que você se sentisse como se não tivesse controle sobre sua vida?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
15. Fizeram com que você se sentisse preocupada com possibilidade de manchar peças íntimas?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
16. Fizeram com que você se sentisse menos produtiva em termos de trabalho?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Durante os últimos três meses, com que frequência seus sintomas relacionados ao mioma uterino ...	**Nunca**	**Poucas vezes**	**Algumas vezes**	**A maior parte do tempo**	**O tempo todo**
17. Fizeram com que você se sentisse com sono ou tontura durante o dia?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
18. Fizeram com que você tivesse a sensação de ganho de peso?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
19. Fizeram com que você sentisse que estava difícil realizar suas atividades habituais?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
20. Interferiram em suas atividades sociais?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
21. Fizeram você perceber sobre o tamanho e a aparência da sua barriga?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
22. Fizeram com que você se sentisse preocupada em manchar roupas de cama?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
23. Fizeram com que você se sentisse triste, desanimada ou desesperada?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
24. Fizeram com que você se sentisse deprimida ou abatida?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
25. Fizeram com que você se sentisse extremamente cansada?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
26. Fizeram com que você se sentisse preocupada ou ansiosa em relação à sua saúde?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
27. Fizeram com que você planejasse suas atividades com mais cuidado?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
28. Fizeram com que você se sentisse incomodada por sempre carregar absorventes, absorventes internos ou roupas extras para evitar "acidentes"?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
29. Causaram-lhe constrangimento?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
30. Fizeram com que você sentisse incerteza em relação ao seu futuro?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
31. Fizeram com que você se sentisse irritada?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
32. Fizeram com que você se sentisse preocupada em relação a manchar a parte externa da roupa?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
33. Afetaram o tamanho das roupas que você costuma usar durante seus ciclos menstruais?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
34. Fizeram com que você sentisse sem controle sobre sua saúde?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
35. Fizeram com que você se sentisse fraca, como se a energia tivesse sido "sugada" do seu corpo?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
36. Diminuíram seu desejo sexual (vontade de ter relação)?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
37. Fizeram com que você evitasse ter relações sexuais?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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Influence of maternal anxiety on child anxiety during dental care: cross-sectional study

Influência da ansiedade materna na ansiedade infantil frente ao atendimento odontológico: estudo transversal

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PALAVRAS-CHAVE:
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Odontologia.
Escala de ansiedade manifesta.

ABSTRACT

CONTEXT AND OBJECTIVES: Anxiety is usually classified as a disorder of neurotic nature and is often related to contexts of stress, which may include worries, motor tension and autonomic hyperactivity. The aim of this study was to assess the influence of mothers' anxiety on their children's anxiety during dental care.

DESIGN AND SETTING: Analytical cross-sectional study conducted at in a private dentistry school in the south of Brazil.

METHODS: Convenience sampling was used. All mothers of children undergoing treatment were invited to participate in this study. Data to investigate anxiety related to dental treatment among the children were collected through applying the Venham Picture Test (VPT) scale. For the mothers, the Corah scale was applied. A self-administered sociodemographic questionnaire with questions about demographic, behavioral, oral health and dental service variables was also used.

RESULTS: 40 mother-child pairs were included in the study. The results showed that 40% of the children were anxious and 60% of the mothers were slightly anxious. Local anesthesia was the procedure that caused most anxiety among the mothers, making them somewhat uncomfortable and anxious (60%). Family income higher than R$ 1,577.00 had an influence on maternal anxiety (75.6%). Maternal anxiety had an influence on child anxiety (81.3%).

CONCLUSION: Most of the children showed the presence of anxiety, which ranged from fear of dental care to panic, inferring that maternal anxiety has an influence on children's anxiety. Dental procedures did not interfere with the mothers' anxiety, but caused positive feelings, whereas they affected the children more.

RESUMO

CONTEXTO E OBJETIVO: A ansiedade é geralmente classificada como um transtorno de caráter neurótico, frequentemente relacionado a contextos de estresse variando entre preocupações, tensão motora e hiperatividade autonômica. O objetivo desta pesquisa foi avaliar a influência da ansiedade materna na ansiedade de seu filho durante o atendimento odontológico.

TIPO DE ESTUDO E LOCAL: Estudo analítico transversal realizado em uma faculdade particular do sul do Brasil.

MÉTODOS: Amostragem por conveniência foi utilizada. Todas as mães das crianças em tratamento foram convidadas a participar da pesquisa. A coleta dos dados para verificar a ansiedade relacionada com o tratamento odontológico nas crianças foi realizada a partir da aplicação da escala "Venham Picture Test" (VPT). Para as mães, foi utilizada a escala de Corah. Também se utilizou um questionário sociodemográfico autoaplicativo sobre variáveis demográficas, comportamentais, de saúde bucal e de serviço odontológico.

RESULTADOS: Foram incluídos 40 pares de mães e crianças. Os resultados mostraram que 40% das crianças estavam ansiosas e 60% das mães estavam levemente ansiosas. A anestesia local foi o procedimento que causou mais ansiedade entre as mães, deixando-as um pouco desconfortáveis e ansiosas (60%). Renda familiar maior de R$ 1.577,00 influenciou a ansiedade materna (75.6%). A ansiedade materna influenciou a ansiedade das crianças (81.3%).

CONCLUSÃO: A maioria das crianças apresentou ansiedade, o que variou do medo ao pânico ao atendimento odontológico, inferindo que a ansiedade materna tem influência na ansiedade dos seus filhos. Os procedimentos odontológicos não interferem na ansiedade das mães, atingindo mais as crianças, porém provocam sentimentos positivos.

INTRODUCTION

Anxiety is usually classified as a disorder of neurotic nature and is often related to contexts of stress, with symptoms that may include worries, motor tension and even autonomic hyperactivity.[1] Anxiety and fear are common in routine dental practice. Pediatric dentists are better trained to provide smoother child care, which contributes towards a better relationship between patients and professionals.[2] Historically, the study by Johnson and Baldwin was one of the first to identify a positive and significant correlation between maternal anxiety and the repertoire of behavior of children who were undergoing treatment.[3] In the 1970s, there were significant improvements regarding equipment, procedures, techniques and materials. However, dental treatment still causes a series of concerns for dentists, especially regarding provision of dental care for children.

Anxiety among patients during their dental treatment is one of the greatest challenges faced by dentists, considering that it hinders implementation of clinical procedures. This situation may lead patients either not to show up or to quit treatment, which usually ends up worsening their oral health condition. Over time, if these patients do not undergo the treatment that they should, the treatment required will become more specialized, with procedures that are more invasive and which also involve higher financial costs.[5] Difficulties in treating children may lead professionals to feel dispirited and incapable. Hence, it is important to first acknowledge patients' anxious behavior, so that techniques for achieving clinical security may be applied.[4]

People are not born with anxiety and fear of dental treatment and/or the dentist. This association occurs through the socialization process. Children are as susceptible to anxiety as adults, and their anxiety is derived from peer communication of reported bad experiences or even from threats that parents make.[5] All of this makes clinical and psychological management more difficult because of children's different understandings. Therefore, it needs to be possible to work beyond a simple approach of advocating regular visits to dentists, with emphasis on the notion that this is a normal everyday activity and that it may even be enjoyable.

Despite extensive technological advances in dentistry and a search for more humanized services focused on support and comprehensive care, there is still a pattern of thought, especially among the Brazilian population, that associates the dental environment with a place that will cause pain and may generate feelings of fear and anxiety. Such values are transferred from one generation to the next, thus creating a cycle of fear and distress in early childhood. These feelings prevail into adulthood, when they will be reproduced again and transferred to future generations.[5]

Lee et al.[6] suggested that the anxious behavior of adults during dental treatment may have been acquired through childhood fears, which would therefore require dentists to properly handle child patients. The importance of their study for Brazilian children's health is that it shows how their anxious behavior during dental treatment and parental influence may be associated, considering that fear of dentists greatly affects children.[7] This fear also seems to be associated with non-collaborative behavior and lack of visits to a dentist.[6] Armfield et al.[8] explored the relationship between dental fear and dental care. They showed that a vicious circle existed, in which people who were very fearful were more prone to delaying their treatment, thereby leading to worsening of their problems and fueling the dental fear that was already present. These occurrences directly involve dental care and negatively affect children's oral health indicators in Brazil.[9]

The topic of fear and anxiety during dental treatment needs to be researched in several populations, in order to minimize the impact of these factors on oral treatments. Moreover, studies on maternal and child influences, in mother-child pairs, are still scarce in the literature.

OBJECTIVE

This study aimed to assess the association between maternal anxiety and child anxiety within dental care.

METHODS

This study was approved by our institution's Ethics Committee for Research on Human Beings, under protocol 1.096.053. It was conducted in compliance with ethical and legal norms.

Study design, setting and participants

The present study had a quantitative approach and cross-sectional design. The sample was obtained according to convenience: all mothers of children undergoing treatment at the Children's Dental Clinic of the Dentistry School of Faculdade Meridional (IMED) were invited to participate in this study, during the months of March and April 2014, as well as their children aged 5 to 10 years. Mothers who were participating in their children's first visit to a dentist, and children of age groups differing from the study proposal were excluded from the study. All the children were interviewed and were given dental treatment by the same professional, who was a dentistry student. All the children were always accompanied by their mothers, and this formed an inclusion criterion.

Description of data collection instruments

Data on the children were gathered by applying the modified Venham Picture Test (VPT), which assesses the emotional reactions of children when they choose the drawing of a human figure that best identifies them at that moment. It is considered easy to apply and clear, and little time is required for giving responses.[10] Originally, the scale presented 42 drawings only with figures of male gender.[10] Later on, the number of figures was reduced, the female gender was included and the scale went through some changes that made it more practical. These changes included the

way in which pairs of figures were presented and the size of heads in relation to the rest of the body.[11]

The questions that the children were asked were standardized and the figures depicted children at the size of half of an A4 sheet of paper (105 mm wide by 98.5 mm long), in color. Drawings of female figures were given to the girls, and male figures to the boys. The test included seven charts with emotional reactions for the different genders.[12] The figures expressed several reactions, and the children were asked to choose which one most reflected their emotions at that moment, such as: low anxiety (image 1 – neutral), no anxiety (image 2 – happy), and presence of anxiety (image 3 – fearful; image 4 – distressed and crying; image 5 – sad; image 6 – angry; and image 7 – panicky).[12]

Information on the reproducibility of the modified VPT data collection instrument was obtained through applying the questionnaire on a second occasion, to five children who had participated in the pilot study, with a one-week interval between the events. The correlation between the two response times was calculated by means of Spearman's correlation coefficient. Among the five participants, four (80%) answered the questionnaire identically on the two occasions. The Spearman's coefficient values varied between 0.07 and 1. Overall, it can be concluded that there was a good correlation between the two times when the mothers answered the questionnaire. Therefore, the reproducibility of this instrument can be considered satisfactory. The mothers and their respective children who participated in the pilot study did not participate in the final study.

The data collection instrument used for the mothers was a self-administered sociodemographic questionnaire, with semi-structured questions that asked for demographic, behavioral, oral health and dental care variables. The questionnaire used was based on another study[5] with the same variables, which were modified and included in the present study.

In addition, the Corah scale was applied to the mothers, with questions about dental situations, in order to investigate anxiety during dental care.[13] The Corah scale has four questions about dental situations: "If you had to go to the dentist tomorrow, how would you feel?"; "When you are in the waiting room of the clinic, waiting to be called by the dentist, how do you feel?"; "When you are in the dentist's chair waiting for him to start the local anesthetic procedures, how you feel?"; and "You are the dentist's chair, already anesthetized. While waiting for the dentist get the instruments to start the procedure, how does it feel?" For each question, there are five answer options, which are: "quiet, relaxed"; "a little uncomfortable"; "tense"; "anxious and afraid"; and "so anxious or afraid that I start to sweat and feel bad."

Description of data collection procedures

The data collection procedures took place in the waiting room before dental care, in a room separated from other patients, in order to maintain the participants' privacy and ensure the confidentiality of responses.

After the mothers had given their consent, the investigation was directed towards the children in an extroverted manner in which the modified VPT scale was presented as a game, so as to make the children comfortable about participating.

The questions were asked by a single examiner, who used a standardized approach to address the children and apply the instrument; she had previously been trained for this. The examiner was a graduate in educational psychology and had had experience of working with children, which facilitated their understanding of questions.

After the scale had been presented to the children, a standardized question specific for this test was also asked: "All of these children are waiting to enter the dentist's office. Look at their faces. Which one looks most like you?" If the child presented signs of not understanding the question, it was asked differently, in a manner that was standardized as a second option: "Do you see the faces of these children? Do any of them perhaps look like you at this moment?" If any children refused to participate or claimed not to look like any of the children on the scale, the researcher could motivate the game by choosing one of the faces herself and asking the children to choose theirs.

After the activity with the child had been finished, the sociodemographic questionnaire and the Corah scale were applied to the mother.

Data analysis

A descriptive data analysis was performed on the results obtained from the modified Venham Picture Test (VPT) that had been applied to the children. Two categories were set up: presence of anxiety or low or no anxiety, according to the code on each figure. The codes ranged from 0 to 6. Code 0 (neutral) represented low anxiety, code 1 (happy) represented no anxiety and codes 2, 3, 4, 5 and 6 represented presence of anxiety. The anxiety-level results obtained from the Corah scale directed towards the mothers were interpreted as follows: patients whose answers totaled less than 5 points were considered to be minimally anxious; from 6 to 10 points, slightly anxious; from 11 to 15 points, moderately anxious; and over 15 points, extremely anxious.

Maternal anxiety was used as the dependent variable, which was categorized as minimally anxious or some degree of anxiety: all mothers with some degree of anxiety were put together in a single group. The independent variables were as follows: child's age; mother's age; child's gender; mother's education level; family income; mother's assessment of dental experience; whether the child had already presented some major medical problem; whether the child had already had caries (cavities); whether the child had already had teeth extracted; and the child's anxiety level.

All data were written down and typed into a specific database for the descriptive and inferential statistical analyses of the present study. Data were electronically processed through the Statistical Package for the Social Sciences software (SPSS), version 17.0. For the

present study, it was decided not to perform multivariate analysis but to use bivariate analysis by means of Pearson's chi-square test (χ^2). This hypothesis test had the aims of finding dispersion values for each of the nominal variables and assessing associations that might exist among qualitative variables. Thus, the association between the dependent variable (maternal anxiety) and the independent variables was tested at a 5% significance level and with a 95% confidence interval, taking the unilateral hypothesis into consideration. The variables deemed to be associated with the outcome were the ones with significance levels less than or equal to 0.05.

RESULTS

Over the study period, 40 consecutive children were treated in the service, which resulted in a sample of 40 mother-child pairs.

Table 1 describes the variables relating to the children, among whom the prevailing age group was from 7 to 10 years. Out of the 40 children, 31 (77.5%) had not presented any major medical problems and the majority (70.0%) had already had dental caries. Most of the children (40.0%) showed presence of anxiety (fear and panic) before dental care.

Table 1. Distribution of child variables

Variables	n (40)	% (100)
Child's age (years)		
5-6	10	25.0
7-8	15	37.5
9-10	15	37.5
Child's gender		
Female	20	50.0
Male	20	50.0
Child had had some major medical problem		
No	31	77.5
Yes	9	22.5
Child had already been to the dentist		
No	0	0
Yes	40	100
Child had already had caries		
No	12	30.0
Yes	28	70.0
Child had already had teeth extracted		
No	23	57.5
Yes	17	42.5
Child's feeling before dental care		
Neutral	10	25.0
Happy	14	35.0
Fearful	3	7.5
Distressed and crying	2	5.0
Sad	5	12.5
Angry	4	10.0
Panicky	2	5.0
Venham Picture Test result		
Low anxiety	10	25.0
No anxiety	14	35.5
With anxiety	16	40.0

The Corah scale results showed that 16 children (40.0%) were minimally anxious; 20 (50.0%) were slightly anxious; three (7.5%) were moderately anxious; and one (2.5%) was extremely anxious. **Table 2** describes the variables relating to the mothers, according to the two categories of mothers' degrees of anxiety.

Inferential data analysis

From the statistical analysis, it could be seen that neither the sociodemographic characteristics (variables of child's age and gender) nor the mother's age and education level were associated with the dependent variable (maternal anxiety). The variables of mother's dental experience and child's experience (of major medical problems, dental caries or tooth extraction) were also not related to the dependent variable.

However, there were significant associations between maternal anxiety and the variables of family income (P = 0.030) and child's anxiety (P = 0.026). These results showed that family income higher that R$ 1,577.00 had an influence on maternal anxiety (75.6%). Moreover, there was a relationship between mothers who presented some level of anxiety and children with anxiety (81.3%), with P = 0.026. These results are shown in **Table 3**.

DISCUSSION

The modified VPT showed that most of the children (40%) were anxious or a little anxious (25%) before dental treatment. Anxiety is frequently correlated with dental treatment because the pain and emotional reactions to this treatment are seen by many patients as threatening their wellbeing.[2] One study that assessed dental fear levels, states of anxiety and physiological distress among children older than six years of age and their mothers during pediatric dental procedures concluded that maternal anxiety before children's dental treatment was significantly associated with children's fear of dental treatment.[14]

Age is one of the factors with a higher impact on the presence of anxiety among children. Younger children tend to be more afraid of the unknown and of abandonment. However, the present study showed that children aged 7 to 10 years were the ones who most presented some level of anxiety. This may be linked to the possibility that because they were older, they may have had previous painful or distressful experiences relating to dental care. In addition, children of this age have a higher level of attention and cognition, and may have correlated the negative experiences of members of their families with their own experiences.

Maternal anxiety was measured in four categories, among which most mothers were classified as slightly anxious (50%) or minimally anxious (40%). These data are corroborated by the results from other studies,[5] in which the majority of legal guardians of children showed low or slight anxiety during dental care. This characterizes the new profile of dental patients, who have become detached from rooted concepts about dental treatment

panic and have start to regard it just as a regular procedure required for oral health maintenance.

From the Corah scale, the present study showed that what makes mothers feel stressed (22.5%) and uncomfortable (22.5%) is the procedure of injected anesthesia. In other study, states of anxiety and physiological distress levels were significantly higher among mothers before their children's dental treatment but not afterwards.[14] Soares et al.[15] showed that 54.8% of the children whose family income was higher than two minimum wages did not present anxiety in relation to dental care. The present study showed a statistically significant association between maternal anxiety and family income. Among the mothers with family income higher than R$ 1,577.00, the majority (75.6%) presented some level of anxiety. This may be explained by the greater ease of access to information on dental procedures among mothers with higher family income.[5]

Table 2. Distribution of maternal variables, according to the two categories of mothers' degrees of anxiety

Maternal variables	Maternal anxiety			
	Very little		Some level of anxiety	
	n (16)	% (40)	n (24)	% (60)
Mother's age (years)				
24 to 31	6	46.2	7	53.8
32 to 40	5	31.3	11	68.8
41 to 50	5	45.5	6	54.5
Mother's education level				
Up to completion of elementary school	5	45.5	6	54.5
High school	9	45.0	11	55.0
Higher education	2	22.2	7	77.8
Monthly family income (in Brazilian reais)				
Less than 788.00	3	18.3	0	0
789.00 – 1,576.00	7	43.8	5	20.8
1,577.00 – 2,364.00	3	18.8	8	33.3
More than 2,364.00	3	18.8	11	45.8
Mother's assessment of dental experience				
Good	15	46.9	17	53.1
Bad	1	12.5	7	87.5
Mother's perception regarding child anxiety				
No	7	43.8	9	37.5
Yes	9	56.3	15	62.5
If required to visit the dentist tomorrow				
Ok, I would not mind	16	100.0	14	58.3
I would be slightly concerned	0	0	5	20.8
I would feel great discomfort	0	0	2	8.3
I would be afraid of what may happen	0	0	2	8.3
I would be very apprehensive and would not sleep well	0	0	2	4.2
Waiting to be called by the dentist in the waiting room				
Calm, relaxed	14	87.5	9	37.5
A little uncomfortable	2	12.5	6	25.0
Stressed	0	0	4	16.7
Anxious or afraid	0	0	5	20.8
Very anxious and afraid, starting to sweat and feeling bad	0	0	0	0
During local anesthesia				
Calm, relaxed	14	87.5	2	8.3
A little uncomfortable	2	12.5	7	29.2
Stressed	0	0	9	37.5
Anxious or afraid	0	0	5	20.8
Very anxious and afraid, starting to sweat and feeling bad	0	0	1	4.2
After anesthesia, waiting for the dentist to pick up instruments				
Calm, relaxed	16	100.0	12	50.0
A little uncomfortable	0	0	8	33.3
Stressed	0	0	3	12.5
Anxious or afraid	0	0	1	4.2
Very anxious and afraid, starting to sweat and feeling bad	0	0	0	0

One of the most controversial points is mothers' presence in the dental office with their children during dental care.[16] Some authors have stated that child anxiety is associated with maternal anxiety, and that this relationship may result in non-collaborative attitudes among children.[4,17] The present study showed that there was a significant association between maternal anxiety and child anxiety, in which the majority of anxious children (81.3%) had mothers with some level of anxiety. This corroborates the findings from another study that aimed to investigate the effects of sociodemographic factors and maternal anxiety levels on the behavior of children undergoing surgery, which concluded that maternal knowledge and experience of anesthesia, and high levels of maternal anxiety, may be related to increased anxiety among children undergoing surgery.[4] Lack of psychological preparation

Table 3. Bivariate analysis on sociodemographic variables associated with maternal anxiety and its relationship to child anxiety

Independent variables	Maternal anxiety						P
	Minimally anxious		Some level of anxiety		Total		
	n	%	n	%	n	%	
Child's age (years)							
5 - 6	5	50.0	5	50.0	10	100.0	0.707
7 - 8	5	33.3	10	66.7	15	100.0	
9 - 10	6	40.0	9	60.0	15	100.0	
Total	16	40.0	24	60.0	40	100.0	
Mother's age (years)							
24 to 31	6	46.2	7	53.8	13	100.0	0.65
32 to 40	5	31.3	11	68.8	16	100.0	
41 to 50	5	45.5	6	54.5	11	100.0	
Total	16	40.0	24	60.0	40	100.0	
Child's gender							
Female	9	45.0	11	55.0	20	100.0	0.374
Male	7	35.0	13	65.0	20	100.0	
Total	16	40.0	24	60.0	40	100.0	
Mother's education level							
Up to completion of elementary school	5	45.5	6	54.5	11	100.0	0.465
High school	9	45.0	11	55.0	20	100.0	
Higher education	2	22.2	7	77.8	9	100.0	
Total	16	40.0	24	60.0	40	100.0	
Monthly family income (in Brazilian reais)							
788,00-1.576,00	10	79.15	5	41.7	15	100.0	0.030
1,577.00 or more	6	24.35	19	75.6	25	100.0	
Total	16	40.0	24	60.0	40	100.0	
Mother's assessment of dental experience							
Good	15	46.9	17	53.1	32	100.0	0.082
Bad	1	12.5	7	87.5	8	100.0	
Total	16	40.0	24	60.0	40	100.0	
Whether the child had already presented major medical problems							
No	14	45.2	17	54.8	31	100.0	0.200
Yes	2	22.2	7	77.8	9	100.0	
Total	16	40.0	24	60.0	40	100.0	
Whether the child had already had caries							
No	3	25.0	9	75.0	12	100.0	0.181
Yes	13	46.4	15	53.6	28	100.0	
Total	16	40.0	24	60.0	40	100.0	
Whether the child had already had teeth extracted							
No	9	39.1	14	60.9	23	100.0	0.576
Yes	7	41.2	10	58.8	17	100.0	
Total	16	40.0	24	60.0	40	100.0	
Child's anxiety							
Little or none	13	54.2	11	45.8	24	100.0	0.026
Some anxiety	3	18.8	13	81.3	16	100.0	
Total	16	40.0	24	60.0	40	100.0	

for children undergoing dental care tends to cause failures of treatment efficiency and makes success impossible.[18]

The present study had some limitations. Firstly, we only investigated children aged 5 to 12 years. Younger children might have other feelings about pictures that are presented to them. In addition, a larger sample of both mothers and children would have allowed inferential statistical analysis with greater extrapolations of the information and conclusions. Future studies should take these limitations into consideration in reaching new conclusions.

CONCLUSION

Based on the results from this study and taking into account that the conclusions from this study are limited by the small number of mother-child pairs, it is possible to conclude that:

1. Most of the children showed some anxiety, ranging from feelings of fear to feelings of panic regarding dental care;
2. Most of the mothers were slightly anxious and the dental procedure that caused most anxiety was the expectation of local anesthesia;
3. Mothers with higher family income were more anxious;
4. Maternal anxiety had an influence on child anxiety.

This information is important in relation to adequate training for dental care professionals, especially for those involved in dental care for children. The psychological aspects and subjective issues of such situations need to be thought of as being as essential as the technical focus.

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Is reiki or prayer effective in relieving pain during hospitalization for cesarean? A systematic review and meta-analysis of randomized controlled trials

O *reiki* ou a oração são efetivos no alívio da dor durante a internação da cesariana? Uma revisão sistemática e metanálise de ensaios clínicos randomizados

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KEY WORDS:
Spirituality.
Delivery, obstetric.
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Review.

PALAVRAS-CHAVE:
Espiritualidade.
Parto obstétrico.
Terapias complementares.
Fatores de risco.
Revisão.

ABSTRACT

CONTEXT AND OBJECTIVE: This systematic review compared reiki and prayer with drug use for relieving pain during hospitalization for cesarean, given that the popularity of integrative medicine and spiritual healing has been increasing. It had the aim of evaluating whether reiki or prayer is effective in relieving pain during cesarean section.

DESIGN AND SETTING: Systematic review with meta-analysis conducted at Botucatu Medical School, UNESP, São Paulo, Brazil.

METHODS: The following databases were searched up to March 2016: MEDLINE, Embase, LILACS and CENTRAL. Randomized controlled trials published in English or Portuguese were included in the review. Two reviewers independently screened eligible articles, extracted data and assessed the risk of bias. A GRADE table was produced to evaluate the risk of bias.

RESULTS: There was evidence with a high risk of bias showing a statistically significant decrease in pain score through use of reiki and prayer, in relation to the protocol group: mean difference = -1.68; 95% confidence interval: -1.92 to -1.43; $P < 0.00001$; $I^2 = 92\%$. Furthermore, there was no statistically significant difference in heart rate or systolic or diastolic blood pressure.

CONCLUSION: Evidence with a high risk of bias suggested that reiki and prayer meditation might be associated with pain reduction.

RESUMO

CONTEXTO E OBJETIVO: Esta revisão sistemática comparou o *reiki* e a oração ao uso de medicamentos, a fim de aliviar a dor durante a internação para cesariana, visto que há um aumento na popularidade da medicina integrativa e cura espiritual. Esta revisão teve como objetivo avaliar se o *reiki* ou oração são eficazes no alívio da dor durante a cesariana.

TIPO DE ESTUDO E LOCAL: Revisão sistemática com metanálise realizada na Faculdade de Medicina de Botucatu UNESP, São Paulo, Brasil.

MÉTODOS: As seguintes bases de dados foram pesquisadas até março de 2016: MEDLINE, Embase, LILACS e CENTRAL. Nesse sentido, foram incluídos ensaios clínicos randomizados publicados em inglês e português. Dois revisores rastrearam independentemente artigos elegíveis, extraíram dados e avaliaram o risco de viés. A tabela GRADE foi realizada para avaliar o risco de viés.

RESULTADOS: Evidências com alto índice de viés encontraram uma diminuição estatisticamente significativa na redução da dor (diferença média = -1,68; intervalo de confiança de 95%: -1,92 a -1,43; $P < 0,00001$; $I^2 = 92\%$), com o uso de *reiki* e oração comparado com o grupo protocolar. Além disso, não houve diferença estatisticamente significativa na frequência cardíaca, pressão arterial sistólica e diastólica.

CONCLUSÃO: Evidência com alto risco de viés sugeriu que *reiki* e meditação oração podem ser associadas com a redução da dor.

INTRODUCTION

Complementary therapies have been practiced since ancient times, but there is still little scientific evidence on their real efficiency. Most of these therapies originated from oriental cultures, such as in India with Ayurveda treatments; China with acupuncture and moxibustion therapies; and Japan with reiki therapy. Moreover, complementary therapies are implemented both alone and alongside conventional medicine. Thus, complementary therapies tend to take a holistic approach in order to treat the entire person, i.e. body, mind and soul. In other words, they use a comprehensive set of techniques, such as meditation, body therapies, energy manipulation, art and music therapy, dietary therapy and other procedures that involve healthcare, according to the National Center for Complementary and Alternative Medicine.[1-5]

Reiki is an ancient Japanese form of hands-on healing. The term comes from combining two Japanese words: rei, a universal spirit; and ki, meaning universal life energy.[1] Despite being a Japanese form of healing, use of reiki has already spread worldwide. It is mainly used for pain relief.[2] Additionally, prayer meditation is also considered to be an adjunctive therapy involving a non-invasive method with a low-cost procedure.[5] Thus, it improves psychological, social, spiritual and physical health by means of nourishing the environment through peacefulness and mindfulness.[6,7]

A previous systematic review of clinical trials[2] compared reiki therapy with the usual care or with placebo among women undergoing breast biopsy, women with abdominal hysterectomies, cancer patients, individuals with depression, and chronically ill patients. However, that review seemed to have serious limitations with regard to its methodological aspects. For example, it presented a variety of conditions, i.e. 12 articles were included and therefore 12 different types of conditions, but there were no data on pregnant women. In other words, the review was quite generalist. Moreover, it did not use the GRADE approach to rate the quality of scientific evidence. Consequently, the review was unable to provide any conclusion about the effectiveness of reiki and the suggestion made was that new studies on this topic would be necessary.

In the literature, a few studies[1-11] have reported that spirituality and complementary therapies have provided improvements regarding quality of life and benefits in relation to several health conditions.[6-8] Moreover, it has been suggested that non-pharmacological practices could be considered in order to reduce excessive use of allopathic medication in obstetrics and consequently to reduce the costs of care.

OBJECTIVE

The aim of this systematic review of randomized controlled trials (RCTs) was to evaluate whether reiki or prayer meditation is effective for controlling pain among women undergoing cesarean section.

METHODS

The Cochrane Handbook for Intervention Reviews[12] guided our choice of methods. Our reporting adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement.[13]

Eligibility criteria

We included RCTs or quasi-RCTs that compared reiki therapy and prayer meditation with the usual care among pregnant women undergoing cesarean section, including any of the following maternal outcomes before and after receiving the intervention or usual care: pain control; heart rate; diastolic and systolic blood pressure; or medication intake. Furthermore, a single study[9] recorded postpartum physical activities through the Milestone questionnaire.

Data source and searches

Pertinent literature was identified through MEDLINE (from 1966 to March 2016); Embase (from 1980 to March 2016); LILACS (from 1982 to March 2016); and Cochrane controlled trials (CENTRAL) (up to March 2016), using the terms spirituality, reiki, prayer, cesarean and labor pain (**Table 1**). The data-gathering was restricted to Portuguese and English-language studies. There were no publication status restrictions. A review of relevant references in previous systematic review articles[1,2] and primary studies[3-11] was conducted.

Selection of studies

Randomized controlled trials or quasi-RCTs published in English or Portuguese were included. Two reviewers, working independently, screened all titles and abstracts that were identified through the literature search. Furthermore, they selected potential studies by obtaining the full-text articles, and then evaluated them, in accordance with the eligibility criteria.

Data extraction and risk-of-bias assessment

Two reviewers independently screened all the potential quantitative results or critical data from some preselected studies, with regard to the participants, interventions, control conditions, outcome measurements and results. Subsequently, disagreements between the reviewers were discussed with the ninth author, in order to reach a consensus.

The reviewers independently assessed risk of bias by using a version of the Cochrane Collaboration's tool for assessing risk of bias.[14] This includes nine domains: adequacy of sequence generation; allocation sequence concealment; blinding of participants and caregivers; blinding of data collectors; blinding for outcome assessment; blinding of data analysts; incomplete outcome data; selective outcome reporting; and presence of other potential sources of bias not accounted for in the previously cited domains. For incomplete outcome data, we stipulated that low risk of bias consisted of loss to follow-up of less than 10% and a difference in missing data between the intervention and control groups of less than 5%.

Table 1. Search strategies used in MEDLINE (via PubMed), CENTRAL, LILACS and Embase

MEDLINE via PubMed	#1"Spirituality"[Mesh] OR Spiritualities OR "Reiki"[Mesh] OR "`Touch, Therapeutic" OR "Laying on of Hands" OR "Prayer Healing"[Mesh] OR "Faith Healing" OR "Prayer"[Mesh] OR Beliefs OR "Spiritual Healing"[Mesh] OR "Spiritual Therapies" #2"Cesarean"[Mesh] OR "Cesarean Sections" OR "Cesarean Section" OR "Abdominal Deliveries" OR "Abdominal Delivery" OR "Caesarean Section" OR "Caesarean Sections" OR C-Section OR "C Section" OR C-Sections OR "Post caesarean Section". #3"Pain, Labor"[Mesh] OR "Obstetric Pain" OR "Pain, Obstetric" STRATEGY: #1 AND #2 AND #3
CENTRAL	"Spirituality" OR "Spiritualities" OR "Reiki" OR ('Touch, Therapeutic" OR "Laying on of Hands" OR "Prayer Healing" OR "Faith Healing") OR "Prayer" OR "Beliefs" OR "(Spiritual Healing" OR "Spiritual Therapies") AND "Cesarean" OR ("Cesarean Sections" OR "Cesarean Section" OR "Abdominal Deliveries" OR "Abdominal Delivery" OR "Caesarean Section" OR "Caesarean Sections" OR C-Section OR "C Section" OR C-Sections OR "Post caesarean Section") AND "Pain, Labor" OR ("Obstetric Pain" OR "Pain, Obstetric")
LILACS	Espiritualidade OR Reiki OR (Toque terapêutico) OR (Superposição de mãos) OR (Cura Espiritual) OR (Terapias espirituais) OR (Cura pela Fé) AND Cesárea OR (Parto Abdominal) OR (Parto Obstétrico) AND (Dor do Trabalho de Parto) OR (Dores do Trabalho de Parto) OR (Dor de Parto) OR (Dores de Parto) OR (Dores do Parto)
Embase	#1 'reiki'/exp OR #2'religion/exp OR 'prayer' #3 'spirituality'/exp #4 'cesarean section'/exp OR 'birth' OR 'abdominal operation' OR 'birth, caesarean' OR 'caesarean birth' OR 'caesarean section' OR 'caesarian birth' OR 'caesarian section' OR 'cesarean delivery' OR 'cesarean section' OR 'repeat; cesarian section' OR 'elective repeat cesarean section' OR 'sectio caesarea' #5 'labor pain'/exp OR 'delivery pain' OR 'labour pain' OR 'pain, delivery' OR 'pain, labor' OR 'pain, labour' STRATEGY: #1 OR #2 OR #3 AND #4 AND #5

Certainty of evidence

The reviewers used the Grading of Recommendations for Assessment, Development and Evaluation (GRADE) methodology to rate the certainty of scientific evidence for each outcome, which was categorized as high, moderate, low or very low.[15] The GRADE approach assessed the following: overall risk of bias,[16] imprecision,[17] inconsistency,[18] indirectness[19] and publication bias.[20] Thus, the results were summarized in an evidence table, i.e. as a GRADE evidence profile.

The reviewers independently assessed eligibility, risk of bias and data abstraction. Disagreements were resolved by reaching a consensus or by obtaining a third reviewer's opinion if needed.

Data synthesis and statistical analysis

We pooled the data to calculate pooled risk ratios (RRs) or mean differences, with 95% confidence intervals (CIs), using a fixed-effect model by considering the last follow-up outcome that had been measured in each study included. We assessed heterogeneity by means of the I^2 statistic and evaluated the quality of the evidence by using the GRADE method. All of the analyses were conducted using the Review Manager (RevMan) software.[21]

RESULTS

Selection of titles

A total of 1,866 titles were identified in the databases cited above, but only 34 studies were selected for detailed evaluation.[12] Ultimately, it was found that only three studies that included 343 patients were eligible for the current review (**Figure 1**).

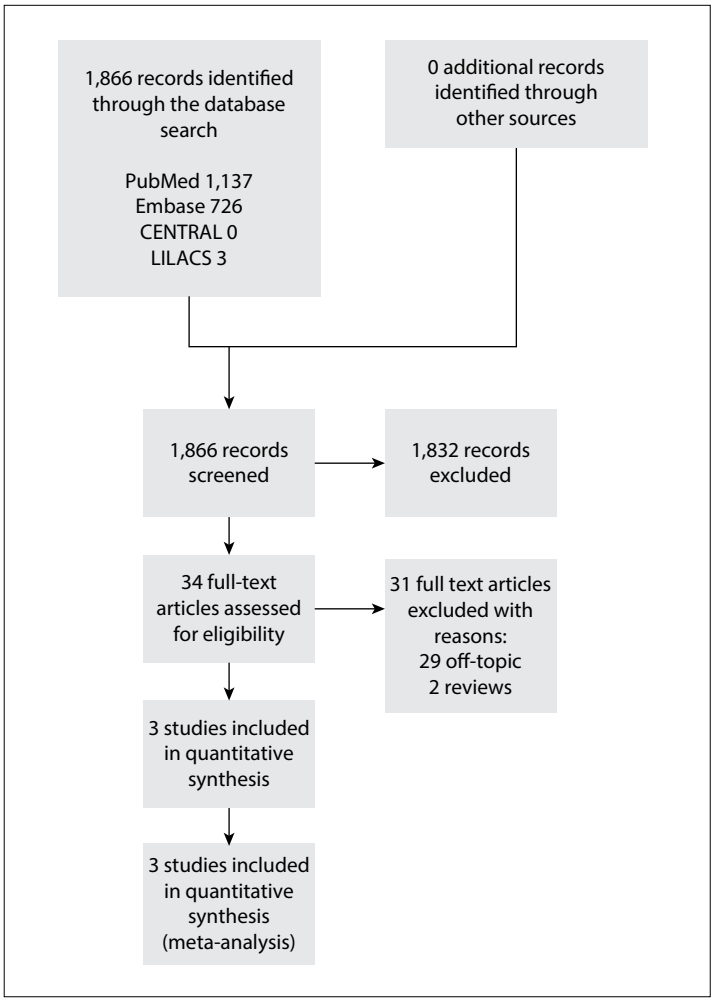

Figure 1. Flowchart for inclusion and exclusion of studies.

These studies presented different interventions, i.e. distant reiki,[9] regular reiki[10] and prayer meditation,[11] but they all presented a similar outcome, i.e. they measured pain through a visual analogue scale (VAS) and also measured heart rate and diastolic and systolic blood pressure. In the literature, all reiki healers consider that distant reiki[9] and regular reiki[10] are the same, with the only difference that in one, the patient`s physical body is absent, while it is present in the other.[1,2,9]

Although prayer meditation[11] may seem to have been the odd one out, we analyzed this study in depth and decided to plot it together with the two reiki studies,[9,10] because all the information from this study with regard to the prayer meditation background, the objectives of the study and the methods used to evaluate the intervention were in line with these other studies.[9,10] Moreover, these factors were in line with our aim in this systematic review, which was to evaluate perceived pain among women undergoing cesarean section. Moreover, both reiki and prayer meditation are non-invasive and non-pharmacological practices, and both of them can be considered to be spiritual interventions.[1,2,9-11]

Study characteristics

Table 2 describes the characteristics of the studies relating to their designs, settings, numbers of participants, interventions and usual care treatments received by the patients; and according to the hospital protocol, mean age, inclusion and exclusion criteria and follow-up after caesarean section. One study was conducted in Canada,[9] and the other two were conducted in the Middle East, in Turkey[10] and Iran.[11] The sample sizes ranged from 40[9] to 80[11] pregnant women aged in their twenties or thirties. All the studies included pregnant women undergoing cesarean section.

The following exclusions of patients were made in one or more of these studies: previous experience with reiki;[9,10] perception of pain on a visual analogue scale (VAS) > 3;[11] not planning to use standard postoperative pain medication;[10,11] not being able to answer the questions;[9-11] visual and hearing impairment;[10,11] any complications during anesthesia or surgery;[10,11] history of drug abuse;[10,11] operation performed under spinal and epidural anesthesia;[10] use of patient-controlled analgesia in the treatment;[10] and presence of any psychiatric disease or allergy to analgesic drugs.[10] The length of the follow-up ranged from 6 hours to 3 days.

Risk-of-bias assessment

Figure 2 describes the risk-of-bias assessment for RCTs. The overall methodological quality of the studies examined was evenly separated into unclear and low risk-of-bias categories. However, the main concern was the risk of bias relating to random sequence generation in the study by Vandervaart et al.[9] Additionally, the allocation concealment and blinding of participants/personnel were uncertain in the studies by Midilli and Eser[10] and Beiranvand et al.[11] Finally, none of the three studies[9-11] showed any certainty with regard to blinding of the outcome assessment.

Effects of reiki and prayer meditation: meta-analysis

Pain score

Regarding the pain scores measured by means of a VAS in the overall analysis, the results from three RCTs[9-11] found a statistically significant difference favoring reiki and prayer meditation over the usual care: mean difference (MD) = -1.68; 95% confidence interval (CI): -1.92 to -1.43; $P < 0.00001$; $I^2 = 92\%$. In relation to the following subcategories, we also found statistically significant differences favoring the integrative practices over the usual care: prayer meditation (MD = -1.70; 95% CI: -2.00 to -1.40; $P < 0.00001$; I^2 = not applicable); and reiki (MD = -2.52; 95% CI: -3.07 to -1.97; $P < 0.00001$; I^2 = not applicable). However, there was no statistically significant difference between the distant and regular reiki groups: MD = -0.20; 95% CI: -0.90 to 0.50; $P = 0.58$; I^2 = not applicable. The certainty of the evidence was downrated to low because of inconsistency and publication bias (**Figure 3**, **Table 3**).

Heart rate

With regard to heart rate in the overall analysis, the results from two RCTs[9,10] did not show any statistically significant difference that favored regular reiki and distant reiki over the usual care: MD = -2.04; 95% CI: -4.93 to 0.84; $P = 0.41$; $I^2 = 0\%$. Therefore, we found no statistically significant difference favoring reiki over the usual care: MD = -3.58; 95% CI: -8.26 to 1.10; $P = 0.17$; I^2 = not applicable. In addition, there was no statistically significant difference between the distant and regular reiki groups: MD = -1.10; 95% CI: -4.76 to 2.56; $P = 0.17$; I^2 = not applicable. The certainty of the evidence was downrated to low because of imprecision and publication bias (**Figure 4**, **Table 3**).

Diastolic blood pressure

For diastolic blood pressure, the results from two RCTs[9,10] did not show any statistically significant difference favoring regular reiki and distant reiki over the usual care: MD = -1.74; 95% CI: -4.18 to 0.70; $P = 0.16$; $I^2 = 0\%$. Therefore, we also found no statistically significant difference favoring reiki over the usual care: MD = -0.58; 95% CI: -4.10 to 2.94; $P = 0.37$; I^2 = not applicable. In addition, there was no statistically significant difference between the distant and regular reiki groups: MD = -2.80; 95% CI: -6.17 to 0.57; $P = 0.37$; I^2 = not applicable. The certainty of the evidence was downrated to low because of imprecision and publication bias (**Figure 5**, **Table 3**).

Table 2. Study characteristics relating to setting, number of participants, mean age, intervention and control groups, inclusion and exclusion criteria, assessed outcomes and follow-up after caesarean section

Author	Location	Participants (n)*	Mean age	Intervention in study group (n)	Intervention in control group (n)	Inclusion criteria	Exclusion criteria	Measured outcomes	Follow-up (hours)
Midilli and Eser[10]	Turkey	100	Reiki: 27.61† Usual care: 27.61†	Usual care (specified)‡ and reiki therapy for 30 minutes for two days (n = 50)	Usual care (specified)‡ and 30 minutes of rest for 2 days (n = 50)	Planned or unplanned cesarean delivery; age between 18-45 years; length of stay of at least two days at the hospital; orientation to place and time; operation performed under general anesthesia; and only using a nonopioid analgesic drug prescribed by a doctor (diclofenac 75 mg/3 ml, intramuscularly).	Operation performed under spinal and epidural anesthesia; any psychiatric disease or allergy to analgesic drugs; hearing and visual impairment; previous experience with reiki; serious complication with the patient or infant(s) during or after cesarean delivery; or use of a patient controlled analgesia in treatment.	Pain intensity from horizontal VAS (0-10); anxiety from STAI; hemodynamic parameter score.	48
Beiranvand et al.[11]	Iran	160	Prayer: 27.4 Usual care: 29.4	Usual care (not specified) and pray meditation therapy for 20 minutes after cesarean section (n = 80)	Usual care (not specified) (n = 80)	Muslim women candidates for cesarean surgery under spinal anesthesia, with mild pain (VAS < 1-3).	Muslim women with VAS > 3; hearing disorder; history of drug abuse; administration of analgesics; any complications during anesthesia or surgery; or being unable to answer the questions.	Pain score from VAS < 3; blood pressure (diastolic and systolic); heart rate; mother´s respiratory rate; PONV; and relaxation.	6
Vandervaart et al.[9]	Canada	83	Reiki: 35.1 Usual care: 32.9	Usual medical and nursing care according to Pfannenstiel protocol in association with distant reiki sessions, one each morning (30 minutes before the cesarean section morning; second and third sessions were administered on the following mornings at about 8 am) (n = 42)	Usual medical and nursing care according to Pfannenstiel protocol (n = 41)	Pregnant women	Patients with previous experience of reiki; or not planning to use postoperative pain medication; or being unable to answer the questions.	AUC for pain (in motion) for days 1-3 from VAS; AUC for pain (in motion) for days 1, 2 and 3, separately; mean VAS (in motion) on days 1-3; mean VAS (at rest) on days 1-3; number of patients in need of opioid pain medication; adverse events to opioids, such as constipation, itchiness; mother´s respiratory rate; heart rate; blood pressure; and time of first activity (e.g. first hunger, first walk) from Milestone questionnaire.	72

n: number; RCT: randomized controlled trial; ml: milliliter, mg: milligram; VAS: visual analogue scale; STAI: State-Trait Anxiety Inventory; cm: centimeters; PONV: incidence of postoperative nausea and vomiting. *Randomized participants; †Patients were equalized according to age into two groups (18-31 and 32-45 years old); ‡Day 1 (24 hours after operation): Patients were given the first dose of the standard analgesic (intramuscularly, 75 mg/3 ml diclofenac at 9.00 a.m. and 9.00 p.m.); Day 2 (48 hours after operation): Patients were given the third dose of the standard analgesic (intramuscularly, 75 mg/3 ml diclofenac at 9.00 a.m. and 9.00 p.m.); Day 3 (72 hours after operation): Minoset® 500 mg, tablet (every 4-6 hours, as needed) was administered in accordance with the analgesic protocol. At 72 hours only, the number of analgesics required by the patient was determined by means of face-to-face interview or telephone call.

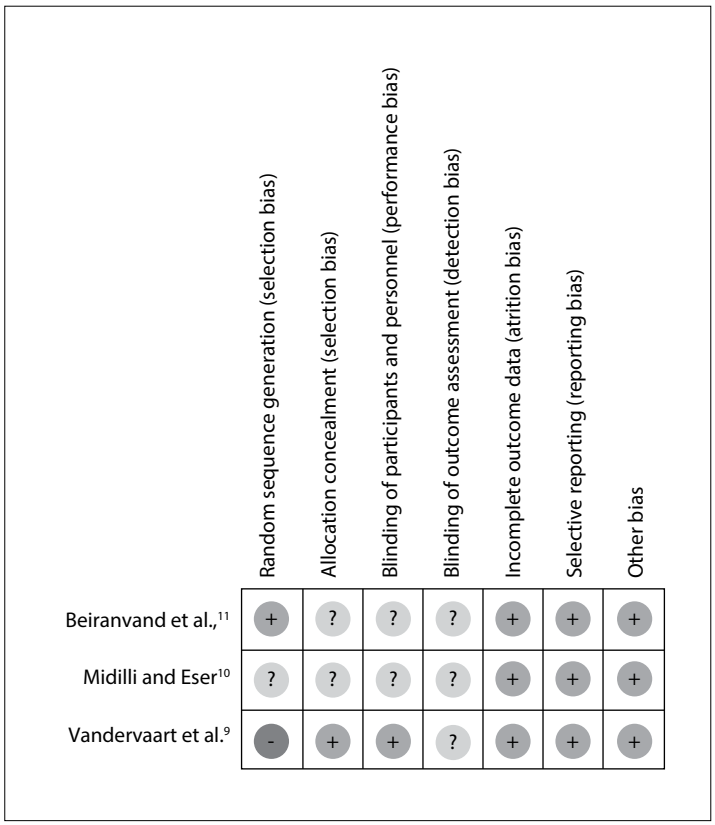

Figure 2. Risk-of-bias assessment.

Systolic blood pressure

In the overall analysis with regard to systolic blood pressure, the results from two RCTs[9,10] showed that there was no statistically significant difference favoring reiki over the usual care: MD = -3.59; 95% CI: -6.79 to 0.39; P = 0.03; I^2 = 26%. Therefore, we also found no statistically significant difference favoring reiki over the usual care: MD = -1.71; 95% CI: -6.21 to 2.79; P = 0.25; I^2 = not applicable. In addition, there was no statistically significant difference between the distant reiki and regular reiki groups: MD = -5.50; 95% CI: -10.04 to -0.96 P = 0.25; I^2 = not applicable. The certainty of the evidence was downrated to moderate because of inconsistency and publication bias (**Figure 6**, **Table 3**).

Effect of first-time activity through the Milestone questionnaire and patients' need for opioids in the Vandervaart study[9]

Regarding the activity milestone questionnaire, which is used among women after elective caesarean to evaluate the rate of healing, a single RCT[9] showed that there was no statistically significant difference between distant reiki and the usual care in any of the following categories: time to first hunger; time to first eating of solid food; time to first flatus; time to first bowel movement; time to first spontaneous voiding; and time to first ambulation (**Figure 1**). Moreover, the same study[9] described the patients' need for opioids, but showed that there was no statistically significant

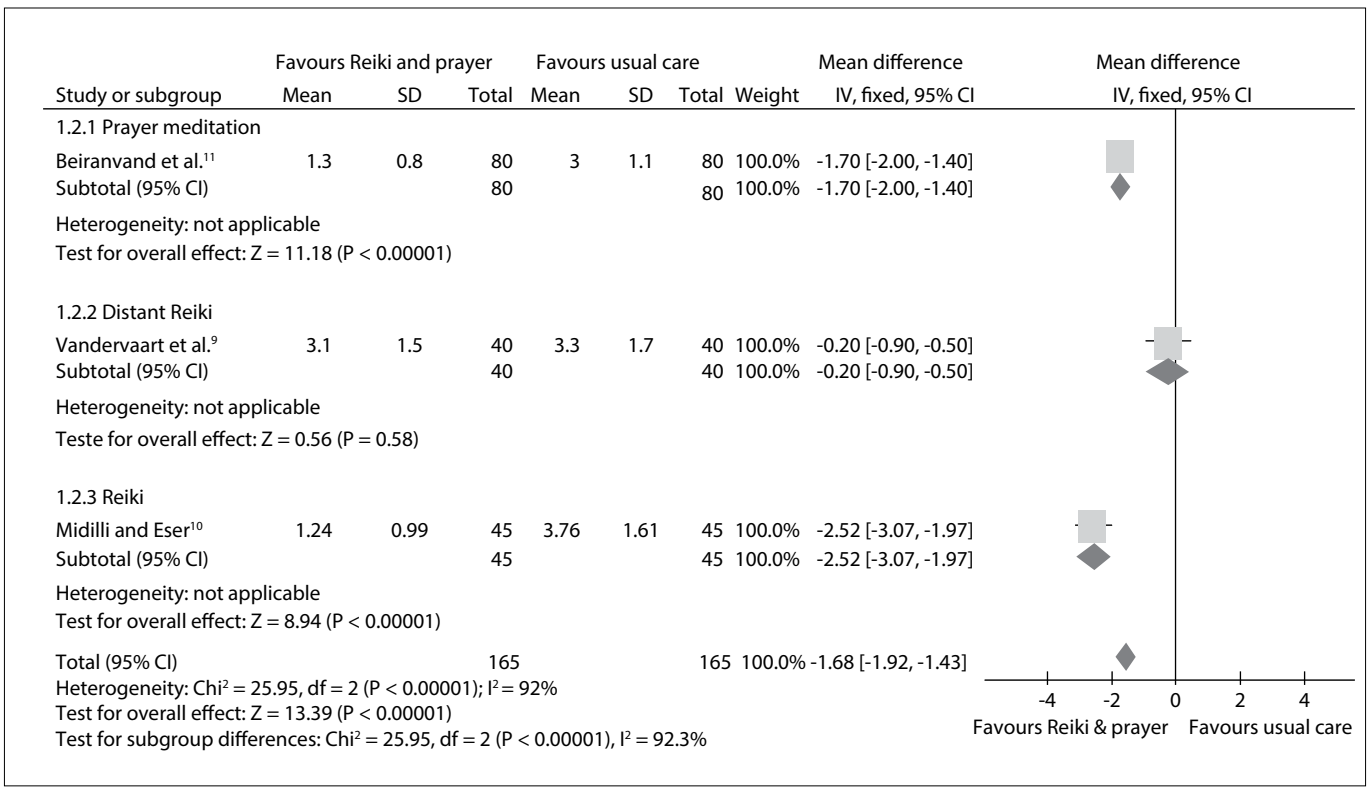

Figure 3. Meta-analysis on mean pain score measured using visual analogue scale (VAS).

Table 3. GRADE evidence profile for continuous outcomes: complementary alternative medicine for cesarean section

Quality assessment						Illustrative comparative risks (95% CI)		
						Assumed risk	Corresponding risk	
Number of participants (number of studies) Range of follow-up time in weeks	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Usual care	Reiki and prayer meditation	Certainty in estimates OR Quality of evidence
Pain score measured using VAS								
343 (3) 6-72	No serious limitation	Very serious limitation*	No serious limitation	No serious limitation	Serious limitation†	The mean change in pain score was 3.0 (SD 1.1)‡	The mean change in pain score in the intervention group was 1.68 lower (1.92 lower to 1.43 lower)	Low
Heart rate								
183 (2) 48-72	No serious limitation	No serious limitation	No serious limitation	Very serious limitation§	Serious limitation†	The mean change in heart rate was 77.6 (SD 8)‖	The mean change in heart rate in the intervention group was 2.04 lower (4.93 lower to 0.84 higher)	Low
Systolic blood pressure								
183 (2) 48-72	No serious limitation	Serious limitation†	No serious limitation	No serious limitation	Serious limitation†	The mean change in systolic blood pressure was 118.31 (SD 9.78)‖	The mean change in systolic blood pressure in the intervention group was 3.59 lower (6.79 lower to 0.39 lower)	Moderate
Diastolic blood pressure								
183 (2) 48-72	No serious limitation	No serious limitation	No serious limitation	Very serious limitation§	Serious limitation†	The mean change in diastolic blood pressure was 67.7 (SD 7.8)‖	The mean changes in diastolic blood pressure in the intervention group was 1.74 lower (4.18 lower to 0.70 higher)	Low

CAM = complementary alternative medicine; SD = standard deviation; std. = standardized; VAS = visual analogue scale.
*There was substantial heterogeneity (I^2 = 92.3%) among the different interventions used in the studies included (i.e. distant reiki; reiki; and prayer meditation); †There was heterogeneity (I^2 = 26%) among the different interventions used in the studies included (i.e. distant reiki; reiki; and prayer meditation). ‡Baseline risk estimates for pain come from control arm of study by Beiranvand et al.[11] (largest randomized trial in the meta-analysis); §95% CI for absolute effects include benefit and harm. ‖Baseline risk estimates for heart rate, systolic and diastolic blood pressure come from control arm of study by Midilli et al.[10] (largest randomized trial in the meta analysis).

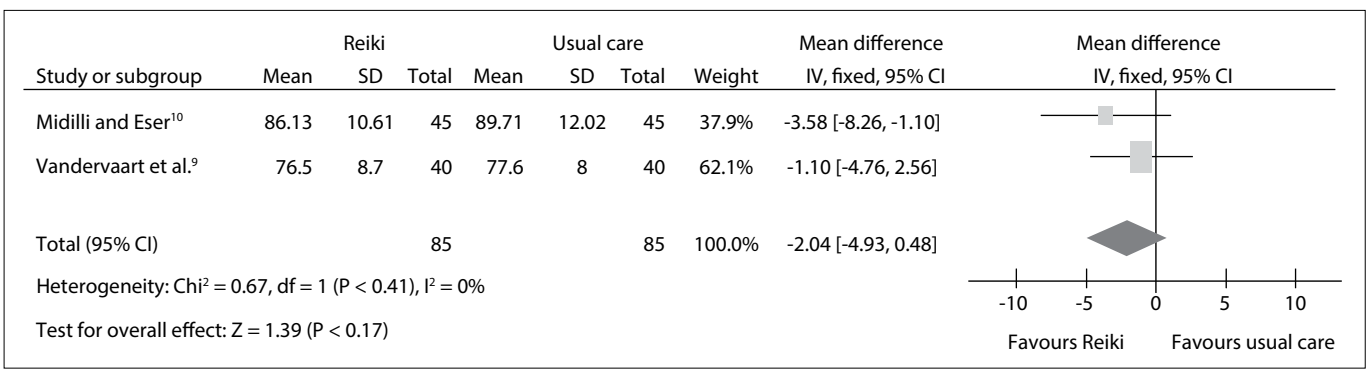

Figure 4. Meta-analysis on heart rate.

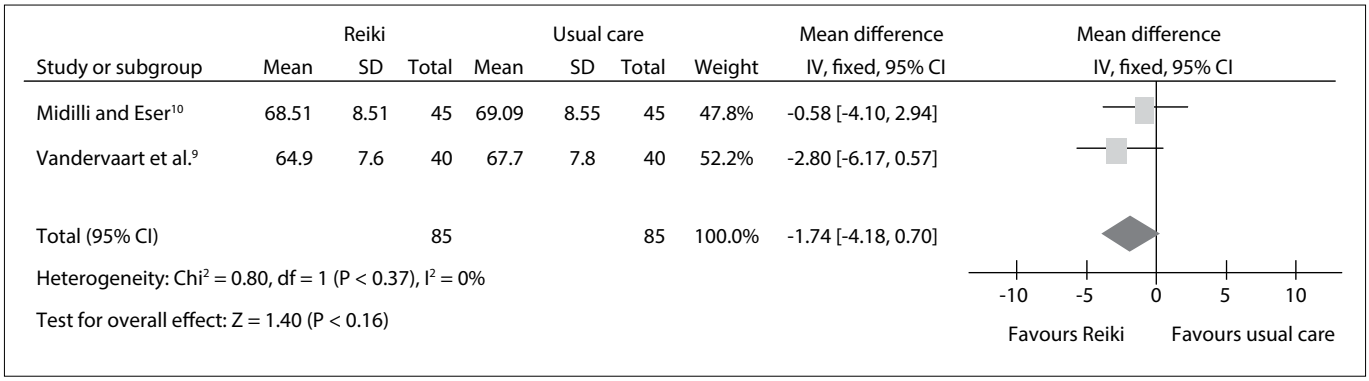

Figure 5. Meta-analysis on diastolic blood pressure.

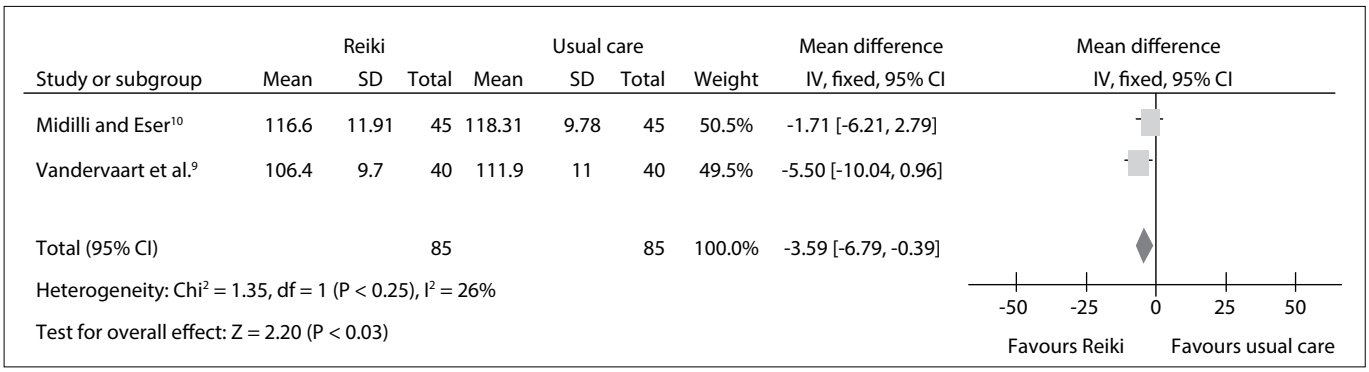

Figure 6. Meta-analysis on systolic blood pressure.

difference between distant reiki and the usual care on the day of admission to hospital (relative risk, RR = 0.81; 95% CI: 0.66 to 1.30; P = 0.64; I^2 = not applicable); or on the next day (RR = 1.22; 95% CI: 0.74 to 1.63; P = 0.65; I^2 = not applicable) (**Figure 2**).

DISCUSSION

This study evaluated the use of reiki and prayer meditation for pain control among women undergoing caesarean section. It was the first-ever study to evaluate spiritual healing in relation to this issue, given that most previous studies and reviews focused on severe chronically ill patients and their quality of life.[1-7]

It should be noted that a language restriction had to be imposed during the selection process due to lack of funds to pay for translation services prior to the search. Thus, it was necessary to restrict the current systematic review to English and Portuguese-language studies were assessed from the outset. However, no study was excluded because it was written in another language, because no such studies were found through the search methods.

A recent study[22] showed that around 26% of women in the United States received spiritual healing treatment if they were non-smokers, non-drinkers or low-risk drinkers, had symptoms of severe tiredness, depression, anxiety, diagnosed cancer or major illnesses. In another study on women in the southern and midwestern United States regions (i.e. extremely religious areas) the proportion that received prayers for health was estimated to be 53%.[23] The fact that the study by Beiranvand et al.[11] presented a significant outcome, i.e. pain control among women undergoing caesarean sections, with high follow-up rates, may have been due the presence of highly religious women in their sample. According to Bell et al.,[23] people who usually use prayer meditation are also likely to use some other form of complementary or alternative medicine (e.g. reiki or acupuncture). Additionally, the more religious the people are, the more likely it is that they will use preventive healthcare.[20]

Among the studies included, some limitations were reported, as follows: the sample size;[9-11] presence of only one reiki therapist;[9] absence of information about the mechanism of action of distant reiki;[9] use of shared rooms;[10] and a noisy environment.[10] In addition to the methodological limitations, this current review also presented a limitation relating to the results obtained through meta-analysis: although the perceived pain seemed to have decreased significantly, the heterogeneity of results was extremely high, i.e. I^2 = 92% (**Figure 3**). This can be explained by the fact that there were three different types of intervention.

On the other hand, regular and distant reiki work in the same way as foundations for this type of therapy[9] and, therefore, they were not different at all. Moreover, prayer meditation is a form of

adjunctive therapy within many cultures.[11,20,23,24] Thus, both prayer meditation and reiki are forms of spiritual healing. According to Benor,[24] spiritual healing is defined as a systematic and purposeful intervention by practitioners that has the aim of helping other people to improve their health condition through focused intention, which can include hand contact or hand movement. Thus, these three studies[9-11] presenting similar methodological aspects and outcomes were plotted together. Within systematic reviews, it is known that meta-analyses that included less than 10 studies cannot to estimate heterogeneity.

The meta-analysis did not show any statistical significant differences from before to after the treatments, either in the intervention or in the usual care group, regarding heart rate (**Figure 4**), diastolic and systolic blood pressure (**Figures 5** and **6**, respectively). In other words, these results were concordant with those of the previous review.[2] However, to reach definitive conclusions regarding the effectiveness of such therapies, larger populations in good RCTs are needed.

With regard to the methodological aspects, the present review noted that there were risks of bias relating to random sequence generation;[9] allocation concealment;[10,11] blinding of participants/personnel;[10,11] and blinding of outcome assessment. This concern corroborates what was reported in the systematic review on reiki conducted by Vandervaart:[2] all of the 12 studies included had failings in at least in one of the following areas: randomization, blinding and accountability of all patients. Therefore, both reviews can be classified as presenting low-quality evidence, and the main issue in this regard is the poor evidence from the RCTs. We sent emails to the respective corresponding authors of the studies selected for this review,[9-11] regarding points in these studies that were unclear to us or not reported, but no replies had been received by the time of submitting this review.

The previous review[2] attempted to evaluate the effectiveness of reiki therapy under several conditions and presented 31 different outcomes within the 12 studies included. Not all of these studies were RCTs; no meta-analysis was performed, and the findings were based on Jadad scores. The previous review also did not include any study on pregnant women undergoing a cesarean section. On the other hand, the present systematic review included three studies[9-11] in which there were similarities regarding methods, outcomes and populations, based our evidence from the GRADE profile for continuous outcomes (**Table 3**), and this review also included a meta-analysis.

Additionally, the major limitation of the current study was that only a very small number of studies considering spiritual healing approaches to pain management after cesarean section have been published. Therefore, there is still a need for high-quality RCTs on this issue, with the aim of assessing the real effectiveness of reiki and prayer meditation in relation to pain control among women undergoing cesarean section.

CONCLUSION

Low-certainty evidence suggested that use of reiki and prayer meditation might be associated with pain reduction.

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Chronic kidney disease — determinants of progression and cardiovascular risk. PROGREDIR cohort study: design and methods

Doença renal crônica — determinantes de progressão e risco cardiovascular. Coorte PROGREDIR: desenho de estudo e métodos

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KEY WORDS:
Renal insufficiency, chronic.
Cardiovascular diseases.
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PALAVRAS-CHAVE:
Insuficiência renal crônica.
Doenças cardiovasculares.
Terapia de substituição renal.
Biomarcadores.
Fatores de risco.

ABSTRACT

CONTEXT AND OBJECTIVE: Chronic kidney disease (CKD) has become an important public health issue. The socioeconomic burden of renal replacement therapy (RRT) is very high, as is CKD-related cardiovascular mortality and morbidity. Preventive and therapeutic measures only have modest impact and more research is needed. Few cohort studies have been conducted on populations with CKD. Our aim was to establish a cohort that would include more advanced forms of CKD (stages 3 and 4). Data collection was focused on renal and cardiovascular parameters.

DESIGN AND SETTING: Prospective cohort study; São Paulo, Brazil.

METHODS: Recruitment took place in Hospital das Clínicas, São Paulo, from March 2012 to December 2013. Data relating to medical history, food-frequency questionnaire, anthropometry, laboratory work-up, calcium score, echocardiography, carotid intimal-medial thickness, pulse-wave velocity, retinography and heart rate variability were collected. A biobank including serum, plasma, post-oral glucose tolerance test serum and plasma, urine (morning and 24-hour urine) and DNA was established.

RESULTS: 454 participants (60% men and 50% diabetics) of mean age 68 years were enrolled. Their mean estimated glomerular filtration rate-CKD Epidemiology Collaboration was 38 ml/min/1.73 m^2. Follow-up is ongoing and the main outcomes are the start of RRT, cardiovascular events and death.

CONCLUSIONS: The PROGREDIR cohort is a promising prospective study that will allow better understanding of CKD determinants and validation of candidate biomarkers for the risks of CKD progression and mortality.

RESUMO

CONTEXTO E OBJETIVO: A doença renal crônica (DRC) tornou-se um problema de saúde pública. A carga socioeconômica da terapia renal substitutiva é muito elevada, assim como a morbimortalidade cardiovascular associada à DRC. Medidas terapêuticas e preventivas têm impacto parcial e novos estudos são necessários. Há poucos estudos de coorte em populações com DRC. Nosso objetivo foi criar uma coorte que contemplasse formas mais avançadas de DRC (estágios 3 e 4). A coleta de dados foi centrada em parâmetros renais e cardiovasculares.

TIPO DE ESTUDO E LOCAL: Estudo de coorte prospectivo; São Paulo, Brasil.

MÉTODOS: O recrutamento ocorreu entre março de 2012 e dezembro de 2013, no Hospital das Clínicas, em São Paulo. Foram coletados dados de história médica, questionário de frequência alimentar, antropometria, exames laboratoriais, escore de cálcio, ecocardiografia, espessura de camada médio-intimal de carótidas, velocidade de onda de pulso, retinografia e variabilidade de frequência cardíaca. Um biobanco incluindo soro, plasma, soro e plasma pós-teste oral de tolerância à glicose, urina (manhã e 24 horas) e DNA foi estabelecido.

RESULTADOS: 454 participantes (60% homens e 50% diabéticos) com idade média de 68 anos foram recrutados. A taxa média de filtração glomerular estimada-Colaboração da Epidemiologia para DRC foi de 38,4 ml/min/1,73 m^2. O seguimento está em andamento e os desfechos principais são: início de terapia renal substitutiva, eventos cardiovasculares e óbito.

CONCLUSÃO: A coorte PROGREDIR é um estudo prospectivo promissor que permitirá melhor compreensão dos determinantes de DRC e a validação de biomarcadores candidatos para o risco de progressão de DRC e de mortalidade.

INTRODUCTION

Chronic kidney disease (CKD) has become an important public health issue worldwide. Increasing prevalence of obesity and diabetes mellitus and today's high life expectancy, particularly among patients with atherosclerosis, are all contributory factors. In addition, CKD progression is still a major challenge, with few new specific therapeutic measures available. The socioeconomic burden on individuals who need renal replacement therapy (RRT) is very high and comes together with CKD-related high cardiovascular mortality and morbidity, with incidence that may in fact even exceed the figures for RRT.[1-7]

In the United States, according to the Annual Report of the United States Renal Data System (USRDS),[8] the prevalence of CKD stages 1-4 was around 14% in the general population and the incidence of end-stage renal disease (ESRD) was 353 cases per million/year in 2012. The prevalence of cardiovascular disease reached 69.8% among CKD patients versus 34.8% among individuals without CKD and the adjusted mortality rates for CKD patients was 76 deaths per 1000 patients, compared with 52 deaths per 1,000 individuals without CKD in 2012. Medicare expenses relating to CKD reach US$ 1700, 3500 and 12,700 per person-year for CKD patients with stages 2, 3 and 4, respectively.[9] Overall, CKD accounts for 6.7% of total Medicare costs.[9]

In Brazil, there were 100,397 patients on dialysis at the end of 2013, with incidence of 170 cases per million/year and an estimated mortality rate of 17.9% per year.[10] In 2013, 5,433 kidney transplantations were performed in Brazil, mostly using public resources.

Preventive measures are highly necessary, and the search for new biomarkers and new therapeutic strategies is intense. While several studies on general populations and cardiovascular cohorts have yielded important contributions towards CKD knowledge, more specific cohorts focusing on CKD progression instead of CKD incidence are necessary within nephrology. In response to this need, several countries like the United States (CRIC study), Germany (GCKD), Canada (CanPREDDICT), Japan, Australia and Uruguay, among others, have ongoing CKD cohort studies.[11]

Along the same lines, the PROGREDIR cohort study was designed to enable better understanding of the determinants of CKD progression and CKD-related mortality, with particular emphasis on mineral metabolism as a cardiovascular risk factor. The cohort comprises people with CKD stages 3 and 4 in São Paulo, Brazil. The cohort was established and baseline data were collected in 2012-2013. Prospective data on hard outcomes such as the start of renal replacement therapy, cardiovascular events and death are currently being gathered. The PROGREDIR cohort is funded by the Research Support Foundation of the State of São Paulo (Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP; 2011-17341-0), São Paulo, Brazil.

OBJECTIVE

The aim of this study was to establish a CKD cohort that would include participants with more advanced forms of this disease (CKD stages 3 and 4), with data collection focused on renal and cardiovascular parameters.

METHODS

Study population and recruitment

Patients attending the outpatient service of Hospital das Clínicas, São Paulo, a public university facility providing quaternary-level care for patients with chronic diseases, were invited to participate in this study. Initially, from the outpatient records, all patients aged ≥ 30 years and at least two measurements of creatinine (with a minimum interval of 3 months) ≥ 1.6 mg/dl for men and ≥ 1.4 mg/dl for women were considered potential candidates. Patients attending oncology, psychiatry, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), viral hepatitis and glomerulonephritis services were excluded. The remaining candidates were then contacted by phone and were invited to participate if they did not meet any exclusion criteria. The exclusion criteria checked by the interviewer were: hospitalization within the last six months, acute myocardial infarction within the last six months, autoimmune diseases, pregnancy, psychiatric diseases, ongoing chemotherapy or immunosuppressive therapy, ongoing RRT, glomerulonephritis, HIV/AIDS infection, hepatitis B or C and any organ transplantation. Recruitment took place between March 2012 and December 2013, and 454 participants were enrolled. The study was approved by two local ethics committees and written informed consent was obtained from all participants.

Sample size estimation

The sample size was calculated using an estimate of the annual incidence of end-stage renal disease (ESRD) of 2% and an annual rate of cardiovascular events of 2-3.5% among diabetic nephropathy patients.[12] By assuming a difference in event rate incidence of 3% between exposed and non-exposed subjects, a sample size of 500 was estimated, with an alpha error of 0.05 and a power of 80%.

Baseline examination and data collection

The baseline assessment lasted approximately six hours and was performed on a single-day visit to our study center. Data collection included all the variables depicted in **Table 1**. Sex and self-declared race were also registered. Anthropometry was performed first, with the participants wearing light clothes, following standard techniques.[13] Blood pressure (BP) was measured using a validated oscillometric device (Omron HEM 705CPINT). Three measurements were made at one-minute intervals. The mean of the last two BP measurements was used as the definition for high blood

pressure. Overnight fasting blood samples and 24-hour and spot urine samples were collected. A standard 75-g oral glucose tolerance test was administered to all participants without known diabetes. Urine and blood aliquots were prepared and stored at -180 °C in nitrogen. DNA extraction was performed and the material was stored at -80 °C. Baseline laboratory measurements were made using conventional techniques (**Table 2**).

Interviews were conducted by trained personal under strict quality control. Data on medical history, socioeconomic variables, family history, medication use, physical activity, smoking and alcohol consumption were obtained. A food-frequency questionnaire was also administered. The food list was defined on the basis of the dietary intake of the Brazilian population and the reproducibility and validity of the questionnaire has been measured elsewhere.[14]

Conventional 12-lead electrocardiograms (ECG) were performed using a digital device (Atria 6100, Burdick, Cardiac Science Corporation, USA) with automated readings of heart rate; P wave, QRS complex and T wave duration, amplitude and axis; and QT, QTc and QT dispersion. All precordial electrodes were positioned after identifying the location for the V4 electrode with a square. The Electrocardiogram Reading Center (ERC) at the Heart Institute of the University of São Paulo (INCOR) provided all ECG readings.

For heart rate variability determinations, a 10-minute continuous ECG was obtained from a single lead (usually D2) using a digital electrocardiograph (Micromed, Brazil) at a frequency of 250 Hz, with subjects in the supine position. Computer software (WinCardio) was used to generate time series of RR intervals that were sent to the central cardiovascular physiology laboratory

Table 1. Baseline assessments in PROGREDIR cohort study

Anthropometry	Body mass index Waist and hip circumferences
Blood pressure	Mean from three seated measurements
Laboratory data	Serum fasting samples Two-hour glucose-tolerance test (75 g oral glucose) Spot urine 24-hour urine (home collected) Biobank (serum, plasma, post-glucose serum, urine and DNA)
Questionnaires	Medical history Food frequency questionnaire
Non-alcoholic fatty liver disease assessment	Measurement of anterior-posterior diameter of right lobe of the liver
Cardiac evaluation	Electrocardiogram Transthoracic echocardiography Heart rate variability
Vascular evaluation	Carotid-femoral pulse-wave velocity (PWV) Carotid intima-media thickness (IMT) Coronary artery calcium score (CAC) Retinography

Table 2. Baseline laboratory tests

Analyte	Test
Whole blood cell count	Automated method (XT 2000)
Creatinine	Enzymatic colorimetric assay (Jaffé) (ADVIA Chemistry)
Urea	Kinetic assay (urease/glutamate dehydrogenase) (ADVIA Chemistry)
Sodium	Potentiometry (ion-selective electrodes) (ADVIA Chemistry)
Potassium	Potentiometry (ion-selective electrodes) (ADVIA Chemistry)
Calcium	Colorimetric assay (ADVIA Chemistry)
Phosphate	Colorimetric assay (phosphomolybdate) (ADVIA Chemistry)
Intact parathormone	Chemiluminescence immune sandwich assay (CENTAUR, ADVIA Chemistry)
Glucose	Hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA)
Glycated hemoglobin (HbA1c)	High-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, California, USA)
Insulin	Immunoenzymatic assay (ELISA) (Siemens)
Total and high-density lipoprotein cholesterol	Enzymatic colorimetric assay (ADVIA Chemistry)
Low-density lipoprotein cholesterol	Calculated by means of the Friedewald equation If triglyceride levels > 400 mg/dl: enzymatic colorimetric assay (ADVIA Chemistry)
Triglycerides	Enzymatic colorimetric assay (glycerol phosphate peroxidase) (ADVIA Chemistry)
Iron	Colorimetric assay (ferrozine) (ADVIA Chemistry)
Ferritin	Chemiluminescence immunoassay (CENTAUR, ADVIA Chemistry)
pH/bicarbonate	Potentiometry/calculated Cobas B121 (Roche)
Albumin	Colorimetric assay (bromocresol green) (ADVIA Chemistry)
High-sensitivity C-reactive protein	Immunochemistry (nephelometry) (BNII; Siemens)
Alkaline phosphatase	Enzymatic colorimetric assay (AMP) (ADVIA Chemistry)
RBP4 (serum)	Turbidimetry with a polyclonal rabbit anti-human RBP antibody (DAKO/Biogen)
Microalbuminuria (spot urine)	Immunochemical assay (nephelometry) (BNII; Siemens)
Urinary creatinine (spot and 24-hour urine)	Enzymatic colorimetric assay (Jaffé) (ADVIA Chemistry)
Urinary urea (spot urine)	Kinetic assay urease/glutamate dehydrogenase) (ADVIA Chemistry)
Urinary RBP4	Immunoenzymatic assay with monoclonal antibody
Urinary sodium and potassium (24-hour urine)	Potentiometry (ion-selective electrodes) (ADVIA Chemistry)
Urinary proteinuria (24-hour urine)	Colorimetric assay (pyrogallol) (ADVIA Chemistry)
Urinary calcium (24-hour urine)	Colorimetric assay (ADVIA Chemistry)
Urinary phosphate (24-hour urine)	Colorimetric assay (ADVIA Chemistry)

(IC-ES). All readings were made through computer software that eliminated artifacts and selected RR intervals lasting 0.5 to 2.0 seconds. Temporal and spectral analyses of heart rate variability (HRV) were then performed using an autoregressive model to identify very low-frequency (VLF, 0 to 0.04 Hz), low-frequency (LF, 0.04 to 0.1 Hz) and high-frequency spectral bands (HF, 0.1 to 0.4 Hz).

Transthoracic echocardiography was performed on all participants using a device (Aplio XG; Toshiba Corporation, Tokyo, Japan) with a 2.5 MHz sector transducer. All examinations were performed by the same echocardiographer. The readings consisted of qualitative analysis of echocardiographic findings and measurements of quantitative parameters such as: left ventricular (LV) geometry and size, left atrial size, LV systolic and diastolic function, segmental LV dysfunction, valvular heart disease and pericardial appearance. Cardiac mass was calculated using the Devereaux formula.[15]

Measurement of the anterior-posterior diameter of the right lobe of the liver was performed by means of ultrasound for quantitative assessment of nonalcoholic fatty liver disease (NAFLD). Liver images were obtained using standard equipment (Toshiba SSA-770A Aplio, Japan) and a broadband convex transducer (PVT-375BT) with a central frequency of 3.5 MHz (2.5-5.5 MHz).[16]

The carotid-femoral pulse-wave velocity (PWV) was measured using a validated automated device (Complior, Artech Medicale, France), with the subject in the supine position in a temperature-controlled room (20-24 °C). First, BP was measured in the right arm with the subject in the supine position using an oscillometric device (HRM Onrom 705 CP). The distance from the sternal furcula to the right femoral pulse was determined using a measuring tape regardless of abdominal curvature. Pulse sensors were positioned in the right carotid and femoral arteries so that pulse waves were recorded and viewed on a computer screen. Computer software that could adequately detect and record pulse waves was used. PWV was calculated by dividing the distance from the sterna furcula to the femoral pulse by the difference between the rise delays of the carotid and femoral pulses. A subject's PWV was the arithmetic average of readings obtained in ten consecutive cardiac cycles at a regular heart rate.

Carotid intimal-media thickness (IMT) was assessed in all patients in a standardized manner using a device (Aplio XG, Toshiba) with a 7.5 MHz linear transducer. The technique used for IMT measurement was as previously published.[17] IMT was measured in the outer wall of a predefined carotid segment of 1 cm in length from 1 cm below the carotid bifurcation, during three cardiac cycles. We considered the images acquired to be valid if they clearly showed three reference points on both sides:

1. anatomical guides for the common carotid arteries;
2. interfaces between the lumen and the far wall of the vessel; and
3. interfaces between the media and adventitia layers of the far wall of the vessel.

We used the MIA software to standardize the readings and interpret the carotid scans as previously described. IMT was then defined as the mean of the right and left carotid measurements.

To determine the coronary artery calcium score, the participants underwent non-contrast computed tomography. The scans were performed using a 64-slice detector computed tomography scanner (Philips Brilliance, Philips, Netherlands). After scout images had been produced, each patient also underwent an ECG-gated prospective calcium score examination with a tube potential of 120 kV and a tube current adjusted to body habitus. Images were reconstructed at 2.5 mm slice thickness using standard filtered back projection. The coronary artery calcium score was expressed in terms of Agatston units and the percentiles were evaluated in a blinded manner by an experienced cardiologist using semi-automated software (Calcium Scoring, Philips Workstation). Coronary calcium scores were not obtained for participants who reported that they had been fitted with coronary stents, since the stent material greatly overestimates the calcium scores.

Retinography was performed using a nonmydriatic retinograph (CR-1, Canon, Japan) with a 10-megapixel digital camera (Canon EOS 40 D). The subjects underwent natural dilation of their pupils through resting in a darkened room for about four minutes, and for each eye two 45° fundus images were obtained: one centered on the optical disk and the second on the maculae. Our institution's central retinography laboratory (IC-RS) developed standardized image acquisition and reading protocols, and DICOM images (approximately 30 MB) and JPEG images (approximately 3 MB) were acquired. The JPEG images were recorded on CD/DVD at the study sites and were mailed to the central retinography laboratory.

Follow-up

The participants are being contacted again annually, for telephone interviews that include questions on hospitalizations, need for RRT and self-rated health. The main clinical endpoints investigated are death, acute myocardial infarction, unstable angina pectoris, cardiac revascularization, heart failure, stroke and RRT. Any cardiovascular and renal clinical events that are reported are then investigated and classified in line with the study protocol, by a panel of physicians that has received training in accordance with international classification criteria.[18] In the event of the participant's death, information regarding this event is sought. Surveillance of clinical events is also conducted through state databases such as the Mortality Registry and the São Paulo State Registry of Dialysis and Transplantation.

RESULTS

Over the two-year recruitment period, 454 participants were enrolled. **Table 3** shows the main clinical and laboratory parameters at baseline. The population recruited mainly had CKD

Table 3. Baseline clinical and laboratory profile of 454 participants in the PROGREDIR cohort

	All	
	n = 454	
Age (years; mean, SD)	67.5	11.9
Sex (men; n, %)	287	63.20%
Race (self-declared white; n, %)	300	66.10%
Hypertension (n, %)	409	90.10%
Diabetes (n, %)	208	45.80%
Acute myocardial infarction (n, %)	147	32.40%
Stroke (n, %)	73	16.10%
Smoking (current + former; n, %)	270	59.50%
Systolic blood pressure (mmHg; mean, SD)	140	24.1
Diastolic blood pressure (mmHg; mean, SD)	76.2	12.9
Body-mass index (mean, SD)	29.4	5.4
Waist-to-hip ratio (mean, SD)	0.97	0.1
Potassium (mEq/l; mean, SD)	4.58	0.52
Urea (mg/dl; median, IQR)	69	54-89.0
Creatinine (mg/dl; median, IQR)	1.7	1.4-2.1
Albuminuria (mg/dl; median, IQR)	80	15.0-640.0
Urinary RBP (mg/g creatinine; median, IQR)	0.29	0.08-1.47
eGFR-CKDEPI (ml/min/1.73 m^2; mean, SD)	38.4	14.6
Phosphorus (mg/dl; mean, SD)	3.6	0.6
Calcium (mg/dl; mean, SD)	9.6	0.6
Parathormone (pg/ml; median, IQR)	93	64.0-143.0
Serum RBP (mg/l; median, IQR)	66.1	55.2-79.8
Glycemia (mg/dl; median, IQR)	104	95.0-126.0
Glycated hemoglobin (%; median, IQR)	6.2	5.8-7.2
Glycemia after OGTT (mg/dl; mean, SD)*	155	51.8
HOMA-IR (median; IQR)*	3.4	2.3-5.8
Insulinemia (mUI/ml; IQR)	16.1	10.4-25.4
Total cholesterol (mg/l; mean, SD)	168.6	39.9
LDL-cholesterol (mg/dl; mean, SD)	91.4	32.2
HDL-cholesterol (mg/dl; mean, SD)	46	14.3
Triglycerides (mg/dl; median, IQR)	142	99.0-192.0
Serum pH (mean, SD)	7.35	0.04
Bicarbonate (mmol/l; mean, SD)	25.6	2.9
Hemoglobin (g/dl; mean, SD)	13.1	1.9
Iron (mcg/dl; mean, SD)	77.1	28.3
Ferritin (ng/ml; median, IQR)	104	52.0-197.0
High-sensitivity C-reactive protein (mg/l; median, IQR)	2.8	1.0-6.5
Albumin (mg/dl; mean, SD)	4.3	0.3
Right hepatic lobe anteroposterior diameter (mm; mean, SD)	104.8	12.9
Pulse wave velocity (cm/s; mean, SD)	12.8	3
Left atrium diameter (mm; mean, SD)	41.4	5.4
Left ventricular wall thickness (mm; median, IQR)	11	10.0-12.0
Ejection fraction (median, IQR)	0.66	0.6-0.7
Intima media thickness (mm; mean, SD)	0.75	0.2
Agatston score (median, IQR)	165	8.0-785.0

*calculated for participants without known diabetes.

SD = standard deviation; IQR = interquartile range; RBP = retinol-binding protein; eGFR-CKDEPI = estimated glomerular filtration rate-Chronic Kidney Disease Epidemiology Collaboration; OGTT = oral glucose tolerance test; HOMA-IR = homeostasis model assessment as an index of insulin resistance; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

in stages 3 and 4, with a mean estimated glomerular filtration rate-CKD Epidemiology Collaboration (eGFR-CKDEPI) of 38.4 (± 14.6) ml/min/1.73 m^2. The albuminuria range was wide, with similar frequencies of normoalbuminuria (35%), microalbuminuria (31%) and macroalbuminuria (34%). The participants' median age was 67 years; 63% were men; 60% were current or past smokers; 45% self-reported diabetes; and 32% reported having had previous myocardial infarction. Coronary artery calcification scores were also high, with more than half of the cohort presenting an Agatston score above 100.

Follow-up is ongoing. Up to the present date, i.e. over the first three years of follow-up, event rates have been high, with a 5-7% mortality rate per year and 2-3% incidence of ESRD and non-fatal cardiovascular events per year. With this event rate, from 2017 onwards, survival analysis will be started, focusing on biomarkers for mineral metabolism.

DISCUSSION

The PROGREDIR cohort was designed specifically to address CKD progression among patients with moderate to advanced disease. Over a two-year recruitment period, we were able to enroll 454 participants, and thus nearly reached the estimated sample size. The baseline characteristics of these participants were in accordance with the profile expected from the inclusion and exclusion criteria: older age, predominance of men and high rates of diabetes and previous cardiovascular disease. In the PROGREDIR cohort, we avoided overrepresentation of glomerulonephritis and other specific kidney diseases such as those relating to HIV, hepatitis C and lupus. Transplantation patients (any organ) were also not included. This decision was mostly related to the fact that PROGREDIR was designed to be a cohort of general CKD cases and not to address mechanisms relating to specific systemic or primary diseases. The eligible participants were originally from a quaternary-level hospital, which might have yielded an excessive number of glomerulonephritis cases if exclusion criteria had not been applied. Other CKD cohort studies have applied similar inclusion and exclusion criteria and have ended up with recruited populations compatible with the profile observed in PROGREDIR.[19-21]

One important accomplishment was to have nearly equal representation of normoalbuminuria, microalbuminuria and macroalbuminuria subpopulations in the baseline profile of the cohort. The prevalence of and interest in normoalbuminuric CKD is increasing,[22-24] since it is now known that 30-45% of diabetic patients may in fact present CKD and normoalbuminuria. It is currently of interest not only to understand the determinants of CKD progression in the normoalbuminuric CKD population, but also to compare the performance of traditional and new risk factors in normoalbuminuric and albuminuric populations, in order to test whether the results can be generalized to a broad spectrum of diseases.

Baseline data were collected in this study in accordance with the study design, covering traditional cardiovascular risk factors and biomarkers for CKD. Surrogate measurements of atherosclerosis and hypertension such as coronary calcium score, cardiac hypertrophy, IMT, PWV and retinography were made, and these will allow understanding and stratification of baseline CKD among the participants. The biobank is wide-ranging and kept under strict quality control, thus providing a source for reliable future measurements.

Follow-up is ongoing and a high event rate is being observed. Follow-up data collection is being centered on three major clinical events: death, non-fatal cardiovascular events and starting of RRT. These events are of particular importance, since CKD is known to be a very important cardiovascular risk factor that makes a significant contribution to high rates of morbidity and mortality.[2] Focusing data collection only on renal events would lead to selection bias, because a significant proportion of the participants might experience cardiovascular events prior to renal events. Thus, to fully address the impact of CKD biomarkers and measurements, it is very important to account for their impact both on renal events such as mortality and on fatal and non-fatal cardiovascular events.

Now that the cohort has been established, the PROGREDIR study can be used for research investigation in two major ways. First, it can be used to test the performance of candidate biomarkers for CKD progression. The current need to promote discovery and validation of biomarkers in CKD is highlighted by the recent launch of a CKD Biomarkers Consortium (BioCon)[25] by the National Institute of Diabetes and Digestive and Kidney Diseases in the United States. Similar approaches are being used by European countries.[26] Secondly, the cohort can be used to test high throughput technologies, which are an innovative approach that may provide new insights on the mechanisms and pathways of complex diseases, as well as enabling identification of novel biomarkers for diseases. As a first step, untargeted metabolomic assessments are currently being performed on baseline serum and the data thus obtained will be analyzed in relation to renal function and clinical events.

Additionally, to contribute towards improvement of scientific knowledge on CKD, the PROGREDIR study will also serve the purpose of being a national data source in which biomarkers can be replicated and validated. Racial factors are known to have an important effect on the risk of diseases, and this has recently been very well illustrated by the discovery of the higher risk attributable to the APOL1 gene in the African-American population.[27] In this regard, it is very important that national datasets should be available, so that the performance of candidate biomarkers can be tested on the Brazilian population, which is known to be highly admixed.

CONCLUSION

In conclusion, the PROGREDIR cohort recruitment and baseline data collection were successfully implemented. In addition to being a national dataset, the PROGREDIR cohort provides promising prospective study material that will allow better understanding of CKD determinants and validation of candidate biomarkers for CKD progression and mortality risk.

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Prevalence of *Helicobacter pylori* infection in an indigenous community in São Paulo and associated factors: cross-sectional study

Prevalência de infecção por *Helicobacter pylori* em uma comunidade indígena em São Paulo e fatores associados: estudo transversal

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Indigenous community of Guarani Mbya *ethnicity, Tekoa Ytu and Tekoa Pyau villages, Jaraguá district, São Paulo (SP), Brazil*

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KEY WORDS:
Prevalence.
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PALAVRAS-CHAVE:
Prevalência.
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Fatores de risco.
Fatores de proteção.

ABSTRACT

CONTEXT AND OBJECTIVE: The prevalence of *Helicobacter pylori* infection is unevenly distributed among different populations. The aim here was to evaluate the factors associated with *Helicobacter pylori* infection among children up to five years of age living in a high-risk community.

DESIGN AND SETTING: Cross-sectional study in an indigenous community of Guarani *Mbya* ethnicity, Tekoa Ytu and Tekoa Pyau villages, Jaraguá district, city of São Paulo (SP), Brazil.

METHODS: 74 children aged 0.4 to 4.9 years (mean 2.9 ± 1.3 years; median 3.1), and 145 family members (86 siblings, 43 mothers and 16 fathers) were evaluated for *Helicobacter pylori* infection using the validated ^{13}C-urea breath test. Clinical and demographic data were collected.

RESULTS: The prevalence was 8.3% among children aged 1-2 years and reached 64.3% among those aged 4-5 years (P = 0.018; overall 31.1%). The prevalence was 76.7% among siblings and 89.8% among parents. There was a negative association with previous use of antibiotics in multivariate analysis adjusted for age (odds ratio, OR: 0.07; 95% confidence interval, CI: 0.01 to 0.66; P = 0.02). The prevalence was higher among males (OR: 1.55), and was associated with maternal infection (OR: 1.81), infection of both parents (OR: 1.5), vomiting (OR: 1.28), intestinal parasitosis (OR: 2.25), previous hospitalization (OR: 0.69) and breastfeeding (OR: 1.87).

CONCLUSIONS: The prevalence was high among subjects older than three years of age, thus suggesting that the incidence of infection was higher over the first three years of life. Previous use of antibiotics was inversely associated with current *Helicobacter pylori* infection.

RESUMO

CONTEXTO E OBJETIVO: A prevalência da infecção pelo *Helicobacter pylori* é desigualmente distribuída entre diferentes populações. O objetivo foi avaliar fatores associados à infecção pelo *Helicobacter pylori* em crianças de até cinco anos de idade morando numa comunidade de alto risco.

TIPO DE ESTUDO E LOCAL: Estudo transversal na comunidade indígena da etnia Guarani *Mbya* das aldeias Tekoa Ytu e Tekoa Pyau do distrito de Jaraguá da cidade de São Paulo (SP), Brasil.

MÉTODOS: 74 crianças de 0,4 a 4,9 anos (média 2,9 ± 1,3 anos; mediana 3,1), e 145 familiares (86 irmãos, 43 mães e 16 pais) foram avaliadas quanto à infecção pelo *Helicobacter pylori* com o teste respiratório com ureia ^{13}C validado. Dados clínicos e demográficos foram coletados.

RESULTADOS: Prevalência foi 8,3% entre 1-2 anos, atingindo 64,3% entre 4-5 anos (P = 0,018; 31,1% globalmente). Em irmãos, foi 76,7% e 89,8% nos pais. Observou-se associação negativa com uso prévio de antibiótico em análise multivariada ajustada para idade (*odds ratio*, OR: 0,07; intervalo de confiança, IC: 95%: 0,01-0,66; P = 0,02). A prevalência foi maior no sexo masculino (OR: 1,55), e foi associada a infecção materna (OR: 1,81), infecção de ambos os pais (OR: 1,5), vômitos (OR: 1,28), parasitose intestinal (OR: 2,25), hospitalização prévia (OR: 0,69) e aleitamento materno (OR: 1,87).

CONCLUSÕES: A prevalência foi alta entre os indivíduos com mais de três anos de idade, o que sugere incidência maior nos três primeiros anos de vida. Uso prévio de antibióticos foi inversamente associado a infecção atual por *Helicobacter pylori*.

INTRODUCTION

Although *Helicobacter pylori* infection is ubiquitous among humans, its prevalence is unevenly distributed among different populations. In developed countries, the incidence of this infection has declined sharply over the past few decades. Recently, prevalence of under 10% among children has been reported. In Brazil, the prevalence of *Helicobacter pylori* remains high among deprived urban settlements, rural communities and indigenous communities. Occurrences of *Helicobacter pylori* are probably due to poor sanitation, allied with poverty.[1-6]

Recently, we reported that there was high prevalence (73.5%) of this infection among children in six indigenous communities in the Amazon Forest.[6] However, there is no study evaluating indigenous communities located in urban areas.

The infection is contracted primarily during childhood, with low incidence among adults. Although the precise mechanism of transmission is largely unknown, there is evidence that person-to-person transmission within households plays a role. Furthermore, in rural areas, transmission may also occur through contaminated food and water, and through close contact with non-maternal caregivers.[7]

Helicobacter pylori infection is associated with long-term morbidity (peptic ulcer disease, gastric cancer and gastric MALT lymphoma), but children may also be affected.[8] Recently, we demonstrated that *Helicobacter pylori* eradication plays a role in treating chronic immune thrombocytopenic purpura in children.[9] Nowadays, the burden of *Helicobacter pylori* infection is mostly borne by developing countries and specific high-risk groups. Studies conducted in such populations allow better knowledge of risk factors, especially if the study targets young children, since most new infections occur at that age.

OBJECTIVE

The present study aimed to evaluate factors associated with *Helicobacter pylori* infection among children up to five years of age in an urban indigenous community of Guarani ethnicity in the city of São Paulo, Brazil.

METHODS

Subjects

Children up to five years of age living in an indigenous community of Guarani *Mbya* ethnicity based in the northwestern region of the city of São Paulo were eligible for inclusion. The total population was estimated as 636 people (demographic census of 2010), with 137 children under the age of 5 years. The community does not have any sewage system, running water or regular electricity supply. Also, the housing is precarious and most of the population lives in houses without toilets or showers. Consequently, most of the children defecate directly on the ground.

The community does not have anywhere for hunting or fishing, and the soil is unsuitable for growing crops. Therefore, the community raises money by selling crafts. Lastly, they have plenty of animals (cats, raccoons, monkeys and, especially, dogs).

Study protocol

All the children up to five years of age and their family members living in the same dwelling (parents and siblings) were invited to participate in the study. Parents who were willing to participate were asked to answer a questionnaire that asked about breastfeeding, dummy/pacifier use, bottle-feeding, bed-sharing, number of household members, episodes of vomiting and/or diarrhea, use of antibiotics over the previous month and previous admissions to hospital. Parents were asked about the use of antibiotics over the previous month among index subjects (children up to five years of age). As the mothers understood the Portuguese language, the interviews were conducted in Portuguese by the principal researcher, always with help from a health agent from the community.

Index subjects and their family members (parents and siblings) performed a ^{13}C-urea breath test so that cases of *Helicobacter pylori* infection could be diagnosed. In addition, all parents were requested to provide a stool sample from the index subject for stool and ova examination, as described below.

^{13}C-urea breath test

After the participants had fasted for four hours, breath samples were collected through one-way valves connected to aluminum bags. Face masks were used to collect samples from children who were unable to blow voluntarily. Samples were collected at baseline and 30 minutes after ingestion of 50 mg of ^{13}C-urea diluted in 100 ml of orange juice without added sugar.

The breath samples were analyzed using an infrared isotope analyzer (IRIS; Wagner Analysen Technik, Bremen, Germany). Delta over baseline (DOB) greater than 4‰ was regarded as positive, as had previously been validated locally among children younger than six years of age (sensitivity 93.3%, with 95% confidence interval, CI: 86.8%-99.7%; specificity 96.2%, with 95% CI: 93.6%-98.8).[10]

Stool ova and parasite test

Every caregiver was given a stool collector (PARATEST brand, Diagnostek) and was instructed to collect freshly evacuated stools. The samples were processed by means of the Hoffman method, with a subsequent search for eggs and cysts using optical microscopy.

Study design and statistics

This was a cross-sectional analysis. The study included all the indigenous children with all their family members living in the same dwelling, and there was no sampling procedure.

All indigenous children up to five years of age and their families were invited to participate in the study. Villages were visited three times a week by the principal investigator and a health worker.

Quantitative variables were described in terms of their means, medians and standard deviations, while categorical variables were described in terms of their proportions. Infection rate variation according to age group was tested by means of the chi-square test for linear trend. Otherwise, associations were assessed using the chi-square test or Fisher's exact test. A binary logistic model was used to assess the association between *Helicobacter pylori* infection and selected variables. The independent variables were the following: sex of the child, vomiting, intestinal parasitosis, previous antibiotic use, previous hospitalization and breastfeeding, as well as infected mother and infected father. Independent variables that were found to be associated with the primary outcome (*Helicobacter pylori* infection in the child, i.e. the dependent variable) at a P-level of less than 0.25 were included in a multivariate logistic model. The fit of the multiple regression was assessed in accordance with the Hosmer-Lemeshow test, and P-values < 0.05 indicated a good fit. P-values < 0.05 were considered statistically significant. The statistical packages SPSS for Windows 17.0 and Stata for Windows version 10.0 were used for statistical analysis.

Ethical considerations

The leaders of the community were given explanations about the study protocol, and they approved it. Moreover, every single subject and their respective legal guardian gave signed consent (assent for children over eight years of age). This study was approved by the Research Ethics Committee of the Paulista School of Medicine, Federal University of São Paulo, by the Ethics Committee of the Municipal Health Department of São Paulo and by the National Council for Research Ethics (Comissão Nacional de Ética em Pesquisa, CONEP).

RESULTS

A total of 74 children (33 males and 41 females; age range 0.4-4.9 years; median age 3.1; and mean age 2.9 ± 1.3) and 145 family members were evaluated. None of the parents actively refused to participate, but 63 of the 137 children (46%) did not show up on the scheduled date. None of these 63 children adhered to the study protocol, despite the various calls made personally in their homes. There was no compliance with the study protocol among these 63 children, in spite of multiple requests made by the study staff.

We do not believe there were any systematic differences between those who participated and those who did not. The family members comprised 86 siblings (46 males and 40 females; age range 5.1 - 16.8 years; mean age 10.9 ± 8.3 years; and median 9.0 years), 43 mothers (age range 15.7-56.4 years; mean 36.1 ± 28.8; and median 30.0) and 16 fathers (age range 22.4-54.9 years; mean 38.6 ± 23.0; and median 27.4) (**Table 1**).

The prevalence of *Helicobacter pylori* among the 74 children was 31.1% (23/74), with a significant direct relationship with age (P = 0.018), such that the prevalence ranged from 8.3% at the ages of 1-2 years to 64.3% at the ages of 4-5 years (**Table 2**). Positive stool and ova tests were not associated with *Helicobacter pylori* infection, given that nearly all the samples were positive (94.1%, 48/51) and 45.8% of them showed multiple infestation. *Endolimax nana* was the parasite most frequently detected concurrently (47.9%), followed by *Entamoeba coli* (35.4%), *Giardia lamblia* (31.3%) and *Ascaris lumbricoides* (31.3%).

The following variables were not associated with cases of *Helicobacter pylori* infection (P > 0.05): infected mother (odds ratio, OR: 1.81; 95% CI: 0.33 to 9.72), male gender (OR: 1.55; 95% CI: 0.58 to 4.19), maternal and paternal infection together (OR: 1.5; 95% CI: 0.27 to 8.29), vomiting (OR: 1.28; 95% CI: 0.41 to 4.03), intestinal parasitosis (OR: 2.25; 95% CI: 0.45 to 11.36), previous hospitalization (OR: 0.69; 95% CI: 0.22 to 2.2) and breastfeeding (OR: 1.87; 95% CI: 0.2 to 17.75) (**Tables 3** and **4**). On the other hand, previous use of antibiotics was inversely

Table 1. Descriptive statistics on the ages of the study population

Age (n)	Range (years)	Mean (years)	Standard deviation	Median (years)
Study group (74)	0.4-4.9	2.9	1.3	3.1
Siblings (86)	5.1-16.8	10.95	8.27	9.0
Mothers (43)	15.7-56.4	36.05	28.78	30.0
Fathers (16)	22.4-54.9	38.65	22.98	27.4

Table 2. Prevalence of *Helicobacter pylori* infection among 74 children younger than 5 years of age

Age (years)	*Helicobacter pylori*		Total
	Positive n (%)	Negative n (%)	
0.4 ¬ 1	1 (12.50)	7 (87.50)	**8**
1 ¬ 2	1 (8.33)	11 (91.66)	**12**
2 ¬ 3	8 (47.05)	9 (52.94)	**17**
3 ¬ 4	4 (17.39)	19 (82.60)	**23**
4 ¬ 5	9 (64.28)	5 (35.71)	**14**
Total	**23 (31.10)**	**51 (68.90)**	**74**

Fisher's exact test: P = 0.018 (association test for linear trend).

Table 3. *Helicobacter pylori* infection and associated factors among 74 children younger than 5 years of age

Helicobacter pylori	Positive n (%)	Negative n (%)	Total n (%)	P*
Hospitalization	17 (29.31)	41 (70.69)	58 (100.0)	0.553
Breastfeeding	22 (31.88)	47 (68.12)	69 (100.0)	1
Shared bed	20 (28.57)	50 (71.43)	70 (100.0)	0.086
Diarrhea	23 (31.50)	50 (68.50)	73 (100.0)	1
Antibiotic use	18 (26.47)	50 (73.53)	68 (100.0)	0.01

*Fisher's exact test.

associated with *Helicobacter pylori* infection (OR: 0.07; 95% CI: 0.01 to 0.66).

In the multivariate analysis, both age (OR: 1.67; 95% CI: 1.05 to 2.67; P = 0.030) and previous use of antibiotics remained significantly associated with the outcome.

DISCUSSION

In this urban community with high prevalence of infectious parasitic diseases, we found that nearly two-thirds of the children had become infected with *Helicobacter pylori* by the age of five years. This high prevalence rate at such a young age highlights the precarious living conditions in this community, with poor sanitation. Comorbidities such as infectious diarrhea were observed in 100% of the children, and intestinal parasites, in almost all of the children examined. In the same city (São Paulo), among children with low socioeconomic status, we previously reported that the seroprevalence of *Helicobacter pylori* infection among children aged 2-4 years was 20.8%, and among those aged 4-6 years, 25%.[5] Furthermore, lower prevalence was found among adolescents (10-16 years of age) in a public school in the city of São Paulo (40.7%).[11] It is clear that this urban indigenous community presented higher prevalence of this infection. The age group at highest risk of contracting the infection was the group younger than three years of age. At this age, around half of the children were infected. This high rate of early acquisition of infection needs to be taken into consideration in designing strategies to prevent infection in high-risk communities.[12]

In a prospective study, Rowland et al. described a baseline prevalence of 8.6%, and an incidence of 7.6% (20/262); 95% (19/20) of the new infections occurred in children younger than five years of age, with a maximum incidence rate at 2-3 years (5.05/100 person-years).[13] On the other hand, in a high-risk population (indigenous children), 60% of the children aged 2-3 years were already infected and the prevalence of infection increased until 4-5 years of age (80.6%), and then increased slightly again at a later age (8-9 years; 85.3%).[6] Thus, both high and low-prevalence populations seem to present the highest incidence in younger children.

Table 4. Factors relating to *Helicobacter pylori* infection among 74 children younger than 5 years of age

Variable	OR	95% CI	P	n
Male gender	1.55	0.58-4.19	0.38	74
Age	1.57	1.03-2.38	0.034	74
Father infected	1	0.07-13.67	1	21
Mother infected	1.81	0.33-9.72	0.49	56
Siblings infected	0.75	0.11-4.95	0.77	53
Infection of both parents	1.5	0.27-8.29	0.64	56
Vomiting	1.28	0.41-4.03	0.67	74
Previous hospitalization	0.69	0.22-2.2	0.53	74
Intestinal parasitosis	2.25	0.45-11.36	0.33	74
Shared bed	0.13	0.01-1.36	0.09	74
Baby-bottle feeding	0.55	0.18-1.62	0.28	74
Breastfeeding	1.87	0.2-17.75	0.58	74
Antibiotic use	0.07	0.01-0.66	0.02	74

OR = odds ratio; CI = confidence interval.

We chose to evaluate children younger than five years of age in a high-risk population because that would be the age group with maximum incidence. Although there was a direct relationship between prevalence and age, children in their fourth year presented lower prevalence (17.39%). It can be hypothesized that spontaneous elimination of *Helicobacter pylori* (*H. pylori)* infection may have occurred in some children up to that age, as was shown by Klein et al., in a cohort of Peruvian children. These authors found that the prevalence among 18-month infants was lower than among 6-month infants (reduction from 71.4% to 47.9%), and they hypothesized that in young infants, *H. pylori* infection may be a reversible process, due to a particular immune response.[14] A longitudinal study might elucidate whether spontaneous elimination of the infection plays any significant role in high-risk populations.

Spontaneous clearance in children has been observed in several studies,[14-16] and there is the possibility that exposure to antibiotics might contribute towards spontaneous elimination of infection particularly in children.[16,17] However, only a few studies have investigated the association between antibiotic use and infection by *H. pylori* among children aged less than four years,[17,18] i.e. the period during which antibiotics are widely used and the incidence of *H. pylori* infection is relatively high.[19] The present study supports the hypothesis that exposure to antibiotics aimed at other infections may be responsible for eradication of *H. pylori* infection in children. According to the Pasitos cohort study, spontaneous elimination of *H. pylori* infection was significantly related to incidental antibiotic exposure, but this only explained a small proportion of the spontaneous clearance rate.[20]

In the present study, it was unclear whether infection in children exposed to antibiotics was eradicated. Because the study group formed part of a small homogeneous indigenous village community, we believe that antibiotic use was not just an epiphenomenon, i.e. a sign of different access to health care. Serological tests might provide further information, since serum samples would remain positive long after eradication, but the lack of sensitivity of these tests in children precluded their inclusion in the present study protocol.[21]

Intrafamilial transmission of *H. pylori* infection could not be evaluated because of loss of parents and siblings from the sample. Only a few parents participated in the study (43 mothers and 16 fathers), which hampered proper assessment of intrafamily transmission. However, the prevalence of *H. pylori* was high among family members, and the chances that children would be infected were slightly greater if the mother was infected (OR: 1.81) or if both parents were infected (OR: 1.5), although these results were not

statistically significant. Our results differed from those reported by Weyermann et al., in which maternal infection was the only strong and significant factor (OR: 13.0; 95% CI: 3.0 to 55.2) in an urban population.[22] Transmission of pathogens between people in the same family is common, especially among children younger than five years of age. Contamination can occur through gastro-oral, oral or oral-fecal-oral transmission, and diarrhea, vomiting or regurgitation may facilitate it.[23] The study population had high hospitalization rates and almost all participants had had an episode of acute gastroenteritis, which may have favored transmission of *H. pylori*.

Recently, a systematic review supported the notion that breastfeeding has a protective role against *H. pylori* infection among children living in economically less developed settings.[24] In the present study, however, breastfeeding was not protective against *H. pylori* infection. Similarly, Rowland et al. reported that there was no difference in the rate or duration of breastfeeding between infected and uninfected children. However, in another study, in a high-prevalence country (77% at 12 months of age), exclusive breastfeeding was associated with reduced risk.[25]

The prevalence of *H. pylori* infection was high over the first five years of life but incidence was higher in the first three years of life, which indicates the importance of implementing immediate public health and sanitation measures. These preventive measures are the key to reducing the prevalence of infection over the initial years of life. In these areas of high risk of infection, the data suggest that antibiotic use is inversely associated with *H. pylori* infection.

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Surgical treatment of neurocysticercosis. Retrospective cohort study and an illustrative case report

Tratamento cirúrgico da neurocisticercose. Estudo de coorte retrospectivo e um caso ilustrativo

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KEY WORDS:
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Cohort studies.

PALAVRAS-CHAVE:
Neurocisticercose.
Hidrocefalia.
Epilepsia.
Derivação ventriculoperitoneal.
Infecções do sistema nervoso central.
Procedimentos neurocirúrgicos.
Relatos de casos.
Estudos de coortes.

ABSTRACT

CONTEXT AND OBJECTIVE: Neurocysticercosis is prevalent in developing countries and manifests with several neurological signs and symptoms that may be fatal. The cysts may be parenchymal or extraparenchymal and therefore several signs and symptoms may occur. Depending on their location, neurosurgical procedures may be required, sometimes as emergencies. The aim here was to review 10-year statistics on all surgical neurocysticercosis cases at a large public tertiary-level hospital.

DESIGN AND SETTING: Retrospective cohort at a large public tertiary-level hospital.

METHODS: All surgical neurocysticercosis cases seen between July 2006 and July 2016 were reviewed. Parenchymal and extraparenchymal forms were considered, along with every type of surgical procedure (shunt, endoscopic third ventriculostomy and craniotomy). The literature was reviewed through PubMed, using the terms "neurocysticercosis", "surgery", "shunt" and "hydrocephalus".

RESULTS: 37 patients underwent neurosurgical procedures during the study period. Most were male (62.16%) and extraparenchymal cases predominated (81%). Patients aged 41 to 50 years were most affected (35.13%) and those 20 years or under were unaffected. Ventricular forms were most frequently associated with hydrocephalus and required permanent shunts in most cases (56.57%).

CONCLUSIONS: The treatment of neurocysticercosis depends on the impairment: the parenchymal type usually does not require surgery, which is more common in the extraparenchymal form. Hydrocephalus is a frequent complication because the cysts often obstruct the cerebrospinal flow. The cysts should be removed whenever possible, to avoid the need for permanent shunts.

RESUMO

CONTEXTO E OBJETIVO: A neurocisticercose é prevalente em países em desenvolvimento e manifesta-se com vários sinais e sintomas neurológicos que podem ser fatais. Os cistos podem ser parenquimatosos ou extraparenquimatosos, portanto vários sinais e sintomas podem estar presentes. Dependendo da sua localização, procedimentos neurocirúrgicos podem ser necessários, às vezes em caráter emergencial. O objetivo foi revisar dados estatísticos de um período de 10 anos de todos os casos cirúrgicos de neurocisticercose num grande hospital público terciário.

TIPO DE ESTUDO E LOCAL: Coorte retrospectiva de um grande hospital público terciário.

MÉTODOS: Todos os casos cirúrgicos de neurocisticercose de pacientes tratados entre julho 2006 e julho 2016 foram revisados. As formas parenquimatosas e extraparenquimatosas foram consideradas, assim como tipo de procedimento cirúrgico (derivação, terceiroventriculostomia endoscópica e craniotomia). A literatura foi revisada por meio da PubMed, utilizando-se os termos "neurocysticercosis", "surgery", "shunt" e "hydrocephalus".

RESULTADOS: 37 pacientes foram submetidos a procedimentos neurocirúrgicos nesse período, a maioria do sexo masculino (62.16%%) e casos extraparenquimatosos predominaram (81%). Pacientes com idade 41-50 anos foram os mais afetados (35,13%) e aqueles com 20 anos ou menos não foram afetados. As formas ventriculares mais frequentemente estiveram associadas a hidrocefalia e necessitaram da realização de *shunts* definitivos na maior parte dos casos (56,57%).

CONCLUSÕES: O tratamento depende da forma de acometimento: o tipo parenquimatoso usualmente não necessita de cirurgia que é mais comum na forma extraparenquimatosa. Hidrocefalia é uma complicação frequente pois muitas vezes os cistos obstruem o fluxo liquórico. A remoção dos cistos deve ser realizada sempre que possível para evitar a necessidade de derivações definitivas.

INTRODUCTION

Neurocysticercosis is caused by central nervous system (CNS) infection due to *Taenia solium* (pork tapeworm) larvae.[1] It constitutes the most common cause of epilepsy[1] and hydrocephalus in adults who live in developing countries.[1,2] This disease may be acquired when a healthy person ingests eggs from the feces of a tapeworm carrier through contaminated water or vegetables. The infection may affect the brain parenchyma (in some cases, it may mimic brain tumors[3]) or it may be extraparenchymal, in the cisternae, subarachnoid space or intraventricular areas. The most frequent location is in the cerebral hemispheres,[1,4] where lesions are initially surrounded by edema and subsequently calcify but remain as epileptic foci.

The incidence of this disease is greater in developing countries, although some large studies have shown increasing incidence in developed countries such as the United States,[5] and it is very variable around of the world. In Latin America, the incidence also varies depending on the urban or rural region, from 121.7 to 138.4 cases per 100,000 individuals per year.[6] Sanitary conditions have a close relationship with neurocysticercosis, and combating this disease is a priority for the World Health Organization (WHO).[2] It is one of the seven neglected endemic zoonoses targeted by WHO. The clinical manifestations of neurocysticercosis have been well known since the late 1800s and early 1900s.[7] Cysticerci in the CNS can cause several neurological manifestations, depending on the cyst location and stage and the numbers of cysts.[4]

The treatments include use of antiparasitic drugs, especially praziquantel and albendazole.[4] However, when ventricular or cisternal forms are present, these drugs are not effective. Either albendazole or praziquantel is effective for killing live cysticerci. Albendazole is currently the drug of choice because of its slightly greater efficacy, better availability and lower cost. It is also very important to treat symptoms such epilepsy using antiepileptic drugs (AEDs). A neurosurgical approach is usually required for ventricular forms, which cause hydrocephalus, and also in cases in which the cerebrospinal fluid (CSF) flow is altered in other regions such as in the cisternae spaces.[4,8]

The prognosis for neurocysticercosis is usually good when timely treatment is instituted. In neurosurgical approaches, the initial aim should be to try to withdraw obstructions, and thus to remove cysts when possible. Sequelae such as adult epilepsy are common and these patients usually require long-term use of AEDs, with follow-up from the infectious disease team.[4,8]

OBJECTIVE

The aim of this study was to describe all the neurosurgical cases (parenchymal and extraparenchymal forms) seen at our institution over the past 10 years, focusing on cisternal impairment.

METHODS

This was an observational longitudinal and retrospective study in which all patients with a diagnosis of neurocysticercosis who underwent any surgical procedure were included. A database was constructed to analyze all neurosurgical cases of neurocysticercosis that were seen at a large tertiary-level hospital in São Paulo, Brazil, over the past ten years (July 2006 to July 2016). The analysis took gender, age, type of impairment (parenchymal or extraparenchymal), presence of hydrocephalus, type of neurosurgical approach proposed and patient's origin (rural or urban area) into consideration.

In addition, a cerebellomedullary case is reported in greater detail, with the clinical picture and intraoperative images.

A detailed review of the literature was also conducted, focusing on neurosurgical approaches towards this disease. An extensive search was performed in PubMed using the terms: "neurocysticercosis", "surgery", "shunt" and "hydrocephalus".

RESULTS

A retrospective analysis was conducted on 37 patients who underwent neurosurgical procedures to treat neurocysticercosis over a ten-year period (2006-2016).

The male gender was more affected (62.16%). Patients between 41 and 50 years were most affected (35.13%), followed by the age groups from 31 to 40 years (21.62%), from 21 to 30 years (16.21%) and finally from 51 to 60 and over 61 years (13.51% each). Children (20 years and under) were unaffected in this sample (**Table 1**). Patients coming from rural areas were clearly more affected (75.67%).

Table 1. Variables considered for epidemiological analysis on 37 consecutive surgical neurocysticercosis cases

Age (years)	Gender		Type of impairment		Type of procedure			Origin	
	Female	Male	Parenchymal	Extraparenchymal	Shunt	Endoscopic third ventriculostomy	Craniotomy	Rural	Urban
0-10	0	0	0	0	0	0	0	0	0
11-20	0	0	0	0	0	0	0	0	0
21-30	2	4	1	5	4	0	2	4	2
31-40	3	5	1	7	3	1	4	5	3
41-50	4	9	2	11	5	2	6	10	3
51-60	2	3	1	4	4	0	1	5	0
≥ 61	3	2	2	3	2	0	3	4	1

The extraparenchymal type predominated, accounting for 81% of all cases. Among these patients, 76% had only the ventricular form, 14% only the cisternal form and 10% both forms.

Regarding neurosurgical approaches, 8% required endoscopic intervention and the other cases were equally divided between craniotomy (46%) and ventriculoperitoneal shunt (VPS) (46%). The procedure depended on the type of impairment, as shown in **Table 2**. For example, the parenchymal form required craniotomy in most cases (71.43%).

An illustrative case of a 31-year-old female patient who presented with epilepsy and signs and symptoms of intracranial hypertension (progressive headache and papilledema) is described here. Complementary investigation revealed eosinophilic meningitis and neuroimaging investigation showed hydrocephalus and cystic lesions in the cerebellomedullary cisterna (**Figure 1**). Suboccipital craniotomy to excise the cysts was proposed (**Figure 2**). After the cisterna had been opened, several cysts that were obstructing the CSF flow could be seen (**Figure 2**).

DISCUSSION

Neurocysticercosis is a CNS infection in which the incidence is closely related to sanitary conditions. For this reason, it is more prevalent in developing countries[1,2] and especially in rural areas of these countries. It is caused by the larval form of *Taenia solium*.

Table 2. Type of procedure versus form of neurocysticercosis, considering all 37 patients.

	Parenchymal	Extraparenchymal		Both forms
		Cisternal	Ventricular	
Craniotomy	71.43%	100%	30.43%	33.33%
Shunt	28.57%	0	56.57%	33.33%
Endoscopic third ventriculostomy	0	0	13%	33.33%

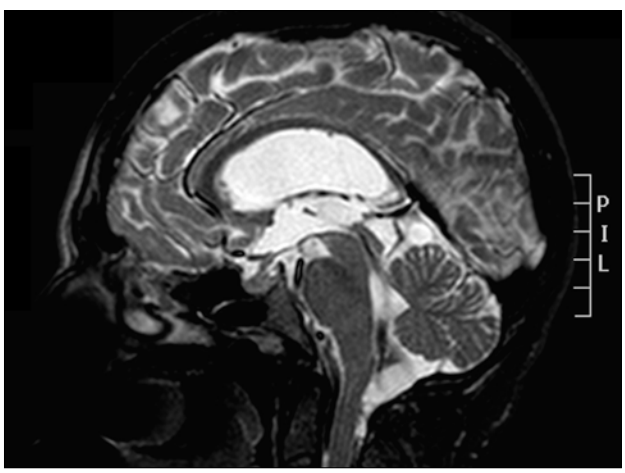

Figure 1. Preoperative sagittal T2-weighted magnetic resonance imaging (MRI) showing several cysts in cerebellomedullary cisterna obstructing cerebrospinal fluid flow, and showing hydrocephalus.

Humans are usually the definitive host, but in some cases, the cycle becomes altered and fecal-oral contamination occurs. In these cases, humans are the intermediate host.[1,2,4]

The cysts may be found in almost any organ or tissue. In most locations, they will not be noticed, but when cysts are located in the CNS, many symptoms may be present and may produce severe and disabling disease, which is sometimes lethal.[8] Because the cysts are initially surrounded by significant degrees of edema, this constitutes an important epileptogenic factor.[4] Even after degeneration of the cysticercus, the irritant remains and the patient might become epileptic.[9] Therefore, epilepsy is the most frequent symptom of the parenchymal type.[1,9] In the extraparenchymal form, in which an obstruction occurs at some point of the CSF flow (**Figures 1** and **2**), patients may present signs and symptoms of intracranial hypertension secondary to hydrocephalus. In one of the cases analyzed in the present study, a patient had an initial presentation of aphasia: she had perisylvian cysts and the initial edema could have affected the speech area. Brain neoplasm was initially considered as primary differential diagnosis[3] and this case was previously reported.[3]

It was observed that patients could present with several different clinical pictures caused by the presence of brain cysts, depending on their locations. The severity of the cases was very variable, from asymptomatic to extremely symptomatic with elevated intracranial pressure and a comatose state.

Two main types of neurocysticercosis have been defined, depending on its location: parenchymal or extraparenchymal.[1,3,10] To institute the correct therapeutic approach, it is essential to differentiate these types. Parenchymal cysticercosis generally has a better prognosis, mainly because it tends to respond to antiparasitic drugs:

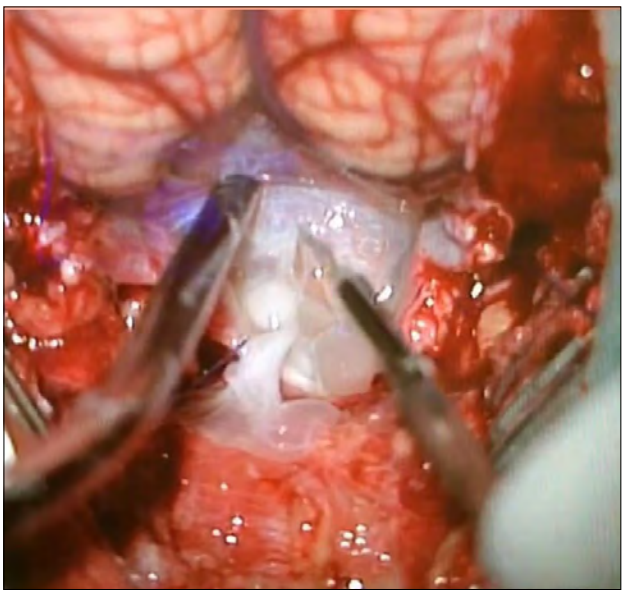

Figure 2. Microsurgical appearance of cerebellomedullary neurocysticercosis.

albendazole (15 mg/kg/day) or praziquantel (50-75 mg/kg/day) for 15 days. During their use, concomitant use of steroids has been advocated,[10] without any need for neurosurgical treatment.[4,11] Surgical intervention is required when this lesion in its racemose form, which leads to a significant mass effect with edema surrounding it. In a few cases, this lesion can mimic a brain tumor (as suggested from neuroimaging),[3] as in one of the cases evaluated in this review. Such lesions are more often associated with epileptic manifestations.[10]

On the other hand, the extraparenchymal form may present as cysts in the subarachnoid and cisternal spaces and intraventricular areas. This is more often related to severe symptoms such as intracranial hypertension (secondary to hydrocephalus due to obstruction of CSF circulation). It is also associated with marked inflammation and increased concentrations of proteins and cells in the CSF, resulting from continued exposure to remnants of parasite membranes.[4,10]

The treatment for this type is essentially neurosurgical, with cyst removal by means of endoscopy or craniotomy, depending on their locations.[9] For example, in the case reported in **Figure 1**, it was decided to use suboccipital craniotomy, to provide complete resection of the cerebellomedullary cysts. Patients may not require permanent shunts such as ventriculoperitoneal valves. The basic principle is to remove the obstruction through removing cysts and adhesions to facilitate CSF flow. However, despite cyst removal, some patients might still require permanent shunts. This could be due to chronic inflammatory processes caused by the parasite.[6] Initially, the patient whose case is reported in **Figure 1** did not require a shunt, but about two weeks after the first surgery, she presented with hydrocephalus and a definitive shunt procedure was performed.

In the present case series, diverging from most studies in the literature,[1,4,6,8] the extraparenchymal form predominated. This may have been because the present study only reported on neurocysticercosis cases that required operations. The parenchymal form, which is generally the most common type, usually does not require neurosurgical intervention, unlike cases in which the disease has an extraparenchymal location.

CONCLUSION

It may present with several signs and symptoms, and therefore the diagnosis is made through clinical examination and neuroimaging (epidemiological factors may also help). A neurosurgical approach is usually required in cases of the extraparenchymal form. Thus, when only surgical cases are considered, the incidence of this type is greater, as in the present review. The aim of surgical procedures should always be to remove the cysts and avoid the need for permanent shunts when hydrocephalus is present. These patients should be followed up for indefinite periods of time, given that many of them remain epileptic after the acute phase.

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Comparative analysis of death by suicide in Brazil and in the United States: descriptive, cross-sectional time series study

Análise comparativa de morte por suicídio no Brasil e nos Estados Unidos: estudo transversal temporal descritivo

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PALAVRAS-CHAVE:
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Saúde Pública.
Saúde Mental.

ABSTRACT

CONTEXT AND OBJECTIVE: The World Health Organization recognizes suicide as a public health priority. Increased knowledge of suicide risk factors is needed in order to be able to adopt effective prevention strategies. The aim of this study was to analyze and compare the association between the Gini coefficient (which is used to measure inequality) and suicide death rates over a 14-year period (2000-2013) in Brazil and in the United States (US). The hypothesis put forward was that reduction of income inequality is accompanied by reduction of suicide rates.

DESIGN AND SETTING: Descriptive cross-sectional time-series study in Brazil and in the US.

METHODS: Population, death and suicide death data were extracted from the DATASUS database in Brazil and from the National Center for Health Statistics in the US. Gini coefficient data were obtained from the World Development Indicators. Time series analysis was performed on Brazilian and American official data regarding the number of deaths caused by suicide between 2000 and 2013 and the Gini coefficients of the two countries. The suicide trends were examined and compared.

RESULTS: Brazil and the US present converging Gini coefficients, mainly due to reduction of inequality in Brazil over the last decade. However, suicide rates are not converging as hypothesized, but are in fact rising in both countries.

CONCLUSION: The hypothesis that reduction of income inequality is accompanied by reduction of suicide rates was not verified.

RESUMO

CONTEXTO E OBJETIVO: A Organização Mundial da Saúde reconhece o suicídio como uma prioridade de saúde pública. Aumentar o conhecimento dos fatores de risco de suicídio é necessário para se poder adotar estratégias eficazes de prevenção. O objetivo deste estudo foi analisar e comparar a associação do coeficiente de Gini, usado para medir a desigualdade, com as taxas de mortalidade por suicídio em um período de 14 anos (2000-2013) no Brasil e nos Estados Unidos (EUA). Aventou-se a hipótese de que a redução da desigualdade de renda é acompanhada da redução das taxas de suicídio.

TIPO DE ESTUDO E LOCAL: Estudo transversal temporal descritivo realizado no Brasil e nos EUA.

MÉTODOS: Dados populacionais, de óbitos e mortes por suicídio foram extraídos do banco de dados do DATASUS, no Brasil, e do Centro Nacional para Estatísticas de Saúde dos EUA. Dados do índice de Gini foram obtidos dos Indicadores de Desenvolvimento Mundial. Foi realizada análise de séries temporais de dados oficiais do Brasil e dos EUA sobre o número de mortes por suicídio, de 2000 a 2013, e do coeficiente de Gini. As tendências de morte por suicídio foram analisadas e comparadas.

RESULTADOS: Brasil e EUA apresentaram convergência no coeficiente de Gini devida principalmente à redução da desigualdade no Brasil na última década. No entanto, as taxas de suicídio não estão convergindo como foi conjeturado; em verdade, elas estão crescendo em ambos os países.

CONCLUSÕES: A hipótese de que a redução da desigualdade de renda é acompanhada da redução das taxas de suicídio não foi verificada.

INTRODUCTION

Suicide is a serious public health problem that needs to be faced openly and in a manner that is as well-informed as possible. In fact, there is a need to distinguish between the achieved act (suicide) and suicide attempts (unsuccessful). Suicide is the act of deliberately killing oneself. Over the last 45 years, suicide rates have increased by about 60% worldwide.[1] Consequently, over this period, suicide has become a public health concern. Over 842,000 people die by suicide every year globally, which is a rate of 11.6 per 100,000 individuals per year, or one death somewhere in the world every 40 seconds. Suicide is the 15th largest cause of death for all age groups in the world.[2]

The World Health Organization (WHO) recognizes suicide as a public health priority. In the Mental Health Action Plan 2013-2020, member states have committed themselves to working towards the global target of reducing the suicide rate by 10% by 2020. The 2014 World Suicide Report "Preventing Suicide: A Global Imperative" aims to increase awareness of the public health significance of suicide and suicide attempts, thereby making suicide prevention a high priority on the public health agenda.[3]

International suicide rates fluctuate between 10 and 15 per 100,000. In some countries, such as Hungary and Korea, the rates reach 21 per 100,000.[4,5] In Brazil, the mortality rate due to suicide is considered comparatively low, at 5.8 deaths per 100,000 inhabitants, but it has been increasing among young adults, particularly among males.[5] Supposedly, social determinants such as decreased income inequality and increased income per capita may have positive associations with decreased suicide rates.[6] A previous study showed that Rio Grande do Sul has the highest suicide rate among Brazilian states, and it has been suggested that ethnicity, culture, social crises and even the local climate might be factors influencing this.[7]

The suicide rate in the United States (US) is 12.1 deaths per 100,000 inhabitants, and this is the 10th largest general cause of death for all ages.[8] Approximately 110 Americans die by suicide every day, which means one death every 12.3 minutes, and over 41,000 lives every year.[8] These numbers represent only the successful suicides: the numbers of suicide attempts are projected to be 20 times higher than this.[9] Moreover, for every death by suicide there are at least six close individuals whose lives are emotionally, socially and economically severely affected. To increase knowledge of suicide risk factors in specific contexts, effective prevention strategies need to be adopted.[6]

Therefore, the objective of the present study was to ascertain whether income inequality, through the Gini coefficient, is associated with mortality due to suicide, specifically by contrasting Brazil and the US. It was hypothesized that reduction of income inequality would be accompanied by reduction of suicide rates, as suggested in the literature.[6] The Gini coefficient (World Bank) was selected particularly because it measures the extent to which the distribution of income (or, in some cases, expenditure on consumption) among individuals or households within an economy deviates from perfectly equal distribution.

OBJECTIVE

This study analyzed and compared the association between the Gini coefficient and suicide death rates over a 14-year period (2000-2013) in Brazil and in the US. It hypothesized that reduction of income inequality would be accompanied by reduction of suicide rates.

METHODS

This was an exploratory, descriptive and retrospective quantitative study. Brazilian mortality and suicide data from 2000 to 2013 were obtained from the Mortality Information System database (Sistema de Informações sobre Mortalidade, SIM) of the Information Technology Department of the Brazilian National Health System (Departamento de Informática do Sistema Único de Saúde, DATASUS), at <http://www2.datasus.gov.br/DATASUS/index.php>, which was accessed on April 22, 2016. Data on the estimated populations were obtained from the Brazilian Institute for Geography and Statistics (Instituto Brasileiro de Geografia e Estatística, IBGE), at <http://www.sidra.ibge.gov.br/cd/cd2010Serie.asp>, which was accessed on April 22, 2016. The US data were obtained from the Disease Control and Prevention platform (CDC-WONDER), at <http://wonder.cdc.gov/cmf-icd10-archive2013.html>, which was accessed on April 22, 2016, covering the same period of time. Suicide mortality rates in Brazil and the US were analyzed. Gini coefficient data were obtained from the World Bank estimates, at <http://data.worldbank.org/indicator/SI.POV.GINI>, which was accessed on April 22, 2016.

The data were tabulated using Microsoft Excel spreadsheets, which were used to make time series comparisons and evaluations of the data. Subsequently, the figures were plotted. Data covering the period from 2000 (when the new the International Classification of Disease, ICD, 10th revision started to be widely used in the US to represent the causes of death) to 2013 were analyzed, using the following indicators: Gini coefficient variation, number of suicide deaths and number of suicides according to age. Total suicide rates were calculated by dividing the absolute number of deaths due to suicide by the total population of that same year, at the same age, and multiplied by 100,000. Age-adjusted suicide rates were calculated using the direct method, which allowed comparison of rates between the US and Brazil. Adjustment was accomplished by multiplying the age-specific suicide rates by age-specific weights. The age-specific total population of the US was used as a standard population for the Brazilian adjusted rate.

RESULTS

The suicide rate in the US increased by roughly 25%, from 10.4 to 13.0 per 100,000 inhabitants, between 2000 and 2013. In Brazil, the rate of increase after age adjustment in relation to the US population was approximately 22.4%, from 4.7 to 5.7 deaths per 100,000 inhabitants over the same period of time. **Table 1** summarizes the populations of Brazil and the US and the numbers of suicides. The last two columns show calculations of suicide rates for each country over the years and the ratio of suicides between the two countries, after age adjustment in relation to the US population. The values in these last two columns grew slightly over the period of time analyzed. **Figure 1** shows how the Gini coefficients rapidly converged over time, mainly due to the reduction of inequality in Brazil. **Figure 2** shows the age-adjusted ratio of suicides, contrasted with the population growth of the two countries. It can be seen that the numbers of suicides, especially in the US, have been growing in large steps since 2006, both as crude numbers and also as rates.

Table 1. Total number of suicides, population and age-adjusted suicide rate per 100,000 inhabitants and ratio between the countries, over the study period

Year	Suicides (United States)	Suicides (Brazil)	Population (United States)	Population (Brazil)	Suicide rate (United States)	Age-adjusted suicide rate (Brazil)	United States/ Brazil ratio
2000	29,319	6,789	281,421,906	173,448,346	10.4	4.7	2.2
2001	30,545	7,738	284,968,955	175,885,229	10.7	5.2	2.1
2002	31,595	7,726	287,625,193	178,276,128	11.0	5.0	2.2
2003	31,422	7,861	290,107,933	180,619,108	10.8	5.0	2.2
2004	32,363	8,017	292,805,298	182,911,487	11.1	5.0	2.2
2005	32,559	8,550	295,516,599	185,150,806	11.0	5.3	2.1
2006	33,200	8,639	298,379,912	187,335,137	11.1	5.2	2.1
2007	34,529	8,868	301,231,207	189,462,755	11.5	5.3	2.2
2008	35,969	9,328	304,093,966	191,532,439	11.8	5.5	2.2
2009	36,837	9,374	306,771,529	193,543,969	12.0	5.4	2.2
2010	38,307	9,448	308,745,538	195,497,797	12.4	5.3	2.3
2011	39,442	9,852	311,591,917	197,397,018	12.7	5.5	2.3
2012	40,531	10,321	313,914,040	199,242,462	12.9	5.7	2.3
2013	41,060	10,533	316,128,839	201,032,714	13.0	5.7	2.3

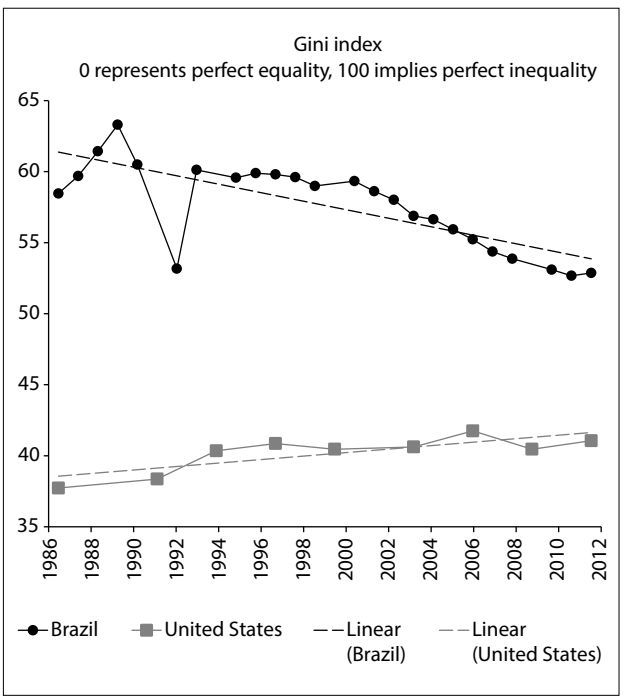

Figure 1. Gini index in Brazil and the United States over the years.

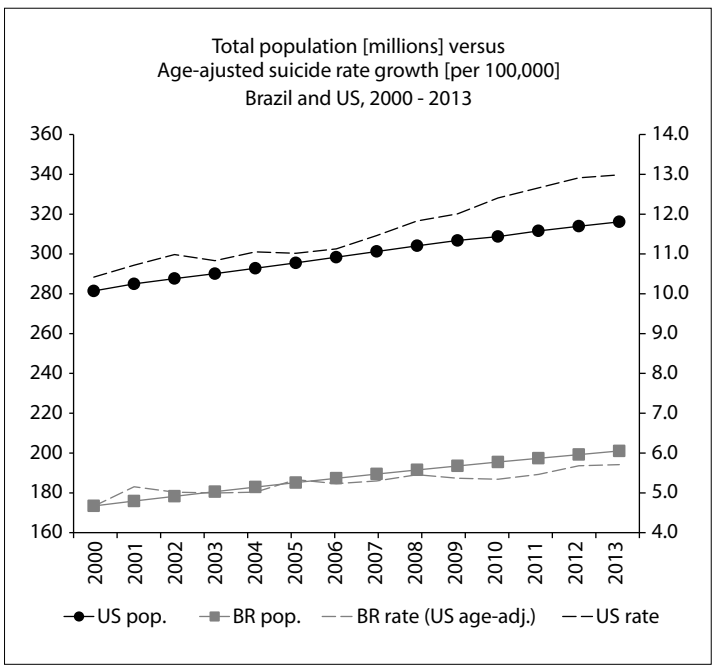

Figure 2. Total population (millions) versus age-adjusted suicide rate growth (per 100,000).

The distributions of deaths due to suicide in Brazil and the US according to age groups over these 14 years are presented in **Figures 3** and **4** respectively. A peak at the age range of 25-34 years can be observed in the Brazilian graph, specifically in 2010. This differed from the US, where more suicides occurred at older age ranges, with a peak that moved to older groups: this peak was in the 35-44 group in 2000 and transferred to the 45-54 group over the years.

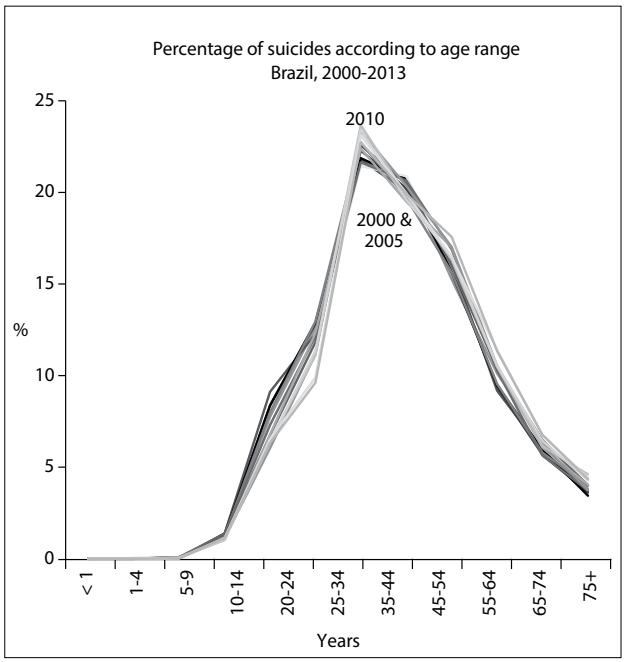

Figure 3. Percentage of suicides distributed according to age range, Brazil, 2000-2013.

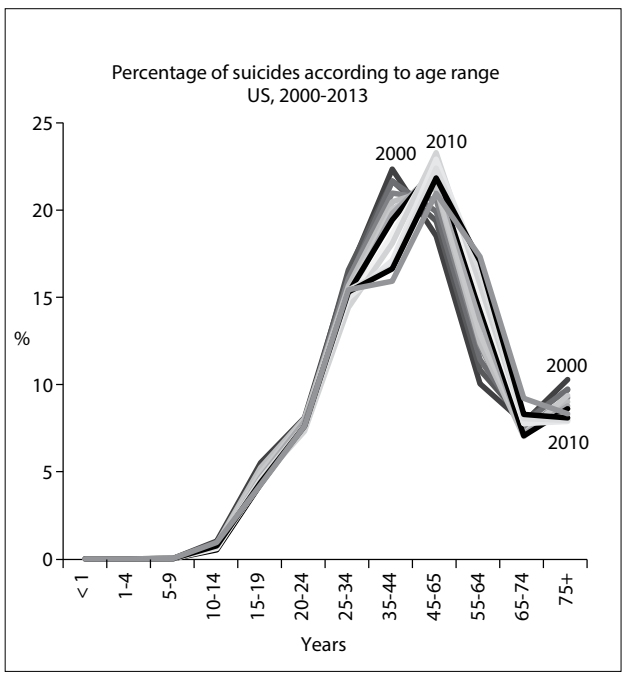

Figure 4. Percentage of suicides distributed according to age range, United States, 2000-2013.

DISCUSSION

Brazil is classified as an emerging country, while the US is a developed country. Although the relevance of comparing the Gini coefficient between Brazil and the US may not appear to be scientifically well-founded, this study took into consideration the fact that these countries have converging Gini coefficients, and thus it was hypothesized that some convergence in the numbers of suicides might also be observed. **Figure 1** shows how the two countries' Gini coefficients have been rapidly converging over time, especially because of Brazil's decreasing inequality, given the significant economic and social changes that have mostly taken place since 1994, with solidification of the democratic regime and attainment of monetary and fiscal equilibrium. Brazil has had superior development due to its thriving economy, which has been reflected in continuous social change. On the other hand, over the period considered, the US faced a serious economic crisis with high unemployment indicators, possibly affecting certain social groups more than others.

Shikida et al.[10] analyzed how economic variables influence suicide rates, according to Brazilian states. These variables are particularly important among the causes of suicides because they take into account the so-called "contagion effect" that has been described in the literature. In this phenomenon, suicidal behavior triggered by one individual can affect the behavior of other individuals who are living under the same psychological and socioeconomic conditions. These authors established that spatial dependence also seems to characterize suicide data distribution, such that it occurs in all directions, but is inversely related to geographical distance.

According to Durkheim,[11] the impact of socioeconomic changes, including industrialization, urbanization, secularization, population growth, social integration, migration and female participation in the labor force, has become central to the theories of suicide. In the view of the Durkheimian School, modernization leads to individualism and egoism in relation to the religious system, educational system, economic system and family system; and this erosion of social control reinforces the potential for suicide.[12-14] Some studies have found support for Durkheimian theories, such as the positive relationships between suicide and urbanization,[15] decreasing religiosity and increasing modernization,[16] population growth[17] and cultural variables relating to individualism.[18] Other findings, however, have posed challenges and revisions. Individual factors that affect the risk of suicide include mental disorders, genetics, drug misuse, psychological states and cultural, family and social situations, and some of these frequently coexist.[5-7] A correlation between suicidal intent and lethality has also been reported in the literature.[19]

Socioeconomic problems such as unemployment, poverty, homelessness and discrimination may also trigger suicidal behavior.[5,20] Suicide is an individual act, although it occurs within the

context of a given society, and certain sociodemographic factors, such as gender, age, migration, housing, marital state and occupation, among others, may be associated with it. Brazil's rapid decline in fertility since the 1960s is the main factor behind the country's slowing population growth rate, aging population and fast-paced demographic transition.[21] Consequently, protective factors may have been affected, such as: pregnancy, sense of responsibility towards the family and presence of children in the family.[20] It is believed that these factors can protect individuals from suicidal behavior, although there is still no scientific evidence to corroborate this assumption.

In most countries, males commit suicide more frequently than females, but the male/female ratio varies from country to country.[5,19,22] Relationships (family and friends) appear to be protective while separation and living alone increase the risk of suicide.[5,20] However, these results are not uniform.[19] The distributions of the deaths due to suicide in Brazil and the US according to age groups over the 14 years studied are shown in **Figures 3** and **4**, respectively. A peak at the age range of 25-34 years can be observed in the Brazilian graph, in 2010. This differed from the US, where more suicides occurred at older age ranges, with a peak that moved to older groups: this peak was in the 35-44 group in 2000, and transferred to the 45-54 group over the years. According to WHO,[5] young people are among those most affected but, as found in this study, the numbers may differ between countries.

In Western countries, the typical male-to-female gender ratio for suicide is high: between 2:1 and 4:1.[5] Data from other studies[23,24] confirm that male suicide rates in Brazil and the US are high. Some of the assumed reasons explaining this difference are: higher alcohol abuse among men; men choosing suicide methods of higher lethality; and women coping better with mental illness and thus seeking psychiatric services earlier than men.[23] Asian countries shows a more balanced ratio, while China is the only country in the world where the suicide rate among women is higher than among men. Social and cultural factors may provide explanations for China's high female suicide rates.[25,26]

Santa Catarina and Rio Grande do Sul are Brazilian states that stand out regarding socioeconomic development rates. However, they present critical suicide rates, above the Brazilian average.[7,27] Studies have shown that a direct relationship between economic development and suicide rates cannot be easily established. However, Machado et al.[6] and Kim[28] concluded that inequality is an important determinant of suicide, both in Brazil and in the US. These studies found linkages between state-level income inequality and the risks of dying due to suicide. Future research should address smaller demographic areas and compare indexes other than the Gini, or even aggregate them with additional healthcare system data measurements, and contrast the combination of such data with suicides and homicides.

It is important to bear in mind that the main racial groups in the US are classified merely as black and white, whereas in Brazil, in addition to these two groups, large numbers of people are grouped as *pardo* (brown/mulatto). Thus, the race categorizations of these two countries are not directly comparable. However, independently of each other, they respect the current census groupings and it is noteworthy that, internally, there are important differences between the rates that can be explored in future research.

Another limitation that it is important to highlight relates to the locations where suicides occurred. It has been suggested in the literature[29-31] that differences between rural and urban areas are significant. Further studies using supplementary sociodemographic variables could be conducted to cover these possibilities. Bando et al.[23] observed that in addition to the higher risk of suicide among singles, divorcees and widowers, compared with married people, foreigners were also at higher risk of suicide. Immigrant refugees are more likely to present serious mental disorders and, thus, commit suicide. Social disadvantage is another putative reason for explaining the higher risk of suicide among immigrants.[23] Lester[32] identified associations between quality of life and rates of personal violence that were only valid for some groups: in particular, for whites and blacks in the US. Prevention programs and strategies need to take these factors into account. Epidemiological analysis is an important tool for identifying population subgroups that are at increased risk of suicide, thus helping to develop prevention and sentinel strategies for high-risk groups.

Common sense suggests that people should become happier as their conditions of life improve. If poverty and forms of oppression such as sexism and racism could be eliminated, if the environment could be cleaned up, and if education and cultural offerings for people could be improved, then society ought to be considerably happier. Unfortunately, one sociological theory predicts the opposite of this. Henry and Short[33] argued that if people have clear external sources that they can blame for failures and unhappiness, then they will feel angry and be outwardly aggressive. In contrast, if there is no clear external source of blame, then people hold themselves responsible. In this case, individuals are more likely to feel depressed and culpable, thus increasing the chance that they might kill themselves. There is evidence to support this idea. In both the United States and South Africa, for example, the oppressed (blacks) have higher homicide rates, and the oppressors (whites) have higher suicide rates.[32] Lester[34] found that the states in the US with the highest quality of life (rated on a variety of aspects) had the highest suicide rates and the lowest homicide rates. Similarly, in a study on various countries, Lester[35] found that countries with the highest quality of life had the highest suicide rates and the lowest homicide rates. It appears that if life is better, suicide becomes more common and homicide less prevalent.

While the Gini coefficients of Brazil and the US are converging over time, suicide rates are simply growing in both countries (**Figure 2**). Conversely, it seems plausible to extend the sociological theory presented by Henry and Short[33] to make comparisons between the low suicide rates in Brazil and the higher rates in the US.

CONCLUSION

Based on the fact that Brazil and the US have converging Gini coefficients, with reduction of inequality in Brazil and slightly increased inequality in the US, this study hypothesized that this reduction of income inequality would extend to reduction of suicide rates. However, despite convergent Gini coefficients, suicide rates are growing in both countries. The supposition that reduction of income inequality would be accompanied by reduction of suicide rates was not verified.

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The role of dietary fatty acid intake in inflammatory gene expression: a critical review

O papel da ingestão dos ácidos graxos da dieta na expressão de genes inflamatórios: uma revisão crítica

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KEY WORDS:
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Inflammation.
Dietary fats, unsaturated.

PALAVRAS-CHAVE
Gorduras na dieta.
Ácidos graxos.
Expressão gênica.
Inflamação.
Gorduras insaturadas na dieta.

ABSTRACT

CONTEXT AND OBJECTIVE: Diet is an important modifiable factor involved in obesity-induced inflammation. We reviewed clinical trials that assessed the effect of consumption of different fatty acids on the expression of inflammation-related genes, such as cytokines, adipokines, chemokines and transcription factors.

DESIGN AND SETTING: Narrative review study conducted at a research center.

METHODS: This was a review on the effect of fat intake on inflammatory gene expression in humans.

RESULTS: Consumption of saturated fatty acids (SFAs) was related to postprandial upregulation of genes associated with pro-inflammatory pathways in peripheral blood mononuclear cells (PBMCs), in comparison with monounsaturated fatty acid (MUFA) or polyunsaturated fatty acid (PUFA) intake. In addition, acute intake of a high-SFA meal also induced a postprandial pro-inflammatory response for several inflammatory genes in subcutaneous adipose tissue. Both high-MUFA and high-PUFA diets showed anti-inflammatory profiles, or at least a less pronounced pro-inflammatory response than did SFA consumption. However, the results concerning the best substitute for SFAs were divergent because of the large variability in doses of MUFA (20% to 72% of energy intake) and n3 PUFA (0.4 g to 23.7% of energy intake) used in interventions.

CONCLUSIONS: The lipid profile of the diet can modulate the genes relating to postprandial and long-term inflammation in PBMCs and adipose tissue. Identifying the optimal fat profile for inflammatory control may be a promising approach for treating chronic diseases such as obesity.

RESUMO

CONTEXTO E OBJETIVO: A dieta é um importante fator modificável envolvido na inflamação induzida pela obesidade. Nós revisamos ensaios clínicos que avaliaram o efeito do consumo de diferentes ácidos graxos sobre a expressão de genes relacionados com a inflamação, tais como citocinas, adipocitocinas, quimiocinas e fatores de transcrição.

TIPO DE ESTUDO E LOCAL: Estudo de revisão narrativa realizado em um centro de pesquisa.

MÉTODOS: Revisão do efeito da ingestão de gordura sobre a expressão de genes envolvidos com inflamação em seres humanos.

RESULTADOS: O consumo do ácido graxo saturado (AGS) foi relacionado com a regulação favorável pós-prandial de genes associados com vias pró-inflamatórias nas células mononucleares de sangue periférico (CMSP), em comparação com a ingestão do ácido graxo monoinsaturado (AGMI) ou do ácido graxo poli-insaturado (AGPI). Além disso, o consumo agudo de uma dieta com alto conteúdo de AGS também induziu uma resposta pró-inflamatória pós-prandial para vários genes da inflamação no tecido adiposo subcutâneo. Ambas as dietas com alto conteúdo de AGMI e AGPI apresentaram perfil anti-inflamatório ou, pelo menos, menor resposta pró-inflamatória em relação ao consumo de AGS. Contudo, os resultados são controversos acerca do melhor substituto para o AGS, devido à grande variabilidade na dose de AGMI (20% a 72% da ingestão energética) e AGPI n3 (0,4 g para 23,7% da ingestão energética) utilizados nos estudos de intervenção.

CONCLUSÕES: O perfil lipídico da dieta pode modular os genes relacionados com inflamação pós-prandial e a longo prazo em CMSP e no tecido adiposo. Identificar o perfil lipídico ideal no controle inflamatório pode ser uma abordagem promissora para o tratamento de doenças crônicas como a obesidade.

INTRODUCTION

Inflammation is a physiological response triggered by infection and injury that has the purposes of eliminating irritating agents and accelerating tissue regeneration.[1,2] In this process, several inflammatory mediators are released, including cell adhesion molecules, cytokines, chemokines and other inflammatory agents (e.g. nitrogen and reactive oxygen species).[3] In order to maintain the homeostatic balance, a controlled inflammatory response is required. On the other hand, excessive or inappropriate inflammation leads to a pathological inflammatory status.[1] Increasingly, there is evidence to suggest that a deregulated inflammatory response plays a pivotal role in the onset and progression of atherosclerosis.[4]

Moreover, excessive adiposity and adiposity-related metabolic diseases (metabolic syndrome, diabetes and atherosclerosis) are attributed to a chronic state of low-grade inflammation. Therefore, diet-induced weight loss is an important factor for reducing pro-inflammatory markers.[5-7] In fact, besides lipid storage, fat cells are capable of producing and secreting chemoattractants such as monocyte chemotactic protein-1 (MCP-1) and pro-inflammatory mediators such as interleukins (IL), for instance IL-1β, IL-6 and tumor necrosis factor (TNF)-α, during adipose tissue expansion, thereby resulting in inflammatory and metabolic deregulation.[8]

Many environmental factors can contribute towards obesity and thus interfere with inflammatory expression, including diet.[9] Nutritional interventions can modulate inflammation, as demonstrated in studies based on a hypocaloric diet or on high consumption of fruits and vegetables. Both interventions have been shown to reduce the expression and synthesis of pro-inflammatory cytokines (IL-6 and TNF-α) and decrease other inflammatory markers such as C-reactive protein (CRP).[10-12] In addition, previous studies have confirmed that high-fat meals reduce leptin concentrations and increase the activation of inflammatory markers such as IL-6 during the postprandial phase.[13,14]

In fact, fatty acids can directly or indirectly modify immune and inflammatory responses. Current evidence suggests that a family of receptors involved in innate immunity, known as Toll-like receptors (TLRs), is connected with the inflammatory response relating to saturated fatty acid (SFA) intake. In this regard, it has been proposed that SFAs are nonmicrobial TLR agonists that promote inflammatory activation.[15] Studies have shown that the SFA lauric acid stimulates pro-inflammatory expression by TLR2 and TLR4, thereby mediating nuclear factor kappa B (NF-κB) and cyclooxygenase-2 activation and expression. In contrast, consumption of fish oil rich in n3 polyunsaturated fatty acid (PUFA) inhibits the TLR4-induced signaling pathways and target gene expression.[16,17] Moreover, SFA intake is known to cause lipemia that is more pronounced than the lipemia due to monounsaturated fatty acids (MUFA) and PUFA, which can lead to a higher pro-inflammatory state associated with SFA consumption.[18] Additionally, SFA palmitate and stearate acids can trigger IL-1β secretion through mechanisms involving NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome activation.[19]

Recently, it was proposed that GPR120 (G protein-coupled receptor 120) mediates the anti-inflammatory effects of n3 PUFA.[20,21] Dietary n3 PUFA has been correlated with inhibition of TLR-induced signaling pathways and target gene expression, probably through disruption of translocation of TLR4 into a lipid raft.[16,17] In combination, these mechanisms can potentially inhibit the signaling pathways that lead to NF-κB activation, thus resulting in downregulation of pro-inflammatory responses through n3 PUFA intake.

OBJECTIVE

Given the above, we aimed to summarize and discuss recent evidence about the effect of consumption of different fatty acids in humans, on inflammation-related gene expression, as evaluated through clinical studies.

METHODS

This was a narrative review of the English-language literature on the effects of fat intake on inflammatory gene expression in humans. It evaluated studies indexed in the Cochrane Library, LILACS and PubMed databases between the time of database inception and March 2016 (**Table 1**). We included original studies that reported on clinical trials on men or women (not pregnant, not in lactation and not in the postmenopausal period) who were not athletes, not undergoing hormonal treatment, not dependent on alcohol or drugs and not suffering from chronic illnesses (such as hepatic, renal, thyroid or cardiac dysfunction) or acute inflammatory processes. Since the objective was to evaluate the effect of fatty acid consumption among humans, only clinical trials were included, given that these are considered to be the mainstay design for causal inferences.

All the papers were checked according to their titles and abstracts (screening). Full papers were obtained from journals available on the CAPES Foundation (Ministry of Education, Brazil) website. Unavailable articles were requested from their authors. Articles presenting potentially relevant studies were read and analyzed to assess the inclusion criteria.

We excluded articles that consisted of *in vitro* or animal studies, articles in which the participants' characteristics did not match those mentioned above, poster session abstracts, review articles and other types of publications (non-standard

Table 1. Database search results

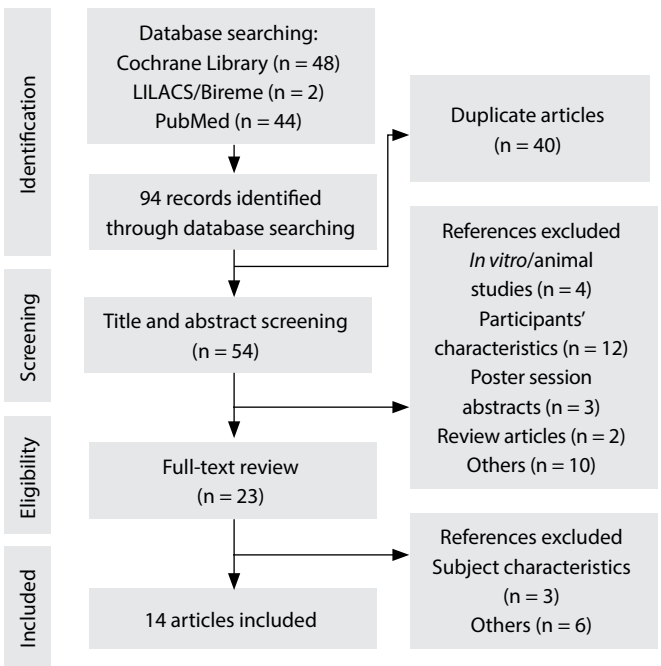
Database	Search	Filters		Results
Cochrane Library	("gene expression" OR "RNA" OR "mRNA" OR "gene") AND (("saturated fatty acid" OR "saturated fatty acids" OR "SFA" OR "SFAs") OR ("monounsaturated fatty acid" OR "monounsaturated fatty acids" OR "MUFA" OR "MUFAs") OR ("polyunsaturated fatty acid" OR "polyunsaturated fatty acids" OR "PUFA" OR "PUFAs")) AND ("inflammation" OR "inflammatory" OR "proinflammatory")	Title, abstract, keywords in trials	48 articles	2 animal/*in vitro* studies 3 poster session abstracts 43 clinical trials
LILACS	("gene expression" OR "RNA" OR "mRNA" OR "gene") AND (("saturated fatty acid" OR "saturated fatty acids" OR "SFA" OR "SFAs") OR ("monounsaturated fatty acid" OR "monounsaturated fatty acids" OR "MUFA" OR "MUFAs") OR ("polyunsaturated fatty acid" OR "polyunsaturated fatty acids" OR "PUFA" OR "PUFAs")) AND ("inflammation" OR "inflammatory" OR "proinflammatory")	No filter	2 articles	2 reviews
PubMed	("gene expression" OR "RNA" OR "mRNA" OR "gene") AND (("saturated fatty acid" OR "saturated fatty acids" OR "SFA" OR "SFAs") OR ("monounsaturated fatty acid" OR "monounsaturated fatty acids" OR "MUFA" OR "MUFAs") OR ("polyunsaturated fatty acid" OR "polyunsaturated fatty acids" OR "PUFA" OR "PUFAs")) AND ("inflammation" OR "inflammatory" OR "proinflammatory")	Title/abstract in clinical trials on humans	44 articles	4 animal/*in vitro* studies 40 clinical trials

dietary interventions; studies on drug therapy; studies without any analysis on inflammation; dietary trial interventions on fatty acid intake along with vitamin or mineral supplementation; studies on heated oils; or studies without any clear differentiation between the total polyunsaturated, monounsaturated and saturated fatty acid content used to compare the interventions). The flowchart for the study selection process is described in **Figure 1**. Other papers were used for contextualization and discussion.

RESULTS

We identified 14 studies that investigated the effect of fatty acid intake on inflammatory gene expression (**Table 2**). Six of these studies had a postprandial design in which an acute inflammatory response to a high-fat meal consumed on a single day was evaluated[22,23] or consisted of a postprandial fat challenge, reflecting fat composition similar to that of a dietary intervention conducted for at least four weeks afterwards.[24-27] Postprandial is a term that was introduced in 1997 and refers to "the time frame after a meal or food intake".[28] Seven studies assessed the inflammatory response after long-term consumption (minimum of 8 weeks).[29-35] Lastly, one study determined both the postprandial and the long-term response to the same dietary intervention.[36] In order to assist comparisons between the studies, dietary interventions were compared according to fat content source (SFA, MUFA or PUFA) and its respective proportion of total energy intake (E%).

Inflammatory genes were analyzed in duodenal tissue, peripheral blood mononuclear cells (PBMCs), subcutaneous or visceral adipose tissue, skeletal muscle and whole blood,



We excluded references if they consisted of an *in vitro* or animal study; because of some characteristics of the participants (pregnancy, lactation, menopause, athlete, hormonal treatment, alcohol or drug dependence, chronic illness such as hepatic, renal, thyroid or cardiac dysfunction, or acute inflammation process); or if they were poster session abstracts, review articles or other types (non-standard dietary interventions; studies on drug therapy; studies without analysis on inflammation; dietary trial intervention with fatty acid intake along with vitamin or mineral supplementation; studies on heated oils; or studies without a clear difference between the total polyunsaturated, monounsaturated and saturated fatty acid content that was used to compare the interventions).

Figure 1. Literature search process.

Table 2. Summary of the studies selected

Year, authors	Subjects	Study design	Dietary intervention	Duration	Postprandial intervention	Inflammatory gene markers
2009, Bouwens et al.[29]	n = 111 M/F: 66/45 Age: 66-80 years	Clinical trial, randomized, double-blind, controlled, parallel	3 groups (N/A E% from fat): - High n3 PUFA (EPA/DHA): 1.8 g/day - Control (sunflower oil): 4.0 g/day	26 weeks	-	RNA microarray analysis (encoding 17,699 genes) in PBMCs, measured in fasting period at baseline and after the intervention period.
2009, van Dijk et al.[33]	n = 20 M/F: 10/10 Mean age: 45-60 years	Clinical trial, parallel	2 groups (N/A E% from fat): - SFA: 19 E% - MUFA: 20 E%	8 weeks	-	RNA microarray analysis (encoding 17,699 genes) in SAT, measured in fasting period at baseline and after the intervention period.
2009, Jiménez-Gómez et al.[24]	n = 20 M/F: 20/0 Mean age: N/A	Clinical trial, randomized, crossover, postprandial	3 groups: High fat (38 E% from fat): - SFA: 22 E% - MUFA: 24 E% Low fat (< 30% E% from fat): - n3 PUFA (ALA): 2 E%	4 weeks	Breakfast (60 E% from fat): - SFA: 35 E% - MUFA: 36 E% - n3 PUFA (ALA): 4 E%	TNF-α, IL-6 and MCP-1 in PBMCs, measured in fasting period and 3, 6 and 9 h after the breakfast.
2011, Meneses et al.[25]	n = 39 M/F: 14/25 Mean age: ~ 57 years	Clinical trial, randomized, controlled, parallel, postprandial	4 groups: High fat (38 E% from fat): - SFA: 16 E% - MUFA: 20 E% Low fat (28 E% from fat): - LFHCC n3: supplement with 1.24 g/day of n3 PUFA - LFHCC: 1.2 g/day supplement of control (sunflower oil)	12 weeks	Breakfast (65 E% from fat): - SFA: 38 E% - MUFA: 43 E% - LFHCC n3: supplement with 1.24 g of n3 PUFA - LFHCC: supplement with placebo capsules	p65, IκBα, IκBβ2, IL-6, MCP-1 and IL-1β in SAT, measured in fasting period and 4 h after the breakfast.
2011, Pietraszek et al.[23]	n = 34 M/F: 11/23 Mean age: ~ 50 years	Clinical trial, randomized, crossover, postprandial	Standard diet: 24 E% from fat	1 day	Breakfast (87 E% from fat): - SFA: 79 E%§ - MUFA: 72 E%	ADIPOR1, ADIPOR2, MCP-1, IL-1β, IL-6, IL-6R, CD16A, LEP, LEPR, RBP4, TLR4, TNF-α AND TNFRSF1A in muscle and SAT, measured in fasting period and 3 h 30 min after the breakfast. Also, ADIPQ was determined in SAT.
2011, Rudkowska et al.[34]	n = 16 M/F: 7/9 Mean age: 57 years	Clinical trial, randomized, crossover	2 groups: - n3 PUFA: 1.8 g/day - n3 PUFA FP: 1.8 g/day + fish protein	8 weeks	-	RNA microarray analysis (encoding 37,804 genes) in PBMCs, measured at baseline and after the intervention period.
2012, Camargo et al.[26]	n = 20 M/F: 10/10 Mean age: 67 years	Clinical trial, randomized, crossover, postprandial	3 groups: High fat (38 E% from fat): - SFA: 22 E% - MUFA: 24 E% Low fat (< 30% E% from fat): - n3 PUFA (ALA): 2 E%	4 weeks	Breakfast (60 E% from fat): - SFA: 35 E% - MUFA: 36 E% - n3 PUFA (ALA): 4 E%	p65, IκBα, MCP-1, MIF-1, MMP-9 and IL-6 in PBMCs, measured in fasting period and 1, 2 and 4 h after the breakfast.
2012, Cruz-Teno et al.[27]	n = 75 M/F: 28/47 Mean age: ~ 56 years	Clinical trial, randomized, controlled, parallel, postprandial	4 groups: High fat (38 E% from fat): - SFA: 16 E% - MUFA: 20 E% Low fat (28 E% from fat): - LFHCC n3: supplement with 1.24 g/day of n3 PUFA - LFHCC: 1.0 g/day supplement of control (sunflower oil)	12 weeks	Breakfast (65 E% from fat): - SFA: 38 E% - MUFA: 43 E% - LFHCC n3: supplement with 1.24 g of n3 PUFA - LFHCC: supplement with placebo capsules	TNF-α, IL-6, IκB-α, p65, MCP-1, MIF, MMP-9 in PBMCs, measured in fasting period and 2 and 4 h after the breakfast.

Continue...

Table 2. Continues

Year, authors	Subjects	Study design	Dietary intervention	Duration	Postprandial intervention	Inflammatory gene markers
2012, van Dijk et al.[30]	n = 49 M/F: 22/27 Mean age: ~ 55 years	Clinical trial, parallel	3 groups (37-40 E% from fat): - SFA: 19 E% - MUFA: 20% E% - MED*: 21 E% from MUFA	8 weeks	-	RNA microarray analysis (encoding 17,699 genes) in PBMCs, measured in fasting period at baseline and after the intervention period.
2012, van Dijk et al.[22]	n = 42 M/F: 42/0 Age: 50-70 years	Clinical trial, randomized, double-blind, crossover, postprandial	Low-fat meal: N/A	1 day	Shake (87 E% from fat): - SFA: 46.5 E% - MUFA: 72 E% - n3 PUFA (EPA/DHA): 23.7 E%	IL-1β, IL-8, MCP-1, NFkB1 and TNF-α in PBMCs, measured in fasting period and 2 and 4 h after the breakfast.
2012, Schmidt et al.[36]	n = 40 M/F: 40/0 Mean age: ~ 40 years	Clinical trial, randomized, double-blind, controlled, parallel, postprandial	2 groups: - n3 PUFA (EPA/DHA): 2.7 g/day - Control (n6 PUFA): 3.05 g/day (LA)	12 weeks	Consumption of capsules of the dietary intervention.	RNA microarray analyses from whole blood at baseline, after 1 and 12 weeks of supplementation (long-term effect). Also 4 h after intake of capsules (postprandial response).
2012, Itariu et al.[31]	n = 55 M/F: 46/9 Mean age: ~ 38 years	Clinical trial, randomized, controlled, parallel	2 groups (30 E% from fat): - n3 PUFA (EPA/DHA): 3.36 g/day - Control (butter): 5 g/day	8 weeks	-	MCP-1, MIP-1α, IL-6, ADIPOQ, HIF1A and TGF-β1, CD68, CD163, MRC1 and CD40 in SAT and VAT, after the intervention period.
2013, Kratz et al.[32]	n = 24 M/F: 8/16 Mean age: ~ 39 years	Clinical trial, randomized, single-blind, controlled, parallel	2 groups (~34 E% from fat): - n3 PUFA: 3.5 E% - Control: 0.5 E% from n3 PUFA	14 weeks	-	TNF-α, IL-6, ICAM-1, CD14, CD206, CD284, MCP-1 and SAA1 in SAT, collected at baseline and after the intervention period.
2013, Labonté et al.[35]	n = 12 M/F: 12/0 Mean age: 54 years	Clinical trial, randomized, double-blind, controlled, crossover	2 groups: - n3 PUFA (EPA/DHA): 3.0 g/day - Control: blend of corn and soybean oil	8 weeks	-	IL-6, IL-18, TNF-α in duodenal samples, collected in fasting period at the end of each intervention.

n = number of subjects; M/F = male/female; N/A = not available; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; n3 PUFA = n3 polyunsaturated fatty acids; LFHCC n3 = high complex carbohydrate supplemented with n3 PUFA; LFHCC = high complex carbohydrate supplemented with placebo; ALA = α-linolenic acid; LA = linoleic acid; E% = % of energy intake; PBMC = peripheral blood mononuclear cells; SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue; ADIPOR = adiponectin receptor; ADIPQ = adiponectin gene; CD14, CD163, CD16A, CD206, CD284, CD40, CD68 = macrophage markers; HIF1α = hypoxia-induced factor 1α; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; IκB = inhibitor of NF-κB; LEP = leptin; LEPR = leptin receptor; MCP-1 = monocyte chemoattractant protein-1; MIF = macrophage migration inhibitory factor; MIP-1 = macrophage inflammatory protein 1; MMP-9 = matrix metalloproteinase 9; MRC1 = mannose receptor C type 1; NFkB1 = nuclear factor kappa-B subunit 1; p65 = nuclear p65 protein; RBP4 = retinol binding protein 4; SAA1 = serum amyloid A1; TGF-β1 = transforming growth factor β1; TLR4 = Toll-like receptor 4; TNF-α = tumor necrosis factor alpha; TNFRSF1A = tumor necrosis factor receptor superfamily member 1A.

*Mediterranean (MED) diet components high in MUFA from extra-virgin olive oil and containing additional MED components (i.e. fatty fish, unrefined grain products, nuts, legumes and red wine).

§The SFA meal was high in coconut oil with 49 E% from medium-chain SFA (predominantly lauric acid) and 30 E% from long-chain SFA (predominantly myristic acid).

mostly using the polymerase chain reaction (PCR), or using a microarray analysis methodology. The main inflammatory genes screened were those that promote expression of adipokines, chemokines, cytokines and transcription factors. Hence, before discussing the role of fatty acid intake in inflammatory gene expression, we firstly contextualize the main markers that have been found in various studies.

Adipokines

Adipose tissue is an active organ involved not only in energy storage control, but also in regulation of complex metabolic and endocrine functions. In this context, adipose tissue releases cytokines and other bioactive mediators. Adiponectin and leptin are known as true adipokines, and are the major adipocyte proteins produced mainly by adipose tissue.[37,38] In particular, adiponectin is an

anti-inflammatory cytokine that might be able to induce production of other anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1RA). At the same time, it may suppress pro-inflammatory cytokine production of interferon (IFN)-γ[37] and may also have a negative correlation with CRP, the systemic inflammatory marker.[39] Moreover, adiponectin can exhibit atheroprotective effects, through attenuating chronic inflammation in vascular walls.[40] On the other hand, leptin correlates directly with body fat mass and adipocyte size, and has a role as a pro-inflammatory cytokine. Leptin stimulates production of several inflammatory mediators such as IL-1, IL-6, IL-12 and TNF.[37] In addition, leptin has been correlated with several obesity-associated diseases such as cardiovascular diseases and diabetes.[41]

Chemokines

Chemokines form a family of small proteins that are secreted in response to signals such as pro-inflammatory cytokines. They play an important role in selectively inducing chemotaxis and chemokinesis of leukocytes.[42] MCP-1, also referred to as chemokine ligand 2 (CCL2), is a potent chemoattractant of monocytes and macrophages to inflammation areas, expressed mainly by inflammatory cells and endothelial cells.[43,44] Macrophage inflammatory protein 1 (MIP-1), or chemokine ligand 3 (CCL3), is another potent chemoattractant of immune cells, particularly macrophages, to inflammatory sites.[43]

Leukocyte extravasation into tissues requires not only chemokines but also matrix-degrading enzymes, in particular matrix metalloproteinases (MMPs). MMP-9, for instance, performs an important role in immune cell functioning and in pathophysiological conditions that involve inflammatory processes. In addition, MMP-9 levels increase in cases of cardiovascular diseases, including hypertension, atherosclerosis and myocardial infarction.[45,46]

Cytokines

Cytokines are proteins that act as intracellular mediators. They play an important role in cell communication and regulation of the immune system. Unlike classical hormones, they are produced by different tissues and cell types rather than by specialized glands. They bind to their cognate receptors on target cells and activate or inhibit cellular functions in a paracrine or autocrine manner.[47,48]

IL-1 was the first interleukin to be identified. It is produced by numerous innate immune cells including monocytes, macrophages and dendritic cells.[49] IL-1 is a key pro-inflammatory mediator involved in hosting responses to pathogens and inflammation. Its synthesis is induced by other inflammatory cytokines such as TNF-α, IFN-γ and IL-2.[50] IL-1 also induces production of pro-inflammatory cytokines, including IL-6.[51] Growing evidence links this cytokine to chronic diseases such as type 2 diabetes and obesity. Furthermore, IL-1 is also related to atherosclerosis development.[52]

IL-6 is known as an inflammatory cytokine secreted mainly (10-35%) by adipose tissue, and also by skeletal muscle and liver.[53] It is an acute-response mediator and consequently increases the plasma concentrations of acute-phase proteins, such as CRP and serum amyloid A.[54] Greater IL-6 concentration is correlated with elevated cardiovascular risk. This can be explained by its correlations with increased adiposity, expressed in terms of body mass index, waist circumference, visceral fat, total body fat and increased risk of insulin resistance.[2,6,55] Furthermore, high consumption of fruit and vegetables followed by good adherence to a calorie-restricted diet based on a Mediterranean dietary pattern can reduce expression and synthesis of this inflammatory marker, as described previously.[10,12]

IL-8 is a pro-inflammatory cytokine produced mainly by monocytes and macrophages. It is responsible for bringing immune cells to sites of inflammation and retaining them there. In addition, IL-8 promotes activation of monocytes and neutrophils. It has been shown to have a potential role in cardiovascular diseases, in particular atherosclerosis.[56]

TNF-α, also known as cachectin, is a strong pro-inflammatory cytokine produced mainly by monocytes and macrophages, via activation of MAPK (mitogen-activated protein kinase) and NF-κB signaling pathways. This process results in release of inflammatory genes and other inflammatory cytokines such as IL-1β and IL-6.[57,58] TNF-α has also been implicated in increased cardiovascular risk, and it is central to the pathophysiology of cancer and chronic inflammatory conditions, including inflammatory bowel diseases, rheumatoid arthritis and psoriasis.[57,59,60] Similarly to IL-6, a reduced-calorie diet and consumption of fruits and vegetables can reduce synthesis and expression of TNF-α.[10,12]

Transcription factors

Nuclear p65 protein is a subunit of NF-κB transcription complex, which plays a crucial role in inflammatory and immune responses. NF-κB is a homo or heterodimer composed of Rel proteins: p65 (RelA), p50 (NFKB1), p52 (NFKB2), c-Rel and RelB.[61] NF-κB is classically activated by pro-inflammatory cytokines such as TNF-α and alternatively by cytokines such as lymphotoxin β. An atypical pathway triggered by DNA damage[62] may also do the activation. Altered NF-kB activation has been demonstrated in tumor development and chronic inflammatory diseases.[62] Interestingly, high intake of fruits and vegetables is inversely associated with mRNA p65 expression in PBMCs of healthy adults.[10]

NF-κB signaling is controlled through NF-κB inhibitors (IκB). This is a family of proteins that can bind NF-κB dimers in the cytoplasm and nucleus, thereby inhibiting the NF-κB transcriptional

response.[63] Certain stimuli result in phosphorylation, and subsequent proteasome-mediated degradation of IkB proteins allows the unbound NF-κB dimers to translocate to the nucleus, thereby regulating the expression of target genes.[62]

Fatty acid intake in inflammatory gene expression

According to the studies reviewed here, the postprandial period resulted in a pro-inflammatory response regarding PBMC gene expression, linked to SFA consumption. In healthy subjects, SFA intake (35 E%) from animal sources resulted in an increased postprandial pro-inflammatory response in PBMCs for TNF-α expression, in comparison with MUFA (36 E%) and n3 PUFA (4 E%) breakfasts consisting mainly of extra-virgin olive oil and fats of vegetable origin (walnuts), respectively. In addition, mRNA IL-6 postprandial expression was higher after the high-SFA meal than after the n3 PUFA meal. However, the increased gene expression did not change the concentration of the inflammatory cytokines.[24] Thus, the length of postprandial assessment time may have been sufficient to detect differences in expression but not in translation of cytokines in PBMCs. These differences between inflammatory gene response and synthesis/secretion of inflammatory markers may have been due to transcriptional and translational process that do not occur simultaneously.[64] Moreover, several translational and post-translational regulatory mechanisms, including miRNA, may be involved and thus may affect the production and release of cytokines,[65] which may also not occur concurrently in the cell and extracellular tissues. In addition, discrepancies can be found between cytokine concentrations and their mRNA expression, probably due to potential confounding factors such as gender, physical activity, smoking and body mass index.[5] However, PBMCs are widely used for determining inflammatory gene expression, given the fact that they are accessible cells. Furthermore, their use is cost-effective and they provide a less invasive alternative to biopsy measurements.[66]

Elderly subjects exhibited a pro-inflammatory response in PBMCs relating to high SFA consumption (35 E%), with higher postprandial inflammatory expression of p65 and MCP-1 genes, compared with MUFA (36 E%) mainly from virgin olive oil. They also presented higher mRNA p65, in comparison with an intervention comprising PUFA (4 E%) from plant origin (walnuts). Additionally, SFA showed downregulated expression of anti-inflammatory genes (IκBα), compared with MUFA, and increased plasma concentration of MCP-1 pro-inflammatory cytokines.[26]

In metabolic syndrome patients, SFA consumption (38 E%) was associated with upregulation of pro-inflammatory genes (MMP-9 and TNF-α) and downregulation of anti-inflammatory genes (IκBα) in a postprandial state, compared with MUFA (43 E%), in PBMCs. Moreover, higher MCP-1 plasma concentration was observed in SFA consumption, compared with MUFA and n3 PUFA (1.24 g). Regardless of the type of ingested fat (SFA, MUFA or n3 PUFA), the postprandial state was associated with increased expression of IL-6, MMP-9 and TNF-α pro-inflammatory genes, as well as higher IL-6 plasma concentrations,[27] thus suggesting that a greater inflammatory response would be expected in these subjects. In fact, non-dietary factors, such as obesity and type 2 diabetes, can increase the extent of fatty acid postprandial inflammatory response.[67] However, the source of fats was not mentioned in the study and it is known that dietary fat sources differ in more aspects than only their fatty acid profiles.

In this regard, olive oil is well known for its potential health-promoting properties, which are due to the presence of high levels of MUFA and other valuable minor components such as phenolics, phytosterols, tocopherols, carotenoids, chlorophyll and squalene.[68] These natural compounds with antioxidant and other potentially important types of bioactivity have a beneficial impact on inflammatory markers.[67] Thus, they represent an important confounding factor in assessing the effect of dietary fat intake on the inflammatory response.

Controversially, high-SFA acute intake (46.5 E%) mainly from plant origin (palm oil) was associated with reduced postprandial inflammatory response regarding PBMC gene expression (MCP-1 and IL-8), compared with MUFA (72 E%) from high-oleic acid sunflower oil and n3 PUFA (23.7 E%) from fish oil interventions.[22] Palm oil use is subject to debate with regard to potential unhealthy effects, because of its high palmitic acid content. An increased inflammatory response (IL-6) relating to a palmitic oil-enriched diet in mice and a similar effect from palmitic acid *in vitro* was shown in one study. However, apart from SFAs, which are mostly from palmitic acid, this plant oil contains oleic and linoleic acids, which are MUFA and PUFA, respectively.[69] Unlike in other studies, a much higher amount of MUFA was used, in comparison with the SFA intervention. In addition, n3 PUFA intake was greater than in other studies.

However, in a long-term dietary trial on the inflammatory response in PBMCs, gene expression remained unchanged after eight weeks of intervention with SFAs (19 E%), among abdominally obese patients.[30] This result may be related to the lower amount of fat provided, in comparison with other interventions. Moreover, presence of the obese phenotype was correlated with a previous abnormal inflammatory profile.[30]

On the other hand, in subcutaneous adipose tissue among abdominally overweight subjects, investigation of a long-term SFA diet (19 E%) regarding the inflammatory response showed that upregulation of genes mainly relating to immune and inflammatory pathways occurred. At the same time, downregulation of anti-inflammatory genes and reduction of plasma adiponectin concentration were also observed.[33] Among healthy subjects, an acute

dietary intervention that was high in medium-chain SFAs (79 E%), i.e. rich in coconut oil, induced a postprandial pro-inflammatory response relating to several inflammatory genes in subcutaneous adipose tissue (CD16a, IL-1β, IL-6, IL-6R and TNF-α) and muscle tissue (MCP-1, IL-6R, CD16a, LEP, TLR4 and TNF-α). Additionally, plasma IL-6 concentration increased in response to medium-chain SFA consumption.[23] In this regard, SFA appears to be able to modulate gene expression in important sources of inflammatory markers, such as PBMCs and adipose tissue.

Regarding the effect of MUFA consumption on inflammatory gene expression in subcutaneous adipose tissue, acute MUFA intake (72 E%) containing macadamia nut oil induced a postprandial anti-inflammatory response (ADIPOQ) in healthy subjects. However, it also increased the pro-inflammatory gene expression (TNFRSF1A), but in a less pronounced manner than did SFA (79 E%) derived from coconut oil intake.[23] Moreover, a long-term MUFA (20 E%) dietary intervention, mainly in the form of refined olive oil, among abdominally obese subjects for eight weeks, also resulted in down-regulation or unchanged expression of pro-inflammatory genes in subcutaneous adipose tissue, compared with a SFA diet (19%).[33] These results indicate that MUFA can also exert a pro-inflammatory response, but only weakly, compared with SFA consumption. In fact, unlike SFAs, unsaturated fatty acids such as oleate acid were unable to activate the NLRP3 inflammasome and thereby stimulate IL-1β production.[19] However, other mechanisms may be involved in the inflammatory response mediated by MUFA intake, which can elicit a pro or anti-inflammatory response.

Among subjects at higher risk of type 2 diabetes, an acute postprandial intervention of MUFA breakfast (72 E%) containing macadamia nut oil showed that several inflammatory genes were upregulated in subcutaneous adipose tissue (MCP-1, IL-1β, IL-6, IL-6R, TNF-α and TNFRSF1A). However, healthy subjects showed upregulation of proinflammatory genes (TNFRSF1A) but also of anti-inflammatory ones such as ADIPOQ.[23] Metabolic syndrome patients also showed an increased postprandial response of inflammatory genes (p65, MCP-1, IL-6 and IL-1β) and anti-inflammatory genes (IκBα) in subcutaneous adipose tissue, regardless of the quality of dietary fat (SFA from animal fat, 38 E%; MUFA mainly from olive oil, 43 E%; or n3 PUFA, 1.24 g).[25] These results suggest that pro-inflammatory expression of adipose tissue would be expected among obesity-related diseases. This has been correlated with overproduction of pro-inflammatory adipocytokines. As mentioned earlier, obesity and type 2 diabetes can elicit a pronounced postprandial inflammatory response.[67]

Furthermore, the major characteristic of the Mediterranean diet is a high amount of MUFA (around 20 E%), mainly from olive oil intake.[70] The Mediterranean dietary pattern has been correlated with reduced cardiovascular morbidity and mortality.[70,71] This diet has been encouraged because of its relationship with an improved cardiovascular profile, including its favorable effect on blood pressure, insulin sensitivity, lipid profiles, lipoprotein particles, oxidative stress, carotid atherosclerosis and inflammation.[11,72] In a study investigating the effect of consumption of a diet rich in extra-virgin olive oil (MUFA; 21 E%) containing additional Mediterranean components (i.e. fatty fish, unrefined grain products, nuts, legumes and red wine), no effect was found on PBMC inflammatory genes. The same result was obtained from a MUFA intervention (20 E%) consisting of refined olive oil added to a Western-type diet. The Mediterranean diet reduced the plasma concentrations of pro-inflammatory proteins (IL-1β and MIP-1α) after an eight-week intervention, but this change did not significantly differ from interventions consisting of MUFA (20 E%) or SFA (19 E%).[30] Higher fruit and vegetable consumption has been correlated with lower plasma concentration of CRP and downregulation of pro-inflammatory genes (ICAM-1, IL1-R1, IL-6, TNF-α and NF-κB1) in PBMCs.[10]

In addition to high MUFA content, olive oil contains other minor biologically active components (e.g. polyphenols and carotenoids),[68] which have been shown to downregulate human genes (IFN-γ, Rho GTPase-activating protein 15 (ARHGAP15) and IL-7R) relating to the inflammatory process.[73] Thus, essential nutrients such as folate, vitamin C and magnesium, and other bioactive compounds (e.g. flavonoids and carotenoids) that can be found in a Mediterranean dietary pattern, along with olive oil, could be responsible for its anti-inflammatory properties, in addition to the MUFA content. Thus, the factors mentioned may not accurately reveal the role of MUFA diets in relation to the inflammatory response.

Polyunsaturated fatty acids (PUFA) such n-3 and n-6 fatty acids are essential nutrients for health. Recent studies have identified potential benefits from n3 PUFA consumption for a wide range of conditions, including enhancement of the lipid profile[74] and reduction of coronary heart disease events[75] and breast cancer risk.[76] Among severely obese patients (≥ 40 kg/m^2) who were scheduled to undergo elective bariatric surgery, n3 PUFA supplementation (3.36 g/day) over an eight-week period showed down-regulation of chemokine promoter genes (MCP-1, MIP-1α, HIF1A and CD40) and a tendency towards reducing IL-6 and increasing anti-inflammatory gene expression of adiponectin in subcutaneous tissue, but not in visceral adipose tissue, in comparison with the control group.[31] Additionally, dyslipidemic subjects exhibited higher numbers of downregulated pro-inflammatory genes after long-term supplementation with n3 PUFA (2.7 g/day) consisting of fish oil, over a 12-week period, in contrast with subjects with normal lipid levels. Moreover, n3 PUFA showed immune-modulatory and anti-inflammatory capability, through downregulating several pro-inflammatory genes and giving rise to balanced up and downregulation of anti-inflammatory genes, particularly regarding

dyslipidemic subjects.[36] Among elderly individuals, long-term n3 PUFA supplementation via fish oil (26 weeks) showed downregulation of inflammatory pathways in PBMC gene profiles, through 1.8 g/day of EPA/DHA.[29]

Thus, consumption of n3 PUFA seems to improve the inflammatory condition associated with metabolic disorders, in relation to obesity, including insulin resistance and hyperinsulinemia. However, consumption of 1.8 g/day of n3 PUFA via fish oil capsules did not affect inflammatory genes in PBMCs or inflammatory markers (CRP, IL-6 and TNF-α) in insulin-resistant subjects with a mean body mass index of 29.9 kg/m^2 (standard error of the mean, SEM, 0.9 kg/m^2), after eight weeks of supplementation.[34] In fact, studies have shown divergences regarding the beneficial effects relating to n3 PUFA consumption among individuals with type 2 diabetes mellitus, probably due to the genetic background associated with ethnicity, considering that Asian individuals appear to benefit from supplementation, unlike those of Western origin.[21,77,78] Furthermore, n3 PUFA intake (3.5 E%) of plant origin (2.1 E%) and marine origin (1.4 E%) over a 14-week period did not affect the adipose tissue inflammation in overweight to moderately obese subjects (28-33 kg/m^2).[32] In duodenal tissue, Labonté et al. evaluated the inflammatory response among obese patients with type 2 diabetes following a n3 PUFA intervention (3 g/day) from fish oil for eight weeks. The results failed to demonstrate any significant effect from n3 PUFA supplementation on the gene expression of pro-inflammatory cytokines in duodenal cells.[35] These authors suggested that the lack of effect was attributable to the low expression of those markers and therefore that they were unlikely to be further modified. However, their study focused on a small number of markers (IL-6, IL-18 and TNF-α) and did not assess any anti-inflammatory markers.

FINAL CONSIDERATIONS

In summary, the studies reviewed here indicate that MUFA intake and n3 PUFA intake exhibit anti-inflammatory profiles or at least a less pronounced pro-inflammatory response, particularly in comparison with SFA consumption. However, some conflicting results have been described in comparing the inflammatory effects between them. The variability in doses of MUFA (20 E% to 72 E%) and n3 PUFA (0.4 g to 23.7 E%) that were used in interventions may have led to these conflicting results. In addition, the variability in intestinal microbiota among individuals seems to be involved in this postprandial inflammatory response. In this regard, the adaptation of gut microbiota over time may be relevant, especially in comparing acute and long-term effects, but this remains to be determined.

Some other limitations that complicate direct comparisons between the studies deserve further attention. There are differences between the specific populations investigated (i.e. in relation to age, sex, genotype, presence of low-grade inflammation and health status). Methodological factors such as study design, dietary intervention (types of oils, percentage fat and dietary components) and intervention period also differed between the studies reviewed here. Moreover, inflammatory responses were assessed in different tissues (adipose tissue, duodenal tissue, muscle, PBMCs and whole blood), and the inflammatory markers that were screened also differed between the studies. Nevertheless, there is a lack of consensus regarding which biomarker is best for determining inflammation in human nutritional studies.[79] In this regard, a combination of multiple inflammatory markers appears to be more informative,[79] although intervention studies have generally focused on a small number of biomarkers instead of several, such as in analyses using microarray methodology. Little emphasis has been placed on anti-inflammatory markers. Thus, regarding the best choice for SFA replacement, limited evidence can support MUFA or PUFA as a better substitute. Identifying the optimal fat profile for inflammatory control may be a promising approach for treating chronic diseases.

CONCLUSIONS

The evidence indicates that inflammatory gene expression is regulated by the type of fat consumed. In this regard, saturated fatty acid (SFA) consumption has been correlated with a pro-inflammatory response upregulating several genes relating to inflammatory pathways, such as CD16A, MCP-1, MMP-9, IL-1, IL-6, TNFα and p65, in PBMCs and adipose tissue. On the other hand, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) consumption exhibit an anti-inflammatory profile and a less pronounced pro-inflammatory response, particularly in comparison with SFAs. Thus, partial replacement of SFA with MUFA or PUFA could be a workable nutritional strategy. However, the evidence for indicating the best unsaturated fatty acid for replacing SFAs remains limited. Identifying the optimal fat profile for inflammatory control may be a promising approach for treating chronic diseases. A larger number of studies is necessary in order to elucidate the beneficial inflammatory modulation induced by consumption of these unsaturated fats.

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Potential mechanisms linking probiotics to diabetes: a narrative review of the literature

Mecanismos potenciais ligando probióticos a diabetes: uma revisão narrativa da literatura

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PALAVRAS-CHAVE:
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ABSTRACT

CONTEXT AND OBJECTIVE: Some studies have suggested a wide range of possible mechanisms through which probiotics may play a role in diabetes prevention and treatment. However, the underlying mechanisms are not fully understood. We conducted this study to review the potential mechanisms suggested for the effect of probiotics in diabetes.

DESIGN AND SETTING: Narrative review conducted at the Food Security Research Center of Isfahan.

METHODS: A search in the electronic databases MEDLINE (PubMed), Cochrane Library, Web of Science and Google scholar was performed up to October 2016.

RESULTS: The initial search yielded 1214 reports. After removing duplicates, 704 titles and abstracts were screened. Finally, out of 83 full-text articles that were reviewed for eligibility, 30 articles were included in the final analysis. The anti-diabetic mechanisms for probiotics reported encompass intraluminal and direct effects on the intestinal mucosa and microbiota (n = 13), anti-inflammatory and immunomodulatory effects (n = 10), antioxidative effects (n = 5), effects on endoplasmic reticulum (ER) stress and expression of genes involved in glucose homeostasis and insulin resistance (n = 6), with some studies pointing to more than one mechanism.

CONCLUSION: The results may throw some light on the capacity of probiotics as a novel approach towards controlling diabetes. However, further human studies are warranted to elucidate and confirm the potential role of probiotics in diabetes prevention and treatment. Also, it needs to be ascertained whether the effectiveness of probiotics in diabetes prevention and treatment is dependent on the strain of the microorganisms.

RESUMO

CONTEXTO E OBJETIVO: Alguns estudos têm sugerido ampla gama de possíveis mecanismos, pelos quais os probióticos podem desempenhar um papel na prevenção e tratamento do diabetes. No entanto, os mecanismos subjacentes não são totalmente compreendidos. Realizamos este estudo para revisar os possíveis mecanismos sugeridos para o efeito dos probióticos na diabetes.

TIPO DE ESTUDO E LOCAL: Revisão narrativa conduzida no Food Security Research Centro de Isfahan.

MÉTODOS: Busca sistemática nas bases de dados eletrônicas MEDLINE (PubMed), Cochrane Library, Web of Science e Google scholar até outubro de 2016.

RESULTADOS: A busca inicial resultou em 1.214 artigos. Após a remoção de duplicatas, foram pesquisados 704 títulos e resumos. Finalmente, de 83 artigos completos revisados para elegibilidade, 30 foram incluídos na análise final. Os mecanismos antidiabéticos relatados dos probióticos abrangem efeitos intraluminais e diretos na mucosa e microbiota intestinal (n = 13), efeitos anti-inflamatórios e imunomoduladores (n = 10), efeitos antioxidativos (n = 5), efeitos sobre o estresse de retículo endoplasmático (RE) e expressão de genes envolvidos na homeostase da glicose e resistência à insulina (n = 6), com alguns estudos apontando para mais de um mecanismo.

CONCLUSÃO: Os resultados podem lançar alguma luz sobre os probióticos como uma nova abordagem no controle do diabetes, no entanto, mais estudos em humanos são justificados para elucidar e confirmar o papel potencial dos probióticos na prevenção e tratamento do diabetes. Além disso, deverá ser determinado se a eficácia dos probióticos na prevenção e tratamento do diabetes é dependente da cepa dos microrganismos.

INTRODUCTION

Probiotics are live microorganisms that may exert beneficial effects regarding the sufficiency of consumption via their impact on the microbial balance of the gut.[1] The most commonly used probiotics are *Lactobacillus*, *Bifidobacterium* and *Saccharomyces boulardii*, which have different effects depending on the dosage, length of therapy and administration route.[2]

Given the influence of the gut microbiota on metabolic conditions including diabetes and on improving host metabolism, the concept of manipulating the gut microbiota has gained considerable interest over recent years. Use of probiotics has been suggested as one of the approaches towards modifying the clonal flora.[3]

Diabetes mellitus is a chronic metabolic disease with major complications largely influenced by glycemic measures.[1] The Global Burden of Disease 2015 study (GBD 2015) showed that diabetes was among the leading causes of years of life lost (YLLs) in most regions.[2] Also, diabetes was shown to be a leading cause of disability-adjusted life years (DALYs), for which the observed burden exceeded expected levels in many localities.[3] The rise in diabetes prevalence is set to pose one of the most important challenges to healthcare systems over the coming years.[4]

A growing body of evidence suggests that favorable associations exist between probiotic consumption and metabolic profile among diabetes subjects.[5] However, the potential mechanisms underlying the effects of probiotics on glycemia-related parameters are not fully understood. One of the main mechanisms postulated may involve increased glucagon-like peptide 1 (GLP-1) secretion from enteroendocrine L-cells to improve carbohydrate metabolism, decrease glucotoxicity and increase insulin sensitivity of target cells.[6] Other proposed mechanisms to explain the action of probiotics on diabetes relate to anti-inflammatory, antioxidant and immunomodulatory effects and alteration of the expression of some genes involved in diabetes.[7-10]

Moreover, probiotic intake affects the structure of the gut flora, which might improve the integrity of the intestinal epithelium, weaken the immune responses and diminish the toll-like receptor 4 pathway, which in turn reduces pro-inflammatory signaling and enhances insulin sensitivity.[11,12]

Given the various statements regarding the effects of probiotics on diabetes that have been made, the aim of the present study was to focus on possible mechanisms for probiotics that might explain some of their beneficial effects in relation to diabetes, in the form of a review.

OBJECTIVE

The aim of the present study was to focus on possible mechanisms for probiotics that might explain some of their beneficial effects in relation to diabetes, in the form of a narrative review.

METHODS

Search strategy

A search of the electronic databases MEDLINE (via PubMed) and Cochrane Library (via Wiley) and the electronic repositories Web of Science and Google Scholar was performed. The search was last performed in October 2016, using combinations of search terms including "probiotics" OR "probiotic" OR "lactic acid bacteria" OR "lactobacillus" OR "lactobacilli" OR "bifidobacterium" OR "bifidobacteria" AND "diabetes mellitus", without any restrictions, in order to find studies focusing on the mechanisms linking probiotics with diabetes.

Eligibility criteria

Studies were included if they assessed the effect of a single or combination of live probiotics on diabetes. On the other hand, studies presented only as abstracts with no full-text available, non-English literature, studies involving patients with other metabolic diseases such as obesity or hypercholesterolemia, studies with no probiotic genus/strains reported, studies using synbiotics (i.e. probiotics combined with prebiotics), study protocols, pilot studies, letters, editorials, obviously irrelevant studies and studies that included non-diabetic patients or animals were all excluded.

Selection strategy

The eligibility of all potential studies identified for inclusion was independently assessed by two reviewers. Discrepancies regarding study inclusion were resolved through discussion with a third reviewer. Initially, titles and abstracts were verified and then an assessment of full texts was conducted. The reference lists of eligible articles or relevant review papers were screened for other eligible papers.

Data extraction

Study characteristics from eligible articles such as the first author's name, year of publication, study design, subjects or animal models, probiotic strain and suggested mechanisms for probiotics on diabetes were extracted by two authors. The details of all eligible articles are outlined in **Table 1**.[10-39]

RESULTS

Our initial search retrieved 1,214 articles. After removing duplicates, 704 titles and abstracts were screened. Then, from among these articles, 83 full texts were assessed for eligibility. Finally, 30 studies were included in this review. A flowchart of the study selection process is illustrated in **Figure 1**.

Local effects of probiotics in the intestine

Endotoxemia (increased circulatory levels of bacterial lipopolysaccharides) has been identified as a triggering factor for insulin

Table 1. Characteristics of the studies included

Study	Study design, animals or participants	Probiotic strain	Mechanisms suggested
Balakumar et al.[10]	Experimental study. Diabetic mice fed on high-fat diet	*Lactobacillus plantarum* MTCC5690 and *Lactobacillus fermentum* MTCC5689	• preventing the translocation of bacterial lipopolysaccharides (LPS) into the systemic circulation • increasing the level of GLP-1 ameliorating ER stress
Dolpady et al.[13]	Experimental study. NOD mice	Lactobacillaceae-enriched probiotic VSL#3	• enriching the local microbiota with Lactobacillaceae strains, increasing Clostridia and Rikenellaceae species and reducing the Bacteroidetes strain S24-7 • producing immunological tolerance in intestinal microenvironment with low expression of inflammatory IL-1β; promoting CD103+ DC differentiation and reducing the Teff/Treg cell ratios within the gut mucosa, mesenteric lymph nodes (MLN) and peripheral lymph nodes (PLN)
Hung et al.[11]	Experimental study. Sprague-Dawley rats with IGT	*Lactobacillus paracasei* subsp. *paracasei* NTU 101	• increasing *Bifidobacterium* spp. and improvement of intestinal environment • preserving gut integrity and preventing translocation of bacterial LPSs into systemic circulation
Tian et al.[12]	Experimental study. High fat diet and streptozotocin-induced type 2 diabetic rats	*Lactobacillus paracasei* subsp. *paracasei* G15 and *Lactobacillus casei* Q14	• reducing the intestinal mucosal permeability and improving the epithelial barrier function through modification of the gut microbiota and preventing translocation of bacterial lipopolysaccharides into systemic circulation
Duan et al.[14]	Experimental study. Diabetic rats	Human lactobacilli engineered to secrete GLP-1(1-37)	• reprograming intestinal cells into glucose-responsive insulin secreting cells
Holowacz et al.[15]	Experimental study. High-fat-diet C57/BL6J mice	Multispecies *Lactobacillus*- and *Bifidobacterium*-containing probiotic mixture	• reducing expression of the gene encoding CCL-2 • preventing macrophage infiltration of adipose tissue and insulin resistance
Le et al.[16]	Experimental study. C57BL/6J mice with streptozotocin (STZ)-induced diabetes	*Bifidobacterium* species (spp.)	• increasing the levels of proteins related to innate immune responses • reducing transcription of target genes such as those of pro- inflammatory cytokines • inducing differentiation of adipocytes into a cell type capable of inducing insulin sensitivity in diabetic mice
Park et al.[17]	Experimental study. C57BL/KsJ-db/db (db/db) mice	*Lactobacillus rhamnosus* GG	• reducing infiltration and activation of macrophage in white adipose tissues • decreasing the expression of ER stress genes in skeletal muscle and alleviating endoplasmic reticulum (ER) stress and lipotoxicity
Stenman et al.[18]	Experimental study. Male C57Bl/6J mice	*Bifidobacterium animalis* ssp. *lactis* 420 (B420)	• increasing the ileum GLP-1 concentration and increasing the amount of insulin released from pancreatic beta cells
Wei et al.[19]	Experimental study. Streptozotocin-induced type 1 diabetic mice	*Lactobacillus kefiranofaciens* M and *Lactobacillus kefiri* K	• stimulating the production of GLP-1 • modulating the gut microbiota by increasing the number of Gram-positive and decreasing the number of Gram-negative bacteria • inhibiting the pro-inflammatory and inflammatory cytokines, and elevating the production of IL-10
Everard et al.[20]	Experimental study. Type 2 diabetic db/db mice	*Saccharomyces boulardii*	• changing the gut microbiota composition
Kim et al.[21]	Experimental study. Rat L6 skeletal muscle cells and KK-A^{y} mouse NIDDM model	*Bifidobacterium lactis* HY8101	• increasing the mRNA expressions of pp-1, GLUT4, and PPAR-γ, and decreasing the mRNA expressions of GSK-3β, and G6PC (all involved in glucose metabolism and insulin sensitivity)
Zhang et al.[22]	Experimental study. HFS diet-induced pre-insulin resistance and a low dose-STZ HFS rats	*Lactobacillus casei*	• microbiota-based bile acid-chloride exchange mechanism: decrease in the number of bile acid 7α-dehydroxylating activity possessing bacteria, bile acid elimination, upregulating of chloride ion-dependent genes (ClC1-7, GlyRa1, SLC26A3, SLC26A6, GABAAa1, bestrophin-3 and CFTR) and prevention of chloride ion loss
Li et al.[23]	Experimental study. Type 2 diabetes in rats	*Lactobacillus plantarum* NCU116	• increasing short-chain fatty acids (SCFA) such as butyric acid in colon which leads to the growth of lactobacilli and bifidobacteria and in lowering intestinal pH and to increased GLP-1 secretion • mRNA upregulation of glucose transporter-4 (GLUT-4) and regulation of the expression of PPAR-α and PPAR-γ
Bejar et al.[24]	Experimental study. Alloxan-induced diabetes in rats	*Lactobacillus plantarum* TN627	• decreasing serum α-amylase activity, thus limiting the process of carbohydrate hydrolysis and absorption

Table 1. Continues...

Study	Study design, animals or participants	Probiotic strain	Mechanisms suggested
Hsieh et al.[25]	Experimental study. High fructose-fed diabetic rats	*Lactobacillus reuteri* GMNL-263	• stimulating GLP-1 secretion
Okubo et al.[26]	Experimental study. KK/Ta mice	*Lactobacillus plantarum* strain No. 14	• reducing the accumulation of visceral fat and preventing low grade inflammation and production of pro-inflammatory adipokines
Yadav et al.[27]	Experimental study. C57J/B6 male mice	Probiotic VSL#3	• increasing the levels of butyrate, thus stimulating release of GLP-1 from intestinal L-cells
Amar et al.[28]	Experimental study. HFD-fed WT mice	*Bifidobacterium animalis* subsp. lactis 420 (B420)	• preventing mucosal bacterial adherence and translocation of live bacteria from the intestine towards adipose tissue and blood
Kingma et al.[29]	Experimental study. Bio-breeding diabetes-prone rats	*Lactobacillus johnsonii* N6.2	• stimulating the innate immune response through activation of membrane-bound receptors expressed in intestinal epithelial cells
Lau et al.[30]	Experimental study. Bio-breeding diabetes-prone rats	*Lactobacillus johnsonii* N6.2	• mediating a TH17 bias within the mesenteric lymph node and retention of the TH17 differentiation state without conversion to Th1 state
Zarfeshani et al.[31]	Experimental study. Streptozotocin-induced diabetic rats	*Lactobacillus casei*	• reducing the onset of inflammation by lowering blood levels of IL6 and CRP and neutrophils
Aumeunier et al.[32]	Experimental study. NOD mice	VSL#3 containing *Bifidobacterium, Lactobacillus* and *Streptococcus*	• stimulating toll-like receptors (TLRs) with immunoregulatory effects on anti-inflammatory cytokines such as interleukin-10 (IL10) and transforming growth factor beta (TGF-β)
Yadav et al.[33]	Experimental study. Rats	Dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei*	• inhibiting the lipid peroxidation and preserving the activity of antioxidant enzymes including SOD, GPx and catalase
Yadav et al.[34]	Experimental study. Rats	Dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei*	• inhibiting the elevation of thiobarbituric acid- reactive substances and decreasing reduced glutathione in the liver and pancreatic tissue
Calcinaro et al.[35]	Experimental study. Female NOD mice	VSL#3 containing bifidobacteria, lactobacilli and *Streptococcus salivarius subsp. thermophilus*	• inducing a change in the cytokine secretion pattern from a pro-inflammatory to an anti-inflammatory profile by means of gut-associated lymphoid tissue (GALT)
Tabuchi et al.[36]	Experimental study. Streptozotocin-induced diabetic rats	*Lactobacillus* GG	• using glucose as a source of nutrition or by controlling the intestinal flora balance and through similar activity such as indigestible fiber, thus affecting glucose absorption • suppressing oxidative stress
Matsuzaki et al.[37]	Experimental study. Alloxan-induced diabetic mice	*Lactobacillus casei*	• preventing nitric oxide production (free radical) and ß-cell destruction in islets of Langerhans
Matsuzaki et al.[38]	Experimental study. KK-Ay NIDDM model mice	*Lactobacillus casei*	• improving the disordered post immune responses via inhibition of the production of IL2 and interferon gamma (INF-γ) and reducing the increase of CD3+ and CD4+ T cells
Ejtahed et al.[39]	Randomized, double-blind, controlled clinical trial with type 2 diabetic patients, 30 to 60 years old	Probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12	• increasing erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity and total antioxidant status (TAS)

resistance in mice and suppression of endotoxemia by probiotic supplementation is considered to be a protective mechanism.[40] In this regard, Balakumar et al.[10] stated that probiotic interventions increased the gene expression profile of the intestinal tight junction markers and gut integrity, thereby preventing translocation of bacterial lipopolysaccharides (LPS) into the systemic circulation.

Furthermore, treatment with *Lactobacillus paracasei subsp. paracasei* NTU 101 may lessen the risk of type 2 diabetes mellitus through increased levels of *Bifidobacterium spp.* and improvement of the intestinal environment. This would preserve gut integrity and prevent translocation of bacterial lipopolysaccharides into the systemic circulation.[18]

Similarly, presence of *Lactobacillus paracasei subsp. paracasei* G15 and *Lactobacillus casei* Q14 in the gut has shown a clear correlation with reduced intestinal mucosal permeability and improved epithelial barrier function, through modification of the gut microbiota. In turn, this has been shown to lower the circulating levels of LPS and inflammatory cytokines, including interleukin (IL)-1β and IL-8, and possibly to alleviate the inflammatory status and islet β-cell dysfunction.[19]

Treatment with the probiotic *Bifidobacterium animalis subsp. lactis* 420 (B420) in another study[14] led to protection against diabetes through prevention of mucosal bacterial adherence and translocation of live bacteria from the intestine towards adipose tissue and blood, which caused inflammation and insulin resistance.

Incretins, especially glucagon-like peptide 1 (GLP-1) secreted by intestinal L-cells, are a group of metabolic hormones that inhibit postprandial hyperglycemia by increasing the amount of insulin released from pancreatic beta cells.[40] Several studies[10,18,25] have shown that the beneficial effects of probiotic interventions on glucose tolerance and insulin sensitivity were related to increased levels of GLP-1.

Also, administration of *Lactobacillus kefiranofaciens M* and *Lactobacillus kefiri K* was found to stimulate GLP-1 production, with a concomitant decrease in the numbers of Gram-negative bacteria, which could trigger inflammation.[19]

The influence of human lactobacilli engineered to secrete GLP-1 on hyperglycemia has been investigated by Duanet al.[14] They showed that these lactobacilli reprogram intestinal cells into glucose-responsive insulin-secreting cells and that they therefore had the ability to ameliorate hyperglycemia and diabetes.

Moreover, in some studies, the effect of probiotics on diabetes has been linked to increases in the levels of short-chain fatty acids (SCFAs), especially butyrate in the colon.[23,27] SCFAs are probably key components in the growth of lactobacilli and bifidobacteria and in lowering intestinal pH. All of these are expected to have beneficial effects on diabetes. In addition, SCFAs have been linked to increased GLP-1 secretion in both animal and human models.

Local changes to the intestinal environment and microbiota have been mentioned as another mechanism relating probiotics to diabetes prevention and treatment. *Saccharomyces boulardii* significantly changes the gut microbiota composition with an increased proportion of Bacteroidetes and decreased quantities of organisms in the phyla Firmicutes, Proteobacteria and Tenericutes. These phyla have been previously correlated with type 2 diabetes in mice.[20]

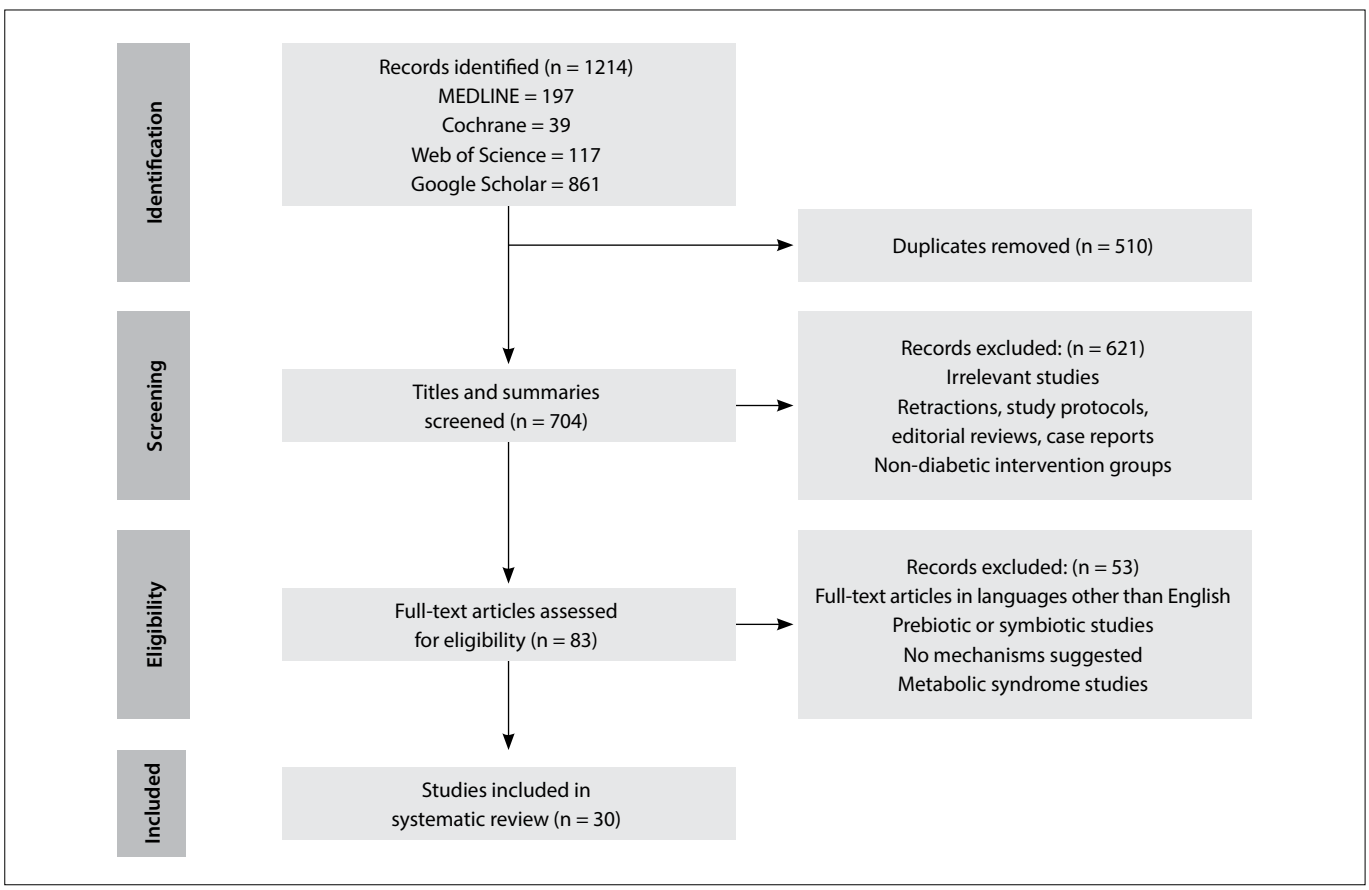

Figure 1. Flow chart of study selection process.

Dolpady et al.[13] also demonstrated prevention of type 1 diabetes (T1D) through enriching the local microbiota with Lactobacillaceae strains and through inducing substantial modifications in the microbiota composition, with increased levels of species of Clostridia and Rikenellaceae and decreased levels of the Bacteroidetes strain S24-7, when a Lactobacillaceae-enriched VSL#3 probiotic was administered. In addition, these modifications generated a protolerogenic intestinal microenvironment with low expression of inflammatory IL-1β. The VSL#3-induced protolerogenic microenvironment promotes CD103+ dendritic cell differentiation and reduces T effectors/T regulatory cell (Teff/Treg) ratios within the gut mucosa, mesenteric lymph nodes (MLN) and peripheral lymph nodes (PLN), which results in autoimmune diabetes prevention.

Pancreatic inflammation caused by type 1 diabetes results in leakage of a-amylase into the bloodstream, thus eliciting higher levels of serum pancreatic a-amylase, a key enzyme involved in carbohydrate digestion. Administration of L. plantarum TN627 to diabetic rats was found to significantly decrease serum α-amylase activity, thus limiting the process of carbohydrate hydrolysis and absorption. Consequently, beneficial effects were observed on the glycemic index.[24]

The effects of probiotics on the inflammatory and immune response pathways

Altered production or function of circulating innate immune proteins, cellular pattern-recognition receptors and inflammatory cytokines have been linked to insulin resistance and diabetes.[41]

Lactobacillus kefiranofaciens M and *lactobacillus kefiri* K were reported to mitigate progression of type 1 diabetes through inhibiting pro-inflammatory and inflammatory cytokines and elevating the production of IL-10. IL-10 inhibits the levels of pro-inflammatory cytokines (tumor necrosis factor-alpha) and Th1 cytokines (IL-1β, IL-2, IL-6) and prevents β cell destruction.[19]

Moreover, administration of *Bifidobacterium spp.* increased the levels of innate immune response proteins, including IκB kinase alpha (IKKα), nuclear factor-kappa B inhibitor alpha (IκBα), extracellular-signal-regulated kinase 2 (ERK2) and protein kinase B (Akt). Akt may affect IKKα and even result in activation of IκBα, which may in turn inhibit the effects of NF-κBand, thus leading to reduced transcription of target genes such as those of pro-inflammatory cytokines. On the other hand, ERK, a widely-expressed protein kinase, is an intracellular signaling molecule involved in functions relating to regulation of cell proliferation, differentiation and survival. Increased ERK2 levels may induce differentiation of adipocytes into a cell type capable of inducing insulin sensitivity in diabetic mice fed with *Bifidobacterium spp*.[16]

Furthermore, *Lactobacillus rhamnosus* GG (LGG) treatment was shown[17] to reduce infiltration and activation of macrophages, which is critical for initiation and amplification of chronic inflammation in white adipose tissues. Hence, the insulin-sensitizing effect of LGG may occur through alleviating this inflammatory pathway.

Another study[26] indicated that administration of *Lactobacillus plantarum* No. 14 prevents development of insulin resistance, mainly through reducing accumulations of visceral fat, which prevents production of pro-inflammatory adipokines. Pro-inflammatory adipokines interfere with the insulin-signaling pathway of peripheral tissues and facilitate development of insulin resistance.

In addition, there is evidence that oral treatment with VSL#3, a probiotic compound containing bifidobacteria, lactobacilli and *Streptococcus salivarius subsp. thermophilus*, induces a change in the cytokine secretion pattern from a pro-inflammatory to an anti-inflammatory profile in the gut-associated lymphoid tissue (GALT), which is associated with qualitative modification of islet-specific destructive autoimmunity and, possibly, diabetes prevention.[35]

Consistent with the abovementioned data, protective action by *Lactobacillus casei* in relation to diabetes was correlated with less frequent onset of inflammation, through lowered levels of IL6, CRP and neutrophils in blood.[31] *Lactobacillus casei* also has the potential to decrease blood glucose levels through improvement of disordered post-immune responses via inhibition of production of IL2 and interferon gamma (INF-γ) and reduction of the increases in CD3+ and CD4+ T cell counts.[38]

Kingma et al.[29] showed that *Lactobacillus johnsonii* (Ljo) N6.2 stimulates the innate immune response through activation of the membrane-bound receptors expressed in intestinal epithelial cells. These receptors activate type 1 interferon (INF), which are key players in innate immunity. Therefore, a higher state of immunological activation would be achieved, thereby preventing diabetes. Moreover, this strain inhibits type 1 diabetes through mediating T-helper 17 (Th17) bias within the mesenteric lymph nodes. Retention of the Th17 differentiation state, without conversion to a Th1 state, which is critical to diabetogenesis, prevents or delays the onset of type 1 diabetes.[30]

Stimulation of toll-like receptors (TLRs), which have immunoregulatory effects on anti-inflammatory cytokines, can prevent the onset of autoimmune diseases. TLR-mediated effects of probiotics involve immune-regulatory cytokines such as interleukin IL-10 and transforming growth factor (TGF)-β and some regulatory T cells, under the experimental conditions that result in protection from spontaneous diabetes.[32]

The effects of probiotics on oxidative stress

In diabetes, the free radicals that are generated cause lipid peroxidation and malondialdehyde (MDA) production. Moreover, the activity levels of reactive oxygen species scavengers are lower in patients with diabetes. Therefore, improvement of oxidative stress status may contribute towards diabetes management.[42,43]

Tabuchi et al.[36] showed that *Lactobacillus* GG lowered the level of MDA per gram of liver weight, which conferred suppression of oxidative stress and improved glucose tolerance.

Other authors concluded that the inhibitory effect of *Lactobacillus casei* on the incidence of diabetes was partially dependent on prevention of nitric oxide production, given that this is a free radical that is involved in the ß-cell destruction process in islets of Langerhans.[37,44]

On the other hand, foods containing probiotics have been shown to protect against indices relating to diabetes. In one study, probiotic yogurt consumption increased the activity levels of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx), which scavenge free radicals, and improved the total antioxidant status (TAS).[39]

Another mechanism that was proposed to explain the action of fermented milk products containing probiotic bacteria on diabetes was through diminishing the elevation of thiobarbituric acid-reactive substances and increasing glutathione levels in the liver and pancreatic tissues of diabetic rats. These findings indicated that this drink had good antioxidant properties.[33]

Probiotic milk has consistently been found to exert antioxidant effects through inhibiting lipid peroxidation and preserving the activity of antioxidant enzymes, including SOD, GPx and catalase (CAT).[34]

The effects of probiotics on gene expression

Some studies on interactions between probiotics and gene expression have suggested that type 2 diabetes in rats is ameliorated through mRNA upregulation of glucose transporter-4 (GLUT-4) through *Lactobacillus plantarum* NCU116 treatment.[23] This has a critical role in glucose uptake.[45] Moreover, NCU can regulate glucose homeostasis and insulin sensitivity in diabetic rats via regulating PPAR-α and PPAR-γ gene expression. These genes play key roles in inflammation and glucose homeostasis.[46]

Bifidobacterium spp. also has an impact on enhanced expression of proteins involved in the insulin-signaling pathway, including IR-β, IRS-1 and Akt. This results in improved glucose uptake and blood glucose reduction.[16]

Zhang et al.[22] postulated that prevention of the onset of type 2 diabetes through using *L. casei* Zhang may occur via a microbiota-based bile acid-chloride exchange mechanism. Hyperglycemia relates to high levels of plasma bile acids and urine chloride ion loss. High intracellular chloride ion levels in β-cells of the pancreas are essential for the electrical activity of the β-cell membrane and for insulin release. *L. casei* Zhang administration was found to cause a decrease in the quantity of bacteria with bile acid 7α-dehydroxylating activity and, therefore, bile acid elimination was enhanced. In turn, chloride ion loss was significantly prevented by *L. casei* via upregulation of chloride ion-dependent genes (ClC1-7, GlyRa1, SLC26A3, SLC26A6, GABAAa1, bestrophin-3 and CFTR).

In addition, discovery of the antidiabetic activity of *Bifidobacterium lactis* HY 8101 has shed new light on the mechanisms for probiotics and their importance in diabetes.[21] Its antidiabetic activity occurs through increasing the mRNA expression of pp-1 (glycogen synthesis-related enzymes), GLUT4 (glucose uptake-related genes) and PPAR-γ (insulin sensitivity-related genes) and decreasing the mRNA expression of GSK-3β (glycogen synthesis-related enzymes) and G6PC (gluconeogenesis-related enzymes), which are all involved in glucose metabolism and insulin sensitivity.

Another investigation[15] also provided evidence that a multispecies mixture of probiotics containing *Lactobacillus* and *Bifidobacterium* reduced expression of the gene encoding CCL-2. The latter is an important chemokine for macrophage infiltration of adipose tissue and contributes towards insulin resistance.[47]

Finally, endoplasmic reticulum (ER) stress has been mentioned as one of the main causes of development of inflammation and insulin resistance. ER stress appears to act directly as a negative modulator of the insulin signaling pathway, but also indirectly by promoting lipid accumulation.[48] Two studies[10,17] showed that probiotic interventions alleviated lipotoxicity and ER stress gene expression in skeletal muscle, which resulted in improvement of glucose tolerance.

DISCUSSION

One significant question regarding clinical use of probiotics is the mechanism underlying the wide range of actions. However, the increasing number of studies that are being conducted with the aim of establishing probiotic mechanisms relating to diabetes conditions indicate that there is a promising future for probiotics in treating this disease. To the best of our knowledge, this is the first review on the mechanisms of probiotic function relating to diabetes. It is hoped that gaining a mechanistic understanding of probiotic action will provide the rationale to support development of new hypothesis-driven studies to define the clinical efficacy of preventive, adjunctive or alternative treatments for diabetes. Also, such efforts could suitably help in selecting strains for specific investigation and applications under these conditions and may uncover novel probiotic functions.

The mechanisms suggested have mostly involved intraluminal and direct effects on intestinal mucosa and microbiota (13 studies). Suppression of endotoxemia, stimulation of secretion of short chain fatty acids (SCFAs) and incretines, and local changes to the gut environment and microbiota were major effects detailed in the present review. In addition, anti-inflammatory and immunomodulatory effects were reported in 10 studies. Prevention of free radical production, increased activity of antioxidant enzymes and inhibition of peroxidation were reported as the main antioxidant

effects of probiotics in relation to diabetes (five studies). Finally, six studies suggested that probiotics might have effects through altering the expression of genes involved in ER stress and glucose homeostasis and insulin resistance.

The strengths of this review include its use of an outcome classification for different possible mechanisms of probiotics in relation to diabetes. However, several limitations need to be taken into account in interpreting our findings. It should be mentioned that, except for one study, all of these mechanisms have been verified in animal studies. Moreover, it seems that such effects depend on the type of bacteria, dose and duration of consumption, manner and frequency of administration, environmental factors and complex interactions between probiotics, cells and metabolic pathways that are rarely mediated by a single mechanism.[49]

In addition, it is important to take into consideration the risk of bias across different studies, such as publication, performance and reporting bias, along with potential conflicts of interest. Such factors might limit the ability to draw robust conclusions from these studies. Given that we only had limited access to some databases such as Embase, and that studies not reported in English were excluded, it is possible that more rigorous reporting of study results would improve the quality of the evidence in further studies.

Nonetheless, elucidation of the mechanisms linking the microbiome to diabetes can provide a rational basis for dietary consumption of probiotic microorganisms in relation to diabetes. In addition, evaluation of the mechanism of action for probiotics both in healthy subjects and in diabetic patients, so as to address the influence of these microorganisms on gene expression for different pathways, is needed in order to better understand the role that probiotics might have in prevention and treatment of diabetes.

CONCLUSIONS

In conclusion, there is some evidence suggesting various potential mechanisms of action for probiotics in relation to diabetes prevention and treatment. Further studies are needed to confirm the underlying pathways involved in the beneficial effects from each strain, along with assessment of other confounding factors.

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Acute WT1-positive promyelocytic leukemia with hypogranular variant morphology, bcr-3 isoform of PML-RARα and Flt3-ITD mutation: a rare case report

Leucemia promielocítica aguda WT1-positivo com morfologia variante hypogranular, isoforma bcr-3 da PML-RARα e mutação Flt3-ITD: relato de caso raro

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KEY WORDS:
Leukemia, promyelocytic, acute.
Fms-like tyrosine kinase 3.
WT1 proteins.
Prognosis.
Lung diseases, fungal.

PALAVRAS-CHAVE:
Leucemia promielocítica aguda.
Tirosina quinase 3 semelhante a fms.
Proteínas WT1.
Prognóstico.
Pneumopatias fúngicas.

ABSTRACT

CONTEXT: Acute promyelocytic leukemia (APL) accounts for 8% to 10% of cases of acute myeloid leukemia (AML). Remission in cases of high-risk APL is still difficult to achieve, and relapses occur readily.

CASE REPORT: Here, we describe a case of APL with high white blood cell counts in blood tests and hypogranular variant morphology in bone marrow, together with fms-like tyrosine kinase-3 with internal tandem duplication mutations (FLT3-ITD), and bcr-3 isoform of PML-RARα. Most importantly, we detected high level of Wilms' tumor gene (WT1) in marrow blasts, through the reverse transcription polymerase chain reaction (RT-PCR). To date, no clear conclusions about an association between WT1 expression levels and APL have been reached. This patient successively received a combined treatment regimen consisting of hydroxycarbamide, arsenic trioxide and idarubicin plus cytarabine, which ultimately enabled complete remission. Unfortunately, he subsequently died of sudden massive hemoptysis because of pulmonary infection.

CONCLUSION: Based on our findings and a review of the literature, abnormal functioning of WT1 may be a high-risk factor in cases of APL. Further studies aimed towards evaluating the impact of WT1 expression on the prognosis for APL patients are of interest.

RESUMO

CONTEXTO: Leucemia promielocítica aguda (LPA) compreende 8% a 10% dos casos de leucemia mieloide aguda (LMA). A remissão em casos de LPA de alto risco ainda é dificilmente conseguida, e recorrência é comum.

RELATO DE CASO: Descrevemos aqui um caso de LPA com glóbulos brancos elevados no exame de sangue e a morfologia variante hipogranular na medula óssea, juntamente com fms-*like* tirosina-quinase-3 com mutações de duplicação em *tandem* interna (FLT3-ITD) e a isoforma bcr-3 de PML- RARα. Mais importante, detectamos alto nível de gene do tumor de Wilms (WT1) em blastos medulares por RT-PCR (*reverse transcription polimerase chain reaction*). Até agora, não há conclusões claras sobre a associação entre os níveis de expressão WT1 e APL. Este paciente recebeu sucessivamente regime de tratamento combinado, de hidroxicarbamida, trióxido de arsênico e idarrubicina e citarabina, alcançando finalmente a remissão completa. Infelizmente, em seguida, ele morreu de repente de hemoptise maciça devido a uma infecção pulmonar.

CONCLUSÃO: Com base em nossos resultados e numa revisão da literatura, a função anormal de WT1 pode ser um fator de alto risco em casos de APL. Novos estudos, com o objetivo de avaliar o impacto da expressão de WT1 no prognóstico dos doentes com APL, são de interesse.

INTRODUCTION

Currently, with therapeutic improvements that have been attained, curative treatments for acute promyelocytic leukemia (APL) can reach complete response rates close to 90% and long-term relapse-free survival of 85%. However, high white blood cell (WBC) counts are among the high-risk factors for APL, and these proven high risk factors are also seen in subtypes of acute myeloid leukemia (AML). Remission in cases of high-risk APL is still difficult to achieve, and relapses occur readily. Despite major advances in treatments for APL, high-risk APL patients often die during the early treatment because of severe complications. The currently known specific risk factors include fms-like tyrosine kinase-3 (FLT3-ITD), hypogranular variant morphology, and the bcr-3 isoform of PML-RARα.[1,2] Here, we describe a case that also expressed high level of Wilms' tumor gene (WT1), harboring a complex karyotype.

CASE REPORT

A 42-year-old male patient was hospitalized with hematuria and high white blood cell counts. Physical examination revealed that he had a pale complexion, scattered petechiae and ecchymosis on his skin, and sternal tenderness.

Routine blood tests showed WBC 117.14 × 10^9/l, hemoglobin (Hb) 68 g/l, platelets (PLT) 23 × 10^9/l and lactic dehydrogenase (LDH) 320 IU/l. Routine urine tests were positive for urinary protein and urinary red blood cells, and the red blood cell count was 143.6/ul. Among the coagulation parameters, prothrombin time (PT) and activated partial thromboplastin time (APTT) were within the normal range, D2 dimers were elevated to 18 mg/l, and a plasma protamine paracoagulation test was positive. A fecal occult blood test was positive too.

There was no abnormality in pulmonary computed tomography (CT) examination (no fluid and no infection). The sonographic findings from superficial lymph nodes showed no abnormality. Abdominal ultrasound examination showed liver cysts and kidney stones (diameter: 0.7 to 1.0 cm). Cardiac ultrasonography was normal.

A bone marrow histological evaluation showed that bone marrow hyperplasia was extremely active. Granulocytes accounted for 99% of the material and, among them, promyelocytes accounted for 91.5%. The cell body was of a different size, containing much cytoplasm and few A particles (also known as azurophilic granules). The nuclear distortion was obvious, typically of butterfly or dumbbell shape. The peroxidase (POX)-positive staining rate was 100%. According to the French-American-British (FAB) criteria, this case was classified as APL with hypogranular variant morphology (M3v) (**Figure 1**). A peripheral blood smear indicated that myeloblasts accounted for 6% and promyelocytes accounted for 92%, and the morphology was similar to what was seen in the bone marrow smear. Flow cytometry detected that the abnormal bone marrow cell population accounted for 93.6%. These abnormal bone marrow cells were positive for CD45, CD117, CD13/CD33, CD64, CD38, MPO, CD34, HLA-DR, CD2, CD4 and CD19 expression, which suggested that this case consisted of AML (B cells$^+$ and T cells$^+$). Fluorescence *in situ* by hybridization (FISH) detection showed that the fusion gene PML/RARα accounted for 94%. PCR experiments were bcr-3 subtype PML/RARα-positive and FLT3-ITD-positive and the WT1 count was 58.8% (**Figure 2**).

The chromosome analysis displayed: 44,XY, t(15;17)(q22;q21), -1,-8 [1]/ 46,XY, t(15;17)(q22;q21) [6]/46,XY [3]. Apart from the typical translocation t(15; 17)(q22;q21), which was the main abnormality in APL, one additional abnormal subclone consisted of loss of a single chromosome on chromosomes 1 and 8 respectively (**Figure 1**). This patient was diagnosed as presenting APL with hypogranular variant morphology (M3v), short (bcr-3) subtype and FLT3-ITD mutation, and was WT1-positive.

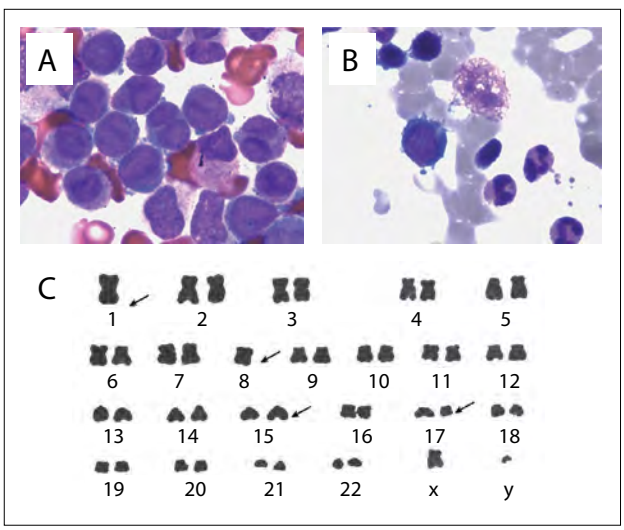

Figure 1. Bone marrow morphology and karyotype analysis: A) Bone marrow smear stained with Wright-Giemsa, showing that this was a rare case of APL with hypogranular variant morphology (M3v). Promyelocytes appeared in the bone marrow aspiration prior to treatment (promyelocytes 91.5%). The cell body was of different size, containing much cytoplasm and few A particles (also known as azurophilic granules). The nuclear distortion was obvious and typically of butterfly or dumbbell shape. B) Complete remission was achieved post-treatment (promyelocytes 1%). C) Karyotype analysis revealed 44,XY, t(15;17)(q22;q21),-1,-8 [1]/ 46,XY, t(15;17) (q22;q21) [6] /46,XY [3]. The clone with the translocation t(15;17)(q22;q21) as the main abnormality was the stem line. One additional abnormal subclone was identified, with loss of a single chromosome on chromosomes 1 and 8 respectively.

The initial treatment for this patient comprised hydroxycarbamide, low-dose cytarabine and arsenic trioxide (ATO; 10 mg/d). To avoid retinoic acid syndrome (RAS), all-trans retinoic acid was not administered at first, during the high white blood cell phase. At the same time, we improved the anemia through transfusion of red blood cells, corrected the abnormal coagulation through plasma transfusion and implemented other symptomatic supportive treatment. After four days of ATO administration, the patient complained of fever (39 °C), chest tightness and shortness of breath. An ultrasound examination showed a small amount of pleural effusion and pericardial effusion. Considering these as side effects of arsenite, we stopped using it and replaced it with idarubicin plus cytarabine/chemotherapy regimen (IA). When IA chemotherapy ended, this patient had developed dyspnea, and blood gas analysis showed type I respiratory failure (pH 7.55; PO_2 59 mmHg). A pulmonary spiral computed tomography (CT) examination showed frosted glass-like changes to the lung fields and medium amounts of pleural effusion (**Figure 3**). Because of the presence of promyelocyte differentiation syndrome, the patient was placed under continuous oxygen therapy, with 80 mg/day of methylprednisolone.

After ten days of hormonal therapy, the symptoms of heart fatigue and shortness of breath showed clear improvement. CT on the lungs showed that a clear reduction of bilateral pleural effusion had occurred and that the right-side pleural effusion had basically been absorbed. One high-density patchy shadow (of dimensions 4.4 x 3.5 cm) was discovered in the left pulmonary hilar (**Figure 3**).

The patient then entered a bone marrow suppression period and contracted repeated high fever. Considering the possibility of fungal infection, we replaced the hormone treatment with voriconazole, based on broad-spectrum antibiotics. On the 17th day after IA chemotherapy ended, the patient still repeatedly presented high fever. Routine blood tests showed WBC 5.34×10^9/l, Hb 62 g/l and PLT 37×10^9/l, Pulmonary CT reexamination showed significant consolidation of lung tissue in the left lower lung lobe, and that the high-density mass had increased to a size of 4 .7 x 3.5 cm. No obvious evidence of

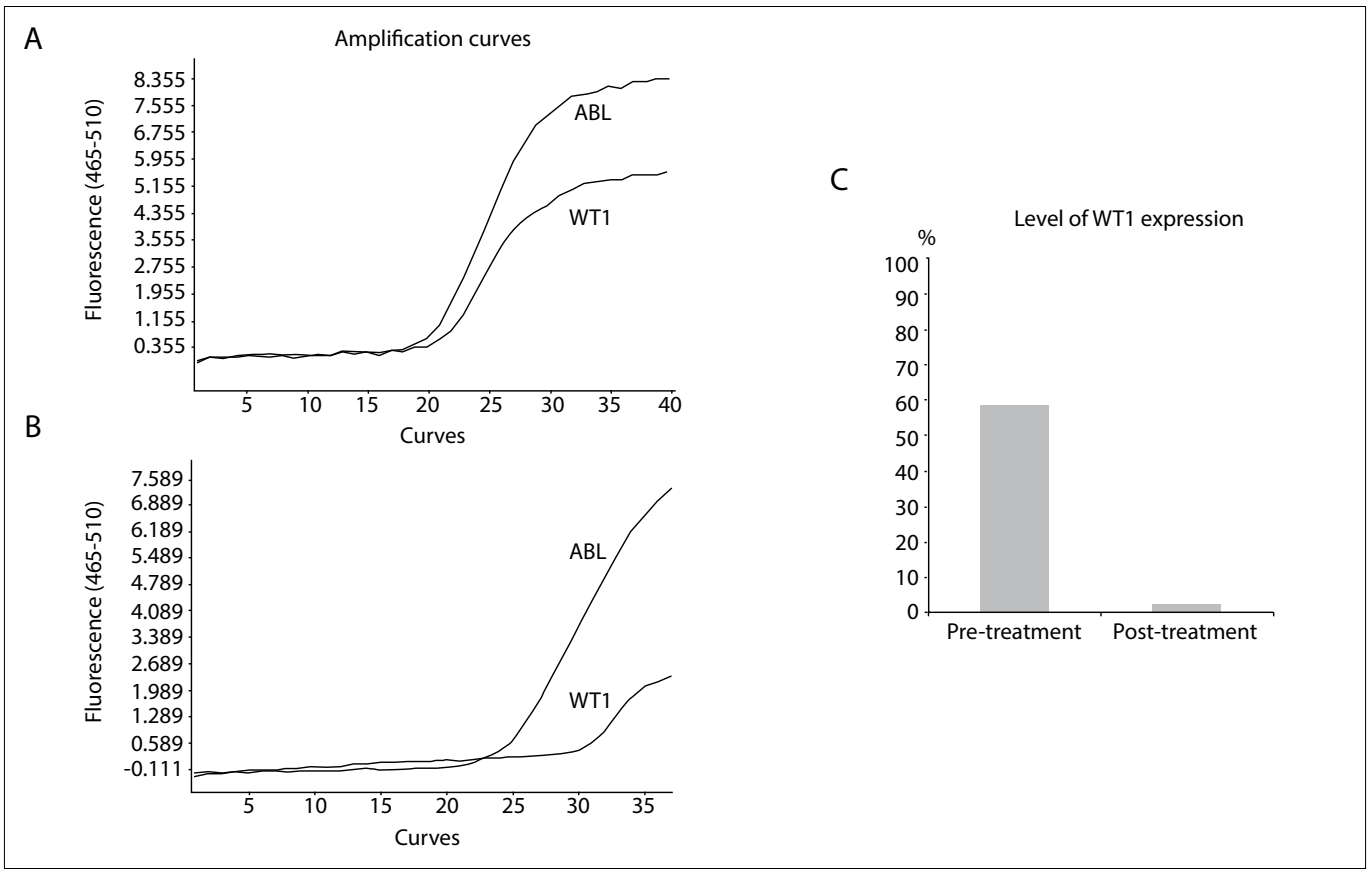

Figure 2. The curves of WT1 genes amplified by means of the real-time polymerase chain reaction (RT-PCR): A) the WT1 value was 641,000 copies at the initial diagnosis and WT1/ABL count of 58.8%; B) the WT1 level reduced to 3630 copies after treatment and WT1/ABL of 2.3%; C) ratio of WT1 to ABL gene before and after treatment.

tuberculosis, bacteria or fungi was found through pleural fluid drainage examination.

On the 26th day after the IA regimen ended, a sputum culture experiment showed the presence of mucosal infection. We replaced the voriconazole with posaconazole for patient and, at the same time, instituted oral retinoic acid therapy.

On the 44th day after chemotherapy, routine blood reexamination showed WBC 5.84×10^9/l, Hb 73 g/l and PLT 404×10^9/l. The morphology of the bone marrow presented complete remission (**Figure 1**). Pulmonary CT reexamination showed massive pleural effusion in the left lung, giving rise to total pulmonary atelectasis (**Figure 3**). In contrast, the right-side pleural effusion was completely absorbed. A tuberculosis antibody test was positive in pleural fluid and blood samples, and investigation of fungus and promyelocytes in pleural fluid was negative. Implementation of diagnostic anti-tuberculosis therapy was then planned. However, the patient suddenly died on the third day after tuberculosis antibody detection, because of massive hemoptysis.

DISCUSSION

APL is a distinctive subtype of myeloid malignancies, characterized by reciprocal translocation between chromosomes 15 and 17. This generates three kinds of PML-RARα fusion genes, referred to as long (L or bcr-1), variant (V or bcr-2) and short (S or bcr-3). The first-line treatment for newly diagnosed APL has mainly been based on a combination of all-trans-retinoic acid (ATRA) and anthracycline drugs, which has achieved good effects.[3,4]

In this paper, we presented one case of the bcr-3 subtype of APL. According to the literature, the efficacy of type bcr-3 is often worse than that of type bcr-1, and patients with bcr-3 tended to have more relapses and shorter survival.[2] In an *in vitro* experiment, in the absence of granulocyte-macrophage colony-stimulating factor (GM-CSF), bcr-3 cells had anti-apoptotic properties and ATRA inhibited the growth of bcr-1 cells more strongly than bcr-3 cells, which suggested that patients with bcr-3 APL may have stronger drug resistance to ATRA.[5] This can be contrasted with cases of retinoic acid syndrome: our patient with high white blood cell counts did not receive ATRA during the first-course treatment.

Some researchers have believed that there is a high-degree correlation between the bcr-3 subtype and FLT3 mutations. Additionally, FLT3 mutations are often associated with high leukocyte states, which are one of the important adverse prognostic markers of APL.[6,7] FLT3 mutations mainly consist of internal tandem repeat (ITD) and tyrosine kinase domain (TKD) mutations. Several reports have mentioned that there is a close association between FLT3-ITD and elevated white blood cell counts, hypogranular variant morphology (M3v) and the bcr-3 isoform of PML-RARα, which is consistent with our report. FLT3-ITD mutation activates tyrosine kinase and downstream signaling pathways such as STAT5, RAS/MAPK and PI3K/AKT, ultimately leading to inhibition of cell apoptosis, but it accelerates excessive proliferation, which results in high white blood cell counts. A high frequency of FLT3-ITD was previously reported in 30-45% of APL patients. The FLT3-ITD mutation of APL had a lower remission rate and shorter overall survival phase.[8,9]

Apart from the FLT-ITD mutation, WT1 expression was also seen in the case of APL that we report here. Overexpression, polymorphisms and mutations of the WT1 gene have been reported in AML and have variably been correlated with the prognosis. Moreover, FLT3 mutations were found in 37% of APL patients and correlated with high WT1 mRNA expression.[10] Recent studies on AML patients have shown that high WT1 expression was specifically correlated with presence of the FLT3-ITD mutation.[10-12] WT1

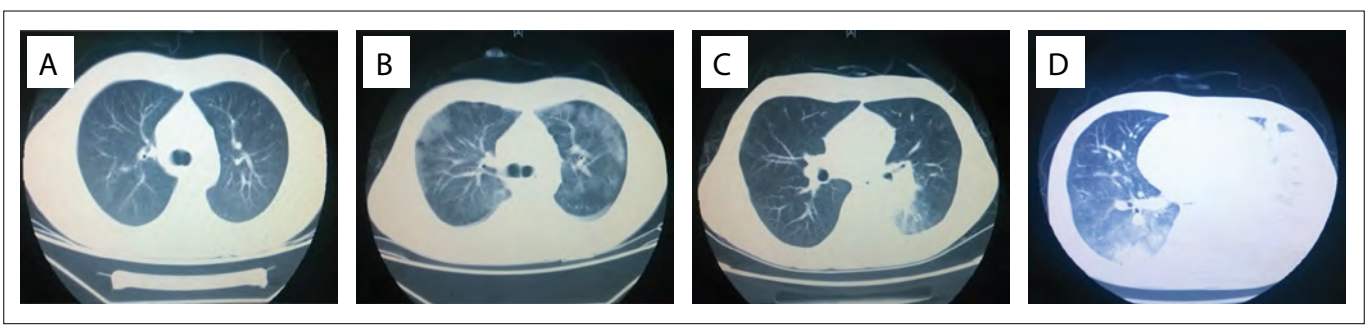

Figure 3. Chest computed tomography scans: A) There were no abnormal changes in the lungs prior to treatment (no pleural effusion and no infection); B) With the promyelocyte differentiation syndrome, computed tomography (CT) scans of the patient's chest showed ground-glass opacity (GGO) in the two lung lobes and pleural effusion; C) After hormonal therapy, CT showed clear reduction of bilateral pleural effusion, such that the right side of the pleural effusion was basically absorbed. However, the lung consolidation with halo signs in the left pulmonary hilum means that fungal infection was likely; D) Massive pleural effusion was seen in the left lung, giving rise to pulmonary atelectasis, with patchy change in the right lung, when there was complete remission from acute promyelocytic leukemia (APL).

mutation is currently recognized as an adverse prognostic factor for AML,[13,14] but information on its impact on the prognosis for APL is lacking. In 2012, Gaur et al.[10] published the first analysis of WT1 gene variations focusing on APL. WT1 mutations were detected in four of the 103 patients with APL, but they found no differences in WT1 expression levels between patients classified as low, intermediate or high risk according to the Sanz score. Hecht et al.[15] found that patients with high WT1 expression achieved a complete remission (CR) significantly faster. There was no difference in the cumulative incidence of relapse and the time until relapse between different WT1 expression groups. Lastly, no clear conclusions about the association between WT1 expression levels and the cause of shorter overall survival (OS) after CR can be drawn. However, the risk of death after CR was nine times higher in the low WT1 group and 10.5 times higher in the high WT1 group than among patients with intermediate WT1 expression. Furthermore, in univariate analysis, high WT1 was also a predictor of shorter relapse-free survival (RFS).[15] Based on our result, the WT1 value was 641,000 copies at the initial diagnosis. When the disease was in complete remission, the polymerase chain reaction (PCR) reexamination showed that the WT1 level reduced to 3630 copies. The ratio of WT1 to the ABL (abelson tyrosine-protein kinase) decreased from 58.8% to 2.3% (**Figure 2**).

In conclusion, there is controversy as to whether high WT1 expression suggests a poor prognosis for AML. However, we believe that high WT1 expression in APL cases indicates an adverse prognosis and should be considered in APL risk stratification. Although the clinical data for further validation is far from plentiful, WT1 inhibitor or WT1-specific cytotoxic cell therapy may be promising in cases of high-risk APL with WT1 overexpression.

We reviewed the literature in MEDLINE, PubMed, Embase and LILACS using the English keywords "acute promyelocytic leukemia", "FLT3-ITD" and "WT1". We found that only two WT1-positive APL patients with FLT3-ITD mutation and bcr-3 isoform PML-RARa expression had previously been reported. Both of them died during early treatment.[16] In addition, Zou et al.[17] and Liu et al.,[18] reported two cases of APL with FLT3-TKD mutation that were WT1-positive, and both of these patients suffered systemic relapse. However, neither the bcr subtype of PML-RARα nor the specific cell morphology was mentioned (**Table 1**).

Our case was similar to that of Greco et al.[16] Moreover, our patient presented a complex karyotype through chromosome analysis and mixed molecular expression according to flow cytometry. After treatment, our patient achieved complete remission, but unfortunately he died of pulmonary infection. The direct cause of death was massive hemoptysis, probably caused by tuberculosis or by fungus eroding blood vessels.

Although the treatment for the pulmonary infection was sufficient and timely, our patient died because of symptoms as mentioned above. Through tracing these symptoms back to their sources, it could be seen that the previous chemotherapy and glucocorticoid applications may have led to serious infection in this patient. This is also a lesson that doctors need to be aware of, regarding the possibility of infection in high-risk APL patients during induction therapy. Although diagnosing and treating high-risk APL are very important, we also learned that severe complications, such as disseminated intravascular coagulation, hydrothorax and serious infection, which may occur especially in cases of high-risk APL, need to be treated as soon as possible. While reducing the tumor burden of APL, lack of care regarding these complications might be fatal.

Table 1. Database search results for acute promyelocytic leukemia, FLT3-ITD and WT1 on August 5, 2016

Database	Search Strategies	Papers found	Papers related
MEDLINE (via PubMed)	'acute promyelocytic leukemia'/exp OR 'acute promyelocytic leukemia' AND 'flt3-itd' AND 'wt1' AND 'case report'	0	0
Embase (via Elsevier)	'acute promyelocytic leukemia'/exp OR 'acute promyelocytic leukemia' AND 'wt1' AND 'case report'	9	3 papers reported FLT3-TKD and WT1 mutation in APL, but FLT3-ITD mutation not mentioned.
LILACS	'acute promyelocytic leukemia'/exp OR 'acute promyelocytic leukemia' AND 'flt3-itd' AND 'wt1' AND 'case report'	0	0

CONCLUSION

We presented a rare case of APL with hypogranular variant morphology, bcr-3 isoform of PML-RARα and Flt3-ITD mutation, which was also WT1-positive. Based on our findings and a review of the literature, abnormal functioning of WT1 may be a high-risk factor for APL. Further studies aiming to evaluate the impact of WT1 expression on the prognosis of APL patients are of interest.

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Liquid silicone used for esthetic purposes as a potentiator for occurrence of post-radiotherapy genital lymphedema: case report

Silicone líquido utilizado com finalidade estética como potencializador da ocorrência de linfedema genital pós-radioterapia: relato de caso

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KEY WORDS:
Lymphedema.
Silicones.
Radiotherapy.
Reconstructive surgical procedures.
Genital diseases, male.

PALAVRAS-CHAVE:
Linfedema.
Elastômeros de silicone.
Radioterapia.
Procedimentos cirúrgicos reconstrutivos.
Doenças dos genitais masculinos.

ABSTRACT

CONTEXT: Lymphedema consists of extracellular fluid retention caused by lymphatic obstruction. In chronic forms, fat and fibrous tissue accumulation is observed. Genital lymphedema is a rare condition in developed countries and may have primary or acquired etiology. It generally leads to urinary, sexual and social impairment. Clinical treatment usually has low effectiveness, and surgical resection is frequently indicated.

CASE REPORT: We report a case of a male-to-female transgender patient who was referred for treatment of chronic genital lymphedema. She had a history of pelvic radiotherapy to treat anal cancer and of liquid silicone injections to the buttock and thigh regions for esthetic purposes. Radiological examinations showed signs both of tissue infiltration by liquid silicone and of granulomas, lymphadenopathy and lymphedema. Surgical treatment was performed on the area affected, in which lymphedematous tissue was excised from the scrotum while preserving the penis and testicles, with satisfactory results. Histopathological examination showed alterations compatible with tissue infiltration by exogenous material, along with chronic lymphedema.

CONCLUSION: Genital lymphedema may be caused by an association of lesions due to liquid silicone injections and radiotherapy in the pelvic region. Cancer treatment decisions for patients who previously underwent liquid silicone injection should take this information into account, since it may represent a risk factor for radiotherapy complications.

RESUMO

CONTEXTO: O linfedema consiste de retenção de fluido extracelular causada por obstrução linfática. Nas formas crônicas, observa-se acúmulo de tecido adiposo e fibrose. O linfedema genital é uma doença rara em países desenvolvidos e pode ter etiologia primária ou adquirida, em geral cursando com disfunções urinária e sexual, bem como com prejuízo do convívio social. O tratamento clínico é, em geral, pouco efetivo, indicando-se com frequência a abordagem cirúrgica, com excisão da área afetada.

RELATO DE CASO: Relata-se o caso de paciente feminina transgênero, encaminhada para tratamento de linfedema genital crônico. Havia antecedente pessoal de tratamento de câncer de canal anal com radioterapia pélvica e de injeções de silicone líquido em glúteos e coxas com finalidade estética. Exames radiológicos mostraram tanto sinais de infiltração tecidual por silicone líquido como granulomas e linfadenopatia como de linfedema. Foi realizado o tratamento cirúrgico da área afetada, com excisão do tecido linfadenomatoso do escroto, preservando o pênis e testículos, com resultado satisfatório. A análise histopatológica mostrou achados compatíveis com infiltração tecidual por material exógeno, bem como com linfedema crônico.

CONCLUSÃO: O linfedema genital pode ser causado pela associação de lesão por injeção de silicone líquido e radioterapia na região pélvica. As decisões no tratamento de neoplasias em pacientes previamente submetidos a injeção de silicone líquido devem levar em conta esse fato, já que pode representar fator de risco para complicações de tratamento radioterápico.

INTRODUCTION

Lymphedema is a condition of fluid retention in subcutaneous tissue caused by lymphatic obstruction. This implies accumulation of water, macromolecules and proteins in the extracellular space, with impaired influx of leukocytes. Consequently, tissue elasticity is lost, connective tissue deteriorates and infectious processes can can spread more easily. In the chronic phase, lymphedema is also characterized by deposition of fat and fibrous tissue.[1,2]

In male individuals, this condition may affect the penis or scrotum, or even the entirety of the genital tissue. It is a condition rarely found in developed countries and may have primary etiology (attributed to hypoplasia of the lymphatic vessels) or secondary etiology, as in cases attributed to malignancies, infections, radiotherapy or lymphadenectomy. In tropical regions, it is commonly associated with infections by the parasite *Wuchereria bancrofti* and it has been estimated that up to 20% of men are affected by lymphedema. Idiopathic lymphedema is rare, and is related to genetic disorders such as Milroy's or Meige's diseases.[3,4] Moreover, genital lymphedema, especially when chronic, affects the patient's social relations and impairs sexual and urinary functions.

Data from the literature regarding treatments of lymphedema show that conservative therapies (such as use of diuretics, elevation of the affected segment and use of scrotal suspenders) provide poor results, especially in chronic forms with significant fibrosis. In 1820, Delpech described the first surgical technique directed towards resection of lymphedematous tissue and reconstruction using local flaps, which represented a viable option for the treatment of chronic genital lymphedema. Subsequently, novel reconstructive alternatives were developed, with use of posterolateral scrotal flaps, as described by Vaught et al.,[5] or a combination of scrotal and perineal flaps, as proposed by Halperin et al.[1]

CASE REPORT

A 36-year-old male-to-female transgender patient was referred to the Plastic Surgery Division with a six-month history of genital lymphedema. She had a past record of AIDS, and had been irregularly treated and followed up at the Infectious Diseases Clinic of our institution for about ten years.

In addition, the patient was attending the Colorectal Surgery Clinic, after being treated for anal canal epidermoid carcinoma. This had been treated non-surgically, with radiotherapy and chemotherapy at the patient's request and in collaboration with the oncology team. A total of 12 radiotherapy sessions were undertaken, finishing one year prior to presentation to our clinic.

The treatment of the anal cancer was successful, with clinically complete remission of the disease. The patient then presented with complaints of inguinal swelling and pain, along with local edema and erythema, but without fever. A potential relapse of the previous malignancy was suspected and computed tomography scans revealed multiple nodules in the gluteal and thigh regions, some partially calcified, along with bilateral inguinal and obturator lymph node enlargement.

The condition was further investigated by means of positron emission tomography, which revealed a diffuse hypermetabolic area affecting the subcutaneous tissue around the pelvis, with exogenous material, and extending to the hypogastric, scrotum and proximal thigh regions. This was compatible with an extensive inflammatory process, and a focal hypermetabolic area was observed in the right inguinal region, probably corresponding to a lymph node. Magnetic resonance imaging (**Figure 1**) showed diffuse subcutaneous edema, more prominently at the scrotum and penis, as well as lipoma-like masses along the left spermatic cord. After the imaging results had been obtained, the patient revealed that she had previously undergone liquid silicone injections to the buttocks and thighs for esthetic purposes.

As there was no sign of recurrent malignancy, the patient was referred for plastic surgery and urology consultations. The initial physical examination showed significant enlargement of the scrotum, with erythematous tender infiltrated skin. The testicles were not palpable and there was loss of penile shaft contour, with no visible transition between its ventral surface and the scrotum (**Figure 2**).

After multispecialty discussion, it was decided with the patient's consent to excise all the affected scrotal tissue while preserving the penis and testicles, and to reconstruct the perineal defect with skin grafts or local thigh flaps. The patient did not desire scrotal reconstruction.

The surgical procedure was performed jointly by the plastic surgery and urology teams, starting with dissection of the testicles and spermatic cords (**Figure 3**). Identification and excision of a hard mass from the left cord was performed, and this mass was

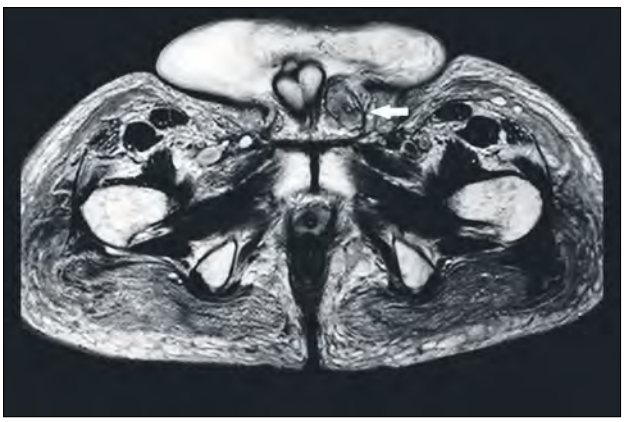

Figure 1. Pelvic magnetic resonance image showing enlargement and infiltration of scrotal soft tissues and presence of nodular images in subcutaneous tissue due to silicone infiltration, such as observed near the base of the penile shaft (arrow).

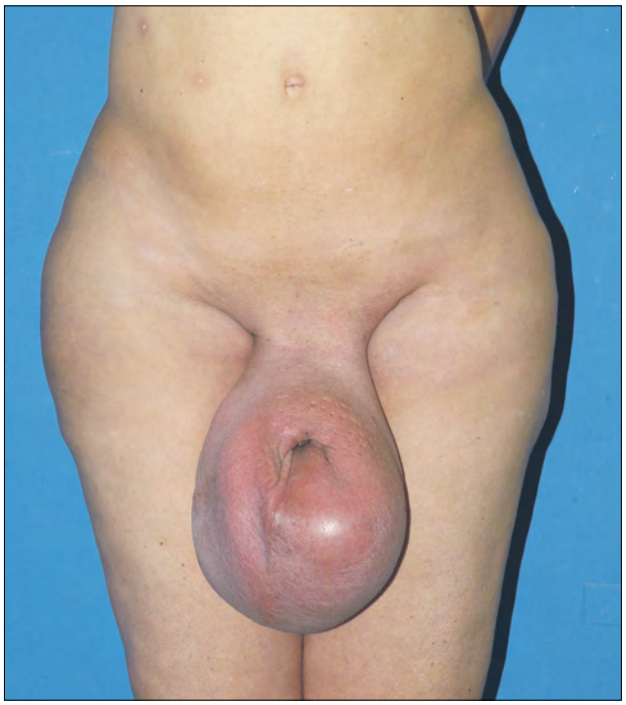

Figure 2. Preoperative appearance of the patient showing massive scrotal enlargement with edematous and erythematous skin and loss of penile shaft contour.

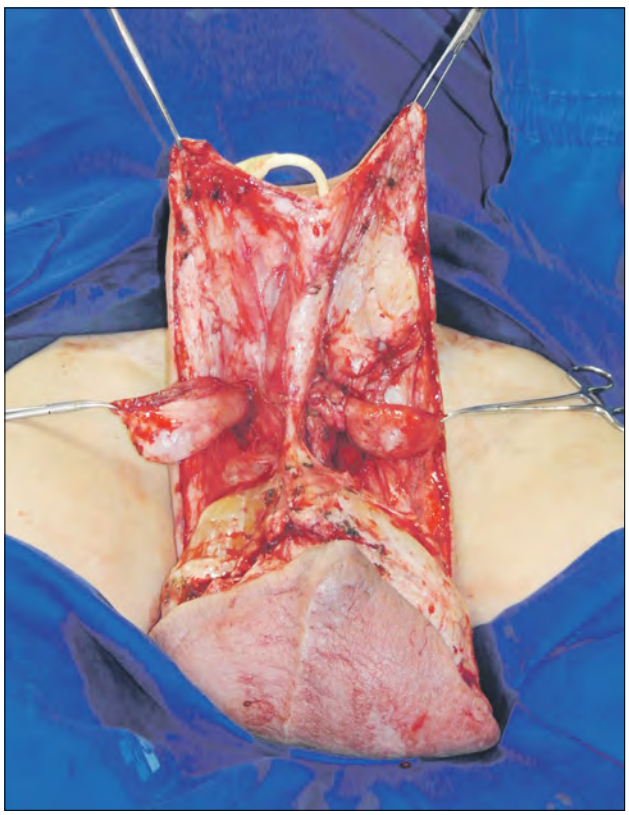

Figure 3. Intraoperative view of the lymphedematous tissue dissection, preserving both testicles, and the lateral skin flap design used for reconstruction.

later shown through pathological analysis to be compatible with chronic lymphedema and stromal alterations relating to previous radiotherapy.

Subsequently, the penile shaft was separated from the scrotal tissue, while preserving lateral unaffected skin flaps. The product of scrotal tissue excision weighed 1,430 g and was also sent for pathological examination. There was no need for skin grafts or thigh flaps for reconstruction, since the remaining lateral skin permitted primary closure of the wound without scrotal reconstruction, as desired by the patient (**Figure 4**).

The histopathological analysis on the resected tissue revealed signs of chronic lymphedema, and presence of frequent lipoblast-like multivacuolated cells, which could be explained by the presence of exogenous material such as liquid silicone phagocytized by macrophages (**Figure 5**). Immunohistochemical analysis revealed that these cells were positive for CD68 and negative for S100 protein. There were also areas with extracellular empty pseudocysts of different sizes and scattered perivascular and interstitial inflammatory infiltrate, predominantly lymphomononuclear. Special stains did not show any mycobacteria or fungus.

The postoperative period was mostly uneventful. The patient presented a single complication consisting of minor dehiscence of the superficial operative wound in the ventral portion of the penis, which was managed with dressings. The final result was considered satisfactory by both the patient and the surgical team, with no signs of lymphedema relapse after nearly one year of follow-up (**Figure 6**).

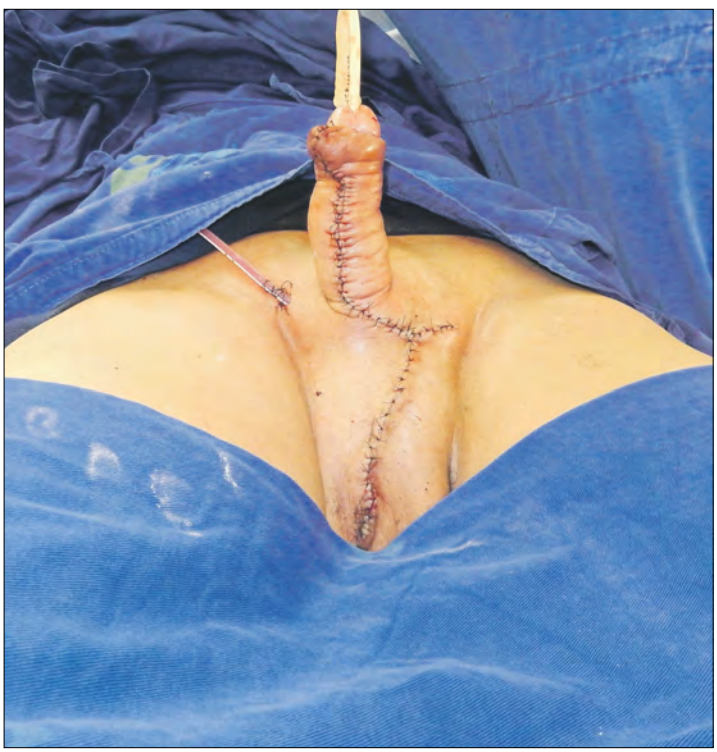

Figure 4. Immediate postoperative result.

DISCUSSION

Genital lymphedema is a disorder that implies complex treatment, especially in its chronic form with tissue fibrosis. It is a debilitating and psychologically stressing condition.[1,6–10] In the present case, the patient presented acquired scrotal lymphedema, probably caused by radiotherapy. The presence of liquid silicone in the subcutaneous tissue and pelvic lymph nodes was also a contributory factor.

Some cases of scrotal lymphedema associated with radiotherapy are presented in the literature, and there is a single case of lower-limb lymphedema in a patient who previously underwent liquid silicone injection to the gluteal and thigh regions for esthetic purposes (**Table 1**).[11] However, to date, there has been no report of these two factors occurring together as a cause of lymphedema, and our case is thus the first one.

It could be postulated that radiotherapy tissue damage acted as a precipitating factor for dysfunction in a lymph drainage system that was already impaired by the presence of liquid silicone. Histopathological findings compatible with infiltration of the scrotal tissue by liquid silicone corroborate the hypothesis that the material would be a contributory factor for development of lymphedema. In the previous description, both the regional lymphadenopathy and the presence of silicone granulomas, which exert vascular extrinsic compression, were reported to be causative mechanisms for compromised lymphatic drainage.[11] Furthermore, in another report, patients who underwent injections in the pelvic area were described as presenting symptoms such as lower-extremity edema and varices, which were attributed to inguinal adenopathy and vascular compression due to local fibrosis.[12]

Although the initial studies demonstrated that injectable silicone is biologically inert, subsequent reports have shown the presence of inflammatory reaction associated with its use as filler.[13] It has been discussed in the literature whether this reaction would take place against the silicone itself, or against its degradation products, or against impurities in or contamination of the substance applied.[13] Histological findings associated with liquid silicone application may include extracellular empty pseudocysts of different sizes, reminiscent of a "Swiss cheese pattern", and macrophages with

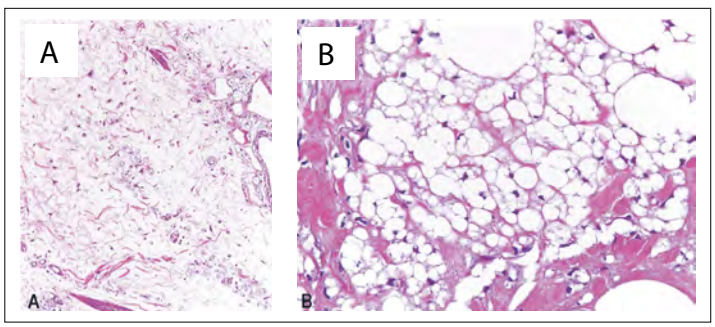

Figure 5. A: marked stromal edema with scattered macrophages containing intracytoplasmic clear vacuoles (100 x, hematoxylin and eosin, HE); B: extracellular empty pseudomicrocysts (top and bottom right) and numerous macrophages with intracytoplasmic clear vacuoles of different sizes, sometimes scalloping the nucleus and thus providing an appearance similar to a lipoblast (400 x, HE).

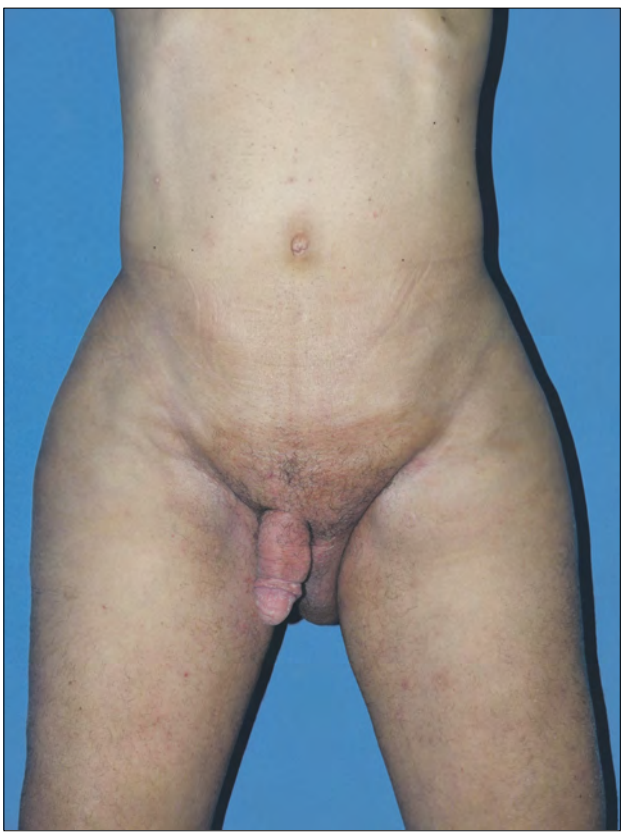

Figure 6. Postoperative result after a 10-month follow-up with no lymphedema relapse.

Table 1. Search strategies used for electronic databases

Database	Search strategy	Articles found	Relevant articles
MEDLINE (PubMed)	((((“Silicones”[Mesh]) OR “Silicone Gels”[Mesh]) OR “Silicone Oils”[Mesh] OR Silicone OR Silicones OR (Silicone Gel) OR (Silicone Gels) OR (Silicone Oil) OR (Silicone Oils))) AND (“Lymphedema”[Mesh]) OR Lymphedema)	17	2
LILACS (BVS)	mh: “Silicones” OR mh: “Silicone Gels” OR mh: “Silicone Oils” OR mh: “Aceites de Silicona” OR mh: “Óleos de Silicone” OR mh: “Geles de Silicona” OR mh: “Géis de Silicone” AND mh: “Lymphedema” OR mh: “Linfedema”	40	1

intracytoplasmic clear vacuoles, sometimes indentating the nucleus and providing an appearance similar to a lipoblast. This last finding may lead to misdiagnosis of liposarcoma, since lipoblasts are often found in this malignancy. Clinical-pathological correlation is important, since this provides information on the history and site of silicone injection.[14] Immunohistochemically, it is expected that pseudolipoblasts present in the silicone foreign body reaction will be negative for S100 protein, unlike many liposarcomas, which present lipoblasts that are positive for this marker. Our patient was indeed S100-negative.

The results from the surgical procedure were deemed satisfactory. Local reconstruction was achieved by means of primary closure in a single operation, without erectile dysfunction or impairment of penile sensitivity. Because the patient is male-to-female transgender, she considered that absence of a scrotal sac was a positive outcome that was more appropriate for her gender identity.

CONCLUSION

The presence of liquid silicone injected for esthetic purposes may represent a risk factor for development of lymphedema in patients who undergo radiotherapy at the same anatomical site, thus potentiating the occurrence of this rare complication.

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Boerhaave syndrome and black esophagus

Síndrome de Boerhaave e esôfago preto

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Dear Editor,

It was with great interest that I read an article by Dr. Dinic and colleagues on Boerhaave syndrome in the latest issue of your journal.[1] They describe a rare case of spontaneous esophageal perforation in the setting of hematemesis in association with duodenal ulcer and black esophagus.

Boerhaave syndrome is an unusual entity in clinical medicine that was historically described by classical physical examination findings of the Mackler triad (vomiting, chest pain and subcutaneous emphysema), Hamman's mediastinal crepitus with heartbeat and pneumomediastinum on X-ray imaging. Computed tomography scans showing Gastrografin extravasation are diagnostic on call to the operating room.[2] Patients typically appear tachypneic and acutely ill.

The case of esophageal perforation presented by Dinic et al. is intriguing for several reasons, primarily because of the presence of a rare finding of distal black esophagus on postmortem examination. Black esophagus or acute esophageal necrosis is a syndrome that is classically characterized by a distal esophagus with circumferential black appearance of varying proximal extent. It presents clinically with gastrointestinal hemorrhage and is often associated with duodenal ulcer disease.[3-5] The patient's clinical history and a four day prodrome may be a clue to the development of black esophagus.

It is true that profound retching with a sudden rise in intraesophageal pressure against a closed cricopharyngeus may result in spontaneous distal esophageal perforation, as seen in Boerhaave syndrome. On the other hand, blind passage of the nasogastric tube in the setting of a necrotic esophagus would potentially cause a longitudinal tear of similar appearance at the weak point just above the gastroesophageal junction. In fact, such a procedure should be avoided.[4] It is also conceivable that repeated vomiting in a patient with black esophagus may result in Boerhaave syndrome. If so, this would be the first such occurrence described in the literature.

It is difficult to retrospectively point out the correct underlying diagnosis in this unfortunate case, but the possibility of an iatrogenic esophageal tear should be considered in this patient with concurrent finding of black esophagus.

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RESPONSE TO LETTER TO THE EDITOR

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Dear Sir,

I am glad that our work has sparked your interest. The study[1] presents a case of a patient with spontaneous rupture of the esophagus caused by vomiting. In Figure 2, we presented the esophagus with its surface covered with blood (not washed). The wall of the esophagus was not necrotized (shown histopathologically), and it was not a black esophagus.[2,3] Setting the nasogastric tube in this case was risky; however, it was necessary in order to evacuate the heavy liquid content of the stomach (detected on abdominal ultrasound), as well as to perform esophagogastroduodenoscopy (EGD). The patient had the same symptoms before placing the probe and after removing it (before EGD), and so we ruled out the possibility that she might have caused the rupture herself.

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What do Cochrane systematic reviews say about interventions for autism spectrum disorders?

O que as revisões sistemáticas Cochrane falam sobre intervenções para os transtornos do espectro autista?

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KEY WORDS:
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PALAVRAS-CHAVE:
Transtorno do espectro autista.
Terapêutica.
Revisão.
Prática clínica baseada em evidências.
Medicina baseada em evidências.

ABSTRACT

CONTEXT AND OBJECTIVE: Autism spectrum disorders (ASDs) include autistic disorder, Asperger's disorder and pervasive developmental disorder. The manifestations of ASDs can have an important impact on learning and social functioning that may persist during adulthood. The aim here was to summarize the evidence from Cochrane systematic reviews on interventions for ASDs.

DESIGN AND SETTING: Review of systematic reviews, conducted within the Discipline of Evidence-Based Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo.

METHODS: We included and summarized the results from Cochrane systematic reviews on interventions for ASDs.

RESULTS: Seventeen reviews were included. These found weak evidence of benefits from acupuncture, gluten and casein-free diets, early intensive behavioral interventions, music therapy, parent-mediated early interventions, social skill groups, Theory of Mind cognitive model, aripiprazole, risperidone, tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI); this last only for adults. No benefits were found for sound therapies, chelating agents, hyperbaric oxygen therapy, omega-3, secretin, vitamin B6/magnesium and SSRI for children.

CONCLUSION: Acupuncture, gluten and casein-free diets, early intensive behavioral interventions, music therapy, parent-mediated early interventions, social skill groups and the Theory of Mind cognitive model seem to have benefits for patients with autism spectrum disorders (very low to low-quality evidence). Aripiprazole, risperidone, tricyclic antidepressants and SSRI (this last only for adults) also showed some benefits, although associated with higher risk of adverse events. Experimental studies to confirm a link between probable therapies and the disease, and then high-quality long-term clinical trials, are needed.

RESUMO

CONTEXTO E OBJETIVO: Os transtornos do espectro autista (TEA) incluem autismo, doença de Asperger e transtorno global do desenvolvimento. As manifestações dos TEA podem ter importante impacto na aprendizagem e funcionamento social, que pode persistir durante a fase adulta. O objetivo foi resumir as evidências de revisões sistemáticas Cochrane sobre intervenções para TEA.

TIPO DE ESTUDO E LOCAL: Revisão de revisões sistemáticas conduzida na Disciplina de Medicina Baseada em Evidências da Escola Paulista de Medicina, Universidade Federal de São Paulo.

MÉTODOS: Nós incluímos e resumimos os resultados de revisões sistemáticas Cochrane sobre intervenções para TEA.

RESULTADOS: Foram incluídas 17 revisões que encontraram evidências fracas de benefícios da acupuntura, dietas isentas de glúten e caseína, intervenção comportamental intensiva precoce, musicoterapia, intervenção precoce mediada pelos pais, grupos de habilidades sociais, modelo cognitivo de Teoria da Mente, aripiprazol, risperidona, antidepressivos tricíclicos, inibidores seletivos da recaptação da serotonina (ISRS); o último apenas para adultos. Nenhum benefício foi encontrado com terapias sonoras, agentes quelantes, oxigenoterapia hiperbárica, ômega-3, secretina, vitamina B6/magnésio e ISRS para crianças.

CONCLUSÃO: Acupuntura, dietas sem glúten e caseína, intervenção comportamental intensiva precoce, musicoterapia, intervenção precoce mediada pelos pais, grupos de habilidades sociais e modelo cognitivo de Teoria da Mente parecem ter benefícios para pacientes com TEA (evidência de qualidade muito baixa a baixa). Aripiprazol, risperidona, antidepressivos tricíclicos e ISRS (o último apenas para adultos) também apresentam algum benefício, embora estejam associados a maior risco de eventos adversos. Estudos experimentais confirmando a relação entre prováveis terapias e a doença, e então ensaios clínicos de alta qualidade e de longo seguimento, são necessários.

INTRODUCTION

Autism spectrum disorders (ASDs) have an estimated prevalence ranging from 3.3 to 116 children per 10,000 and it is widely accepted that autism affects approximately 1% of children worldwide.[1] This may reflect increased access to diagnosis, concern among healthcare professionals and parents, more sensitive diagnostic criteria and/or a true increase in prevalence.[2]

ASDs include autistic disorder, Asperger's disorder and pervasive developmental disorder. Patients with these conditions present deficits in communication, social interaction and cognitive function; problems with feeding; hypo or hypersensitivity; and, sometimes, self-harmful behavior.[3] With greater severity of ASDs, activities of daily living are limited and the impairments of ASDs can have an important impact on learning and social functioning that may persist during adulthood.[4]

Healthcare and social care for people with ASDs is frequently complex, since they are more likely to have mental health comorbidities and suicidal ideation.[5] Assessments need to be multidisciplinary and developmental, and early detection is determinant. Therapeutic interventions need to be personalized, focusing on the specific clinical features presented by each patient.[6]

Individual or parent-based psychosocial interventions are frequently used in clinical practice, with the aim of improving clinical features relating to communication, cognition, behavior and relationships, for instance. Pharmacological therapy is an option for mental health comorbidities. New therapeutic approaches include genetic and pharmacological strategies for reducing synthesis of specific proteins and inhibiting pleiotropic growth factors.[7] Concerning the time for starting the treatment, randomized controlled trials have added new evidence that, for many children aged up to three years, early intervention can improve outcomes, thus increasing the potential benefits of early diagnosis facilitated by early screening.[8,9]

Despite recent advances in this area, the evidence regarding autism remains limited and this condition continues to be a challenge for researchers, healthcare professionals, patients, parents and caregivers.

OBJECTIVES

To map out and summarize all Cochrane systematic reviews on interventions for autism spectrum disorders, and present the results on the basis of the quality of the evidence.

METHODS

Design

Review of Cochrane systematic reviews on interventions for ASD.

Setting

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

Criteria for including reviews

Types of study

We included completed Cochrane systematic reviews, with no restriction regarding date of publication. Protocols for systematic reviews and reviews signaled as "withdrawn" in the Cochrane Database of Systematic Reviews (CDSR) were not considered. We included only the latest version of each review.

Types of participants

Individuals with ASD.

Types of interventions

All types of interventions (pharmacological and non-pharmacological) aiming to treat ASD.

Type of outcomes

Clinical and laboratory outcomes were considered, as presented by the systematic reviews.

Searching for reviews

We conducted systematic searches in the Cochrane Database of Systematic Reviews (CDSR) (via Wiley) using a sensitive search strategy (**Table 1**).

Selecting reviews

Three reviewers evaluated the titles and abstracts of records that had initially been retrieved on the basis of the inclusion criteria. The full texts of records with the potential for inclusion were read to confirm whether they should be included (reasons for exclusions were recorded and presented). Divergences between reviewers were resolved through reaching a consensus.

Presenting the results

We used a narrative structure (qualitative synthesis) to present the results from the systematic reviews included.

RESULTS

Search results

The initial search retrieved 24 reviews. From these, we excluded six reviews addressing other clinical situations (X fragile syndrome,

Table 1. Search strategy and results from Cochrane Database of Systematic Reviews (conducted on November 22, 2016)

#1	"autism spectrum disorder" in Title, Abstract, Keywords
#2	"autism" in Title, Abstract, Keywords
#3	"Asperger" in Title, Abstract, Keywords
#1 OR #2 OR #3	(in Cochrane Reviews)

prematurity and attention deficit hyperactivity disorder) and one that was a previous version of a review that we included. Thus, we included 17 systematic reviews.[4,10-25]

Results from systematic reviews

A summary of each systematic review is presented narratively below. In addition, **Table 2**[4,10-25] presents the issues addressed, the main findings from each systematic review and the quality of the evidence (based on the GRADE approach).[26]

1. Acupuncture

The review[4] considered randomized controlled trials (RCTs) and quasi-randomized controlled trials and included 10 studies (from low to high methodological quality) on 390 children aged from 3 to 18 years who presented ASDs and received acupuncture for periods ranging from four weeks to nine months. The following results were found:

- Needle acupuncture (by means of manual or electrical stimulation) versus sham: no difference in core autistic features. On the Ritvo-Freeman Real Life Rating Scale (RFRLRS): mean difference (MD) = 0.09; 95% confidence interval (CI) = -0.03 to 0.21; P = 0.16. Acupuncture seemed to result in better language comprehension, cognition, selfcare and social functioning, and a higher probability of improvement in overall functioning greater than or equal to 25%.
- Needle acupuncture (by means of manual or electrical stimulation) versus no treatment: acupuncture seemed to improve autistic features, speech, cognition, overall functioning, selfcare, sensory functioning, imitation, repetitive behavior and physical health.
- Acupressure versus no treatment: acupressure might result in improvement in overall functioning, communication attitude, non-verbal communication and matching, and in language and social interaction.

Most of the favorable outcomes were observed only in a single study and few outcomes were supported by pooled results from more than one study. The majority of the effect size estimates for the significant outcomes had imprecisions with a wide confidence interval. These limitations mean that the significant result was not sufficiently robust to enable a reliable conclusion.

Therefore, the authors concluded that the current evidence did not support use of acupuncture for treating ASDs. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD007849.pub2/abstract.

2. Aripiprazole

Antipsychotics have been used as medications for ASD-related irritability. The review[10] included three RCTs on children and adolescents (aged from 9 to 17 years): two were short-term studies (eight weeks) on the effects of aripiprazole on behavioral problems among 316 children/adolescents; one was a longer-term study (up to 16 weeks) in which 85 children/adolescents whose symptoms initially improved through aripiprazole were withdrawn from the medication to assess whether their behavioral problems would recur. All three RCTs were supported by Bristol-Myers Squibb (Princeton, NJ, United States) and Otsuka Pharmaceutical Company, Ltd. (Tokyo, Japan), and had editorial support from Ogilvy Healthworld Medical Education and Bristol-Myers Squibb. The results from a meta-analysis showed that there was an improvement favoring aripiprazole, in comparison with placebo, for the following outcomes:

- Aberrant Behavior Checklist (ABC) - irritability subscale (MD = -6.17 points; 95% CI = -9.07 to -3.26; two RCTs; 308 participants; moderate-quality evidence);
- ABC hyperactivity subscale (MD = -7.93 points; 95% CI = -10.98 to -4.88; two RCTs; 308 participants, moderate-quality evidence);
- ABC stereotypy subscale (MD = -2.66 points; 95% CI = -3.55 to -1.77; two RCTs; 308 participants, moderate-quality evidence).

In terms of side effects, aripiprazole was associated with greater increase in weight (MD = 1.13 kg; 95% CI = 0.71 to 1.54; two RCTs; 308 participants; moderate-quality evidence), higher risk of sedation (risk ratio [RR] = 4.28; 95% CI = 1.58 to 11.60; two RCTs; 313 participants; moderate-quality evidence) and tremor (RR = 10.26; 95% CI = 1.37 to 76.63; two RCTs; 313 participants; moderate-quality evidence). The discontinuation RCT study showed no difference in relapse rate, with regard to symptoms of irritability (hazard ratio [HR] = 0.57; 95% CI = 0.28 to 1.12; 85 participants; low-quality evidence).

The authors concluded that aripiprazole might be effective as a short-term intervention for some behavioral aspects of ASDs among children/adolescents. However, they stated that notable side effects, including weight gain, sedation, drooling and tremor, needed to be considered. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009043.pub3/abstract.

3. Auditory integration training and other sound therapies

Auditory integration therapies (including Tomatis therapy and Samonas sound therapy) are techniques for improving abnormal sound sensitivity in individuals with behavioral disorders including ASDs. The review[11] identified six RCTs that used auditory integration therapy and one that used Tomatis therapy (182 participants aged three to 39 years). Meta-analysis was not possible due to the huge heterogeneity among the RCTs or the reports of data in unusable forms. Three RCTs did not show any benefit from auditory

Table 2. Characteristics and main results from systematic reviews included

Intervention	Details of intervention	Results regarding effectiveness and safety	Quality of evidence (GRADE approach)*
Acupuncture[4]	Manual or electrical acupuncture (four weeks to nine months) versus sham or no intervention	Acupuncture seemed to result in better language comprehension, cognition, selfcare, social functioning and overall functioning. Acupressure seemed to improve some autistic features, speech, cognition, overall functioning, self-care, sensory functioning, imitation, repetitive behavior, physical health, communication attitude, nonverbal communication and matching, and language and social interaction.	Very low
Aripiprazole[10]	Aripiprazole 5, 10 or 15 mg/day for eight weeks	Aripiprazole can be effective over short term for some behavioral aspects of ASDs in children/adolescents. Weight gain, sedation, drooling and tremor were more frequent with aripiprazole. Aripiprazole discontinuation was not associated with lower relapse rates (irritability symptoms)	Moderate to low
Auditory integration training and other sound therapies[11]	Auditory integration training or Tomatis	No evidence suggesting benefits	Very low
Chelating agents[12]	Multiple doses of oral dimercaptosuccinic acid for up to six months for children with ASDs who excrete high levels of heavy metals	No evidence suggesting benefits	Very low
Early intensive behavioral intervention[13]	Early intensive behavioral intervention over a period of 14 months to 36 months	Evidence from non-randomized trials suggesting benefits for adaptive behavior, intelligence quotient, expressive language, receptive language, daily communication skills, socialization and daily living skills	Low
Gluten and casein-free diets[14]	Diet for 12 weeks or 12 months	Evidence suggesting benefits for overall autistic traits and overall ability to communicate and interact	Very low
Hyperbaric oxygen therapy[15]	Hyperbaric oxygen therapy (pressure of 1.5 ATA with 100% oxygen), 20 one-hour sessions held on weekdays over 10 weeks	Evidence suggesting no improvement in social interaction and communication, behavioral problems, communication and linguistic abilities, or cognitive function. More adverse events in hyperbaric oxygen group.	Low
Music therapy[16]	Music therapy for one week to seven months	Evidence suggesting benefits for: social interaction within the therapy context, general social interaction outside therapy context, nonverbal communicative skills within the therapy context, verbal communicative skills, initiating behavior, social-emotional reciprocity, social adaptation, joy and quality of parent-child relationships No benefits regarding nonverbal communicative skills outside of the therapy context None of the studies reported adverse events.	Low to moderate
Omega-3 fatty acids[17]	Capsule or paste, at doses ranging from 1.3 g/day (0.7 g of eicosapentaenoic acid and 0.46 g of docosahexaenoic acid) to 1.5 g/day (0.84 g of eicosapentaenoic acid and 0.7 g of docosahexaenoic acid), for six to 12 weeks.	No benefits for social interaction, communication, stereotypy or hyperactivity	Low
Parent-mediated early intervention[18]	Home-based and/or center or clinic-based interventions, delivered on a one-to-one basis or to groups of parents or self-training from a manual and videotapes.	No benefit for most of the primary outcomes (language and communication, child initiations in observed parent-child interaction, child's adaptive behavior and parents' stress). Strong and statistically significant benefits for shared attention and parent synchrony. Evidence suggestive of improvement in child vocabulary comprehension, and reduction in the severity of autism characteristics.	Low

Continue...

Table 2. Continuation

Intervention	Details of intervention	Results regarding effectiveness and safety	Quality of evidence (GRADE approach)*
Risperidone[19]	Risperidone (2.5 mg to 10 mg/day) for 8 to 12 weeks.	Evidence suggesting benefits for irritability, repetition and social withdrawal. Higher risk of adverse events, especially weight gain.	Not assessed
Secretin (intravenous)[20]	Porcine or synthetic human secretin in single or repeated doses, ranging from 2 IU/kg, synthetic secretin in a single dose of 4 IU/kg dose.	No benefits for core features of ASDs	Not assessed
Selective serotonin reuptake inhibitors[21]	• Fluoxetine (10 to 80 mg/day for 8 to 12 weeks) • Fluvoxamine (up to 300 mg/day for 9 to 12 weeks) • Fenfluramine (twice-daily divided dose totaling 1.5 mg/kg, for 3 months) • Citalopram (up to 30 mg/day)	No evidence of benefits for children and limited evidence for adults.	Not assessed
Social skill groups[22]	One to 25 sessions per week, lasting 60 to 90 min, for 5 to 20 weeks.	Evidence suggesting benefits for overall social competence, friendship quality and loneliness. No benefit for emotional recognition, social communication relating to understanding idioms, or child or parental depression.	Low
Theory of Mind cognitive model[23]	Theory of Mind cognitive models delivered by computer program, specially-designed cartoons, one-to-one therapist-led intervention, teacher-training in classroom, for two weeks to six months.	Evidence suggesting benefits for communication based on individual, social interaction and general communication.	Low to very low
Tricyclic antidepressants[24]	Clomipramine or tianeptine for 5 to 7 weeks (no specified doses)	Tianeptine could be effective over short term for reducing irritability, hyperactivity, inadequate eye contact and inappropriate speech (according to parents' assessment, but clinician ratings did not confirm these findings) Tianeptine was associated with significant adverse effects, including drowsiness and reduced activity levels. Clomipramine could be effective for improving autistic symptoms, irritability and obsessive-compulsive disorder symptoms.	Not assessed
Vitamin B6 plus magnesium[25]	Vitamin B6 30 mg/kg/day (up to 1 g) and magnesium 10 mg/kg/day (up to 350 mg/day)	No benefits for social interaction, communication, compulsivity, impulsivity, or hyperactivity.	Not assessed

ASD = autism spectrum disorders; *GRADE = Grading of Recommendations Assessment, Development and Evaluation. Tool used for assessing the quality of the body of the current evidence. High quality: low probability that further studies will change the confidence regarding the existing evidence. Moderate quality: some probability that further studies will change the confidence regarding the existing evidence. Low quality: high probability that further studies will change the confidence regarding the existing evidence. Very low quality: there is much uncertainty about the information, precluding any valid interpretation.[26]

integration therapy over control interventions. Three RCTs reported improvements from auditory integration therapy on the Aberrant Behavior Checklist, after three months, but they used a total score rather than subgroup scores, which is questionable. The study addressing Tomatis therapy did not find any difference between the treatment and control conditions regarding language.

The authors concluded that, considering the methodological limitations of the existing RCTs, no evidence supporting the use of auditory integration therapy or other sound therapies was available up to that time. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003681.pub3/abstract.

4. Chelating agents for autism spectrum disorders (ASDs)

It has been suggested that the severity of autism spectrum disorder (ASD) symptoms is associated with the levels of serum or

stored toxic metals, and that chelating agents improve symptoms. The review[12] aimed to assess the effects of these agents on ASDs and included one randomized clinical trial (RCT) with 49 children. This RCT compared multiple doses of oral dimercaptosuccinic acid (DMSA), for up to six months versus placebo among children who excreted high levels of heavy metals.

The authors concluded that there was no evidence to suggest that multiple rounds of oral DMSA had any effect on ASD symptoms. Considering previous reports of serious adverse events, including hypocalcemia, renal impairment and reported death, they stated that the risks involved in chelation to treat ASDs currently outweighed the benefits. Evidence supporting a causal relation between heavy metals and autism would be needed before further trials were conducted. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD010766.pub2/abstract.

5. Early intensive behavioral intervention

Early intensive behavioral intervention (EIBI) is based on the principles of applied behavioral analysis on interventions delivered over many years for 20 to 40 hours per week. For the review,[13] the participants needed to have been less than six years of age at treatment onset and to have been assigned to their study condition prior to starting treatment. This review included one RCT and four non-randomized trials (203 participants) that used a treatment-as-usual comparison group. Positive effects favoring EIBI were found for all the following outcomes:

- Adaptive behavior (standardized mean difference using Hedges g = 0.69; 95% CI = 0.38 to 1.01; P < 0.0001);
- Intelligence quotient, IQ (g = 0.76; 95% CI = 0.40 to 1.11; P < 0.0001);
- Expressive language (g = 0.50; 95% CI = 0.05 to 0.95; P = 0.03);
- Receptive language (g = 0.57; 95% CI = 0.20 to 0.94; P = 0.03);
- Daily communication skills (g = 0.74; 95% CI = 0.30 to 1.18; P = 0.0009);
- Socialization (g = 0.42; 95% CI = 0.11 to 0.73; P = 0.0008);
- Daily living skills (g = 0.55; 95% CI = 0.24 to 0.87; P = 0.0005).

The authors concluded that there was some evidence that EIBI was effective for children with ASDs. However, they considered that the current state of the evidence was limited because the data came from non-randomized studies. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009260.pub2/abstract.

6. Gluten and casein-free diets

It has been hypothesized that peptides from gluten and casein may participate in the origins of autism and that that physiology and psychology of autism might be explained in terms of excessive opioid activity linked to these peptides. The review[14] included two small RCTs (35 participants) assessing the effects of gluten and casein-free diets for ASDs. No meta-analysis was possible, due to different outcome measurements. There were significant treatment effects favoring the dietary intervention for overall autistic traits (MD = -5.60; 95% CI = -9.02 to -2.18; P = 0.001) and the overall ability to communicate and interact (MD = 1.70; 95% CI = 0.50 to 2.90). No adverse events were reported.

The authors concluded that, despite the high rates of use of gluten and/or casein-free diets for children with ASDs, the current evidence was not robust enough to support these practices. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003498.pub3/abstract.

7. Hyperbaric oxygen therapy

It has been hypothesized that hyperbaric oxygen therapy might reduce the biochemical dysfunction and clinical symptoms of ASDs. The review[15] included one RCT (60 children) comparing hyperbaric oxygen therapy and sham treatment, and the results showed that there were no improvements in social interaction and communication, behavioral problems, communication and linguistic abilities, or cognitive function. There were more adverse events (odds ratio [OR)] = 3.87; 95% CI = 1.53 to 9.82) and more children who experienced adverse events (OR = 4.40; 95% CI = 1.33 to 14.48) in the hyperbaric oxygen group.

The authors concluded that up to that time, there was no evidence that hyperbaric oxygen therapy improved the core symptoms and associated symptoms of ASDs. They stated that it was important to consider that adverse events could occur. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD010922.pub2/abstract.

8. Music therapy

The review[16] is an updated version of a review published in 2006 that aimed to assess the effects of music therapy for ASD. Ten RCTs (165 participants) examined the short and medium-term effects of music therapy over periods of one week to seven months for children with ASDs. Music therapy was better than "placebo" therapy or the usual care for the following outcomes:

- Social interaction within the therapy context (standard mean difference [SMD] = 1.06; 95% CI = 0.02 to 2.10; one RCT; 10 participants);
- General social interaction outside of therapy context (SMD = 0.71; 95% CI = 0.18 to 1.25; three RCTs; 57 participants; moderate-quality evidence);
- Nonverbal communicative skills within the therapy context (SMD = 0.57; 95% CI = 0.29 to 0.85; three RCTs; 30 participants);
- Verbal communicative skills (SMD = 0.33; 95% CI = 0.16 to 0.49; six RCTs; 139 participants);

- Initiating behavior (SMD = 0.73; 95% CI = 0.36 to 1.11; three RCTs; 22 participants; moderate-quality evidence);
- Social-emotional reciprocity (SMD = 2.28; 95% CI = 0.73 to 3.83; one RCT; 10 participants; low-quality evidence);
- Social adaptation (SMD = 0.41; 95% CI = 0.21 to 0.60; four RCTs; 26 participants);
- Joy (SMD = 0.96; 95% CI = 0.04 to 1.88; one RCT; 10 participants); and
- Quality of parent-child relationships (SMD = 0.82; 95% CI = 0.13 to 1.52; two RCTs; 33 participants; moderate-quality evidence).

There was no difference in nonverbal communicative skills outside of the therapy context (SMD = 0.48; 95% CI = -0.02 to 0.98; three RCTs; 57 participants; low-quality evidence). None of the studies reported any adverse events. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD004381.pub3/abstract.

9. Omega-3 fatty acid supplementation

The review[17] included two RCTs (37 children) comparing omega-3 fatty acid supplementation versus placebo. No evidence was found that omega-3 supplements had any effect on social interaction (MD = 0.82; 95% CI = -2.84 to 4.48), communication (MD = 0.62; 95% CI = -0.89 to 2.14), stereotypy (MD = 0.77; 95% CI = -0.69 to 2.22) or hyperactivity (MD = 3.46, 95% CI = -0.79 to 7.70).

The authors concluded that, up to that time, there was no high-quality evidence that omega-3 fatty acid supplementation was effective for children with ASDs. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD007992.pub2/abstract.

10. Parent-mediated early intervention

Approaches that help parents develop strategies for interactions and management of behavior can be useful in cases of ASDs. The review[18] included 17 RCTs on 919 children with ASDs. The following results were found:

- No benefit for most of the primary outcomes (language and communication, child initiations in observed parent-child interaction, child's adaptive behavior and parents' stress);
- Strong and statistically significant benefits for patterns of parent-child interaction: shared attention (SMD = 0.41; 95% CI = 0.14 to 0.68) and parent synchrony (SMD = 0.90; 95% CI = 0.56 to 1.23);
- Evidence suggestive of improvement of child vocabulary comprehension reported by parents (MD = 36.26; 95% CI = 1.31 to 71.20) and reduction in the severity of children's autism characteristics (SMD = -0.30; 95% CI = -0.52 to -0.08).

For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009774.pub2/abstract.

11. Risperidone

The review[19] included three RCTs (211 adults or children) comparing risperidone (2.5 mg to 10 mg/day) with placebo over periods of eight to 12 weeks. Some benefits were found regarding irritability, repetition and social withdrawal. However, these needed to be considered in relation to the adverse events, especially weight gain.

The authors concluded that risperidone might be beneficial in relation to some features of autism, but that the evidence was limited because the existing studies had small sample sizes with short follow-ups, and because of the lack of a single standardized outcome measurement. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD005040.pub2/abstract.

12. Secretin

Secretin, a gastrointestinal hormone, has been suggested as an effective treatment for ASDs based on anecdotal evidence. The review[20] included 16 heterogeneous RCTs (two of these were conducted by Repligen, a pharmaceutical company) in which over 900 children were recruited to receive intravenous secretin (porcine or synthetic, in single or multiple doses) or placebo. Twenty-five established standardized outcome measurements assessing communication, behavior, visuospatial skills, effects and adverse events were reported.

The authors concluded that there was no evidence that secretin was effective and that therefore currently it could not be recommended and should not be administered as a treatment for ASDs. They stated that further RCTs would only be justifiable if there was any new high-quality replicable scientific evidence proving a link between secretin and ASDs. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003495.pub3/abstract.

13. Selective serotonin reuptake inhibitors (SSRIs)

The review[21] included nine heterogeneous RCTs (320 adults or children) assessing fluoxetine (three RCTs), fluvoxamine (two RCTs), fenfluramine (two RCTs) and citalopram (two RCTs). Varying inclusion criteria were used with regard to diagnostic criteria and the participants' intelligence quotient. Due to heterogeneity, the data were unsuitable for meta-analysis, except for one outcome (proportional improvement). The results did not show any evidence of positive effects from citalopram for children with ASDs (one high-quality RCT). Three small RCTs on adults showed positive outcomes for clinical global impression (CGI)

and obsessive-compulsive behavior (OCB); one RCT showed improvements in aggression; and another in relation to anxiety.

The authors concluded that there was no evidence of effects from SSRIs on children and that the evidence relating to adults was limited due to the small size of the studies, with unclear risk of bias. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD004677.pub3/abstract.

14. Social skill groups

Major difficulties in social interactions have been found to be a defining feature of individuals with ASDs. The review[22] included five RCTs (196 participants aged 6 to 21 years). The results showed that social skill groups improved overall social competence (SMD = 0.47; 95% CI = 0.16 to 0.78; P = 0.003), friendship quality (SMD = 0.41; 95% CI = 0.02 to 0.81; P = 0.04) and loneliness (SMD = -0.66; 95% CI -1.15 to -0.17; one RCT). No differences were found regarding emotional recognition (SMD = 0.34; 95% CI = -0.20 to 0.88; two RCTs), social communication in relation to understanding idioms (SMD = 0.05; 95% CI = -0.63 to 0.72; one RCT) or child or parental depression. No adverse events were reported. Given the nature of the intervention and the outcome measurements selected, the risks of performance and detection bias were high. There was only limited external validity for these results, since all the RCTs were conducted in the United States, focusing mainly on children aged 7 to 12, and the participants were all of average or above average intelligence.

The authors concluded that there was some evidence that social skill groups might improve social competence for some children and adolescents with ASDs. They stated that further research was needed in order to reach conclusions that would be more robust, especially with regard to improvements in quality of life. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008511.pub2/abstract.

15. Theory of Mind cognitive model

The Theory of Mind (ToM) model suggests that people with ASDs have difficulty in understanding the minds (emotions, feelings, beliefs and thoughts) of other people. Interventions to teach ToM for patients could provide some benefits for ASDs. The review[23] included 22 heterogeneous RCTs (695 participants). Evidence of some benefit was found for communication based on individual results (three RCTs; very low-quality evidence), social interaction (11 RCTs; low-quality evidence), general communication (4 RCTs; very low-quality evidence) and ToM ability (4 RCTs; very low-quality evidence). The meta-analysis showed that interventions targeting recognition of emotions across age groups and working with people within the average range of intellectual ability had a positive effect on the target skill (through a test using photographs of faces; mean increase = 0.75 points; 95% CI = 0.22 to 1.29 points; four RCTs; 105 participants). It was found that therapist-led joint-attention approaches could improve joint attention behavior within adult-child interactions (mean increase = 0.55 points; 95% CI = 0.11 to 0.99 points; two RCTs; 88 participants). However, further analysis undermined this conclusion by demonstrating that there was no clear evidence that intervention could improve joint-attention initiations as measured using a standardized assessment tool (mean increase = 0.23 points, 95% CI = -0.48 to 0.94 points; three RCTs; 92 participants). No adverse effects were observed.

The authors concluded that there was some evidence that teaching ToM to people with ASD seemed to present benefits. However, inconsistencies in the findings and measurements meant that that the evidence was of very low or low quality, which reduced the confidence in these findings. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008785.pub2/abstract.

16. Tricyclic antidepressants

Because of the impact of tricyclic antidepressants (TCAs) on serotonins, they have been used to treat ASDs. The review[24] included three RCTs assessing clomipramine (two RCTs) or tianeptine (one RCT), for children and young adults with ASDs. Due to heterogeneity among the study participants, types of TCAs and outcomes measured, no meta-analysis was performed. One study showed that tianeptine could be effective over the short term for reducing irritability, hyperactivity, inadequate eye contact and inappropriate speech, but clinician ratings found that it did not have any significant impact on these symptoms. There were also significant adverse effects, including increased drowsiness and reduced activity levels. For clomipramine, there was evidence of improvement in autistic symptoms, irritability and obsessive-compulsive disorder symptoms. However, there was conflicting evidence in relation to hyperactivity across the two studies on clomipramine, and no significant changes were found regarding inappropriate speech. There were significant dropout rates in the clomipramine arm of one study.

The authors concluded that since there was limited and conflicting evidence regarding the effects and side effects of TCAs for this purpose, further research would be required before TCAs could be recommended for ASDs. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008372.pub2/abstract.

17. Vitamin B6 plus magnesium

The review[25] included three small heterogeneous RCTs (33 participants) and pooling the data was not possible. One RCT did

not report sufficient data to be analyzed. The second RCT did not find any significant differences between the treatment and placebo groups regarding social interactions, communication, compulsivity, impulsivity or hyperactivity. The last RCT focused on a subgroup of children with pervasive developmental disorders (PDDs) who exhibited clinical features similar to those with pyridoxine-dependent epilepsy. This small study (n = 8) only measured IQ and social quotient and found that there was a statistically significant benefit from vitamin B6 plus magnesium for IQ (MD = 5.2; 95% CI = 0.2 to 10.3).

The authors concluded that due to the small number of studies, the methodological quality of studies and the small sample sizes, no recommendations could be made regarding use of vitamin B6 plus magnesium for ASDs. For further details, the original abstract can be consulted, available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003497.pub2/abstract.

DISCUSSION

This review found that despite increasing prevalence of ASDs, there are still few systematic reviews and there is a scarcity of high-quality randomized trials addressing interventions for improving the main clinical features of ASDs. None of the reviews included here provided high-quality evidence for any outcome. Even more surprisingly, some of the interventions studied do not have any proven pathophysiological link with the disease or any well-known potential therapeutic mechanism (such as gluten and casein-free diets, omega-3 fatty acids and chelating agents). Furthermore, many of the off-label interventions used present no benefit for patients and could also increase the risk of adverse events.

Overall, the primary studies from each of the systematic reviews included had limited methodological quality, small sample sizes, short-term measurements of outcomes and poor reporting of adverse events. All of these facts increase the uncertainty surrounding the effects of the pharmacological and non-pharmacological interventions that are frequently used in clinical practice as options for patients with ASDs.

Regarding the implications for practice, some non-pharmacological interventions seem to be useful, given that they present some beneficial effects with few adverse events (albeit based on very low to low-quality evidence). These include gluten and casein-free diets, acupuncture, early intensive behavioral intervention, music therapy, parent-mediated early intervention, social skill groups and the Theory of Mind cognitive model. Their use needs to be discussed with patients, parents and caregivers in order to clarify the uncertainties regarding the results, time taken and costs.

Among the pharmacological options, aripiprazole, risperidone tricyclic antidepressants (clomipramine and tianeptine) and selective serotonin reuptake inhibitors (this last category only for adults) seem to have some benefits for specific symptoms, but the adverse events that have been reported need to be carefully considered before making decisions.

Regarding the implications for further research, this review of reviews makes it clear that much needs to be done on the therapeutics of ASDs. Firstly, experimental research exploring physiopathological mechanisms needs to be conducted in order to support further clinical studies. Subsequently, good-quality long-term clinical trials are required.

CONCLUSION

This review included 17 Cochrane systematic reviews. None of them provided high-quality evidence for any autism-related outcome. Acupuncture, early intensive behavioral intervention, gluten and casein-free diets, music therapy, parent-mediated early intervention, social skill groups and the Theory of Mind cognitive model seem to have benefits for patients with autism spectrum disorders (very low to low-quality evidence). Aripiprazole, risperidone, clomipramine, tianeptine and selective serotonin reuptake inhibitors are pharmacological options that seem to have some benefits (this last one only for adults), but all of them are associated with high risks of important adverse events. Experimental research to confirm the links between therapeutic options and the disease is needed, followed by high-quality long-term clinical trials.

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3. Full name of each author (the editorial policy of the São Paulo Medical Journal is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full or omitted, without using abbreviations); his/her background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or undergraduate student); and his/her position currently held (for example, Master or Doctoral Student, Assistant Professor, Associate Professor or Professor, but not Head of Department, Dean, Provost or Rector), in the department and institution where he/she works, and the city and country (affiliations).
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5. Date and venue of the event at which the paper was presented, if applicable, such as congresses or dissertation or thesis presentations.
6. Sources of support in the forms of finance for the project, study bursaries or funding for purchasing equipment or drugs. The protocol number for the funding must be presented.
7. For Brazilian authors, all grants that can be considered to be related to production of the manuscript must be declared, such as fellowships for undergraduate, master and doctoral students; along with possible support for postgraduate programs (such as CAPES) and for the authors, such as awards for established investigators (*Produtividade* - CNPq), accompanied by the respective grant numbers.
8. Description of any conflicts of interest held by the authors. We recommend that the item "Conflicts of interest" at http://www.icmje.org should be read to obtain clarifications regarding what may or may not be considered to be a conflict of interest.
9. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). The author should also indicate a postal address, e-mail address and telephone number that can be published together with the article.

Main document

Second page: abstract and keywords

The second page must include the title and a 250-word abstract in English (case reports with 100 words). Do not cite references in the abstract.

Use the following headings:

1. Background: Describe the rationale for the study including the research question or the scientific hypothesis.
2. Design and setting: Declare study design correctly,[11] and the setting.
3. Methods: Describe methods briefly.
4. Results: Describe primary results with quantitative results describing the sampling strategy.
5. Conclusions: Make a succinct statement of data interpretation answering the research question presented previously.
6. Clinical Trial Registration. Mandatory for clinical trials, optional for observational studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.

Insert 3 to 5 key words after the abstract, with terms differing from the title. The words must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh.

Text

- Typical main headings include Introduction, Methods, Results, Discussion and Conclusion. The authors can use short subheadings too.
- Number the pages.

- Abbreviations must be avoided.
- A maximum of 3000 words in the main text, from the Introduction to the Conclusions; 1000 words for short communications.
- Maximum number of figures and/or tables is 5
- Maximum number of references is 35 (except for systematic reviews).

References

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

The reference list should be inserted after the conclusions and before the tables and figures. In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the journal's readers will be taken to the exact document cited, and not to a general website.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent a resolution of 300 DPI and/or minimum size of 2500 pixels (width) and be recorded in ".jpg" or ".tif" format. Do not attach images inside Microsoft PowerPoint or Microsoft Word documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Graphs prepared in Microsoft Excel (do not send them in image formats) spreadsheets must be accompanied by the tables of data from which they have been generated.

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

All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded to indicate the magnification used. The staining agent should be specified in the figure legend.

Original articles

Clinical trials; cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies; case series (i.e. case reports on more than three patients analyzed together); and systematic reviews with or without meta-analysis, are considered to be full-text original articles, with a maximum of 3000 words.

Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured in the same way as original articles.

Short communications and case reports must be limited to 1000 words (from the introduction to the end of the conclusion). The abstracts in short communications should not be structured and have a maximum of 100 words.

Authors will be required to comply with the guidelines for writing each type of original article, as follows:

1. Observational articles: STROBE Statement[5,6]
2. Clinical trials: CONSORT Statement[2]
3. Accuracy studies on diagnostic tests: STARD Statement[8,9]
4. Systematic reviews of the literature and meta-analyses: PRISMA[4]
5. Case reports: CARE[7]

São Paulo Medical Journal supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, since 2008, manuscripts on clinical trials have only been accepted for publication if they have received an identification number from one of the clinical trial registers (the options are stated at http://www.icmje.org). The identification number should be declared at the end of the abstract. Authors of randomized clinical trials must thus register their studies before submitting them for publication in the São Paulo Medical Journal.

Results from cases with DNA sequences must be deposited in appropriate public databases. The protocol number or URL can be requested at any time during the editorial review. Publication of other research data in public repositories is also recommended, since it contributes towards replicability of research, increases article visibility and possibly improves access to health information.

Short communications, case reports, case series and narrative reviews

Short communications and case reports must be limited to 1000 words (from the introduction to the end of the conclusion), a maximum of five references and one figure or table. They should be structured in the same way as original articles. Individual case reports should contain the following sections: Introduction, Case Report, Discussion and Conclusion. Reports on case series constitute observational studies and these should be structured in accordance with the norms of the STROBE Statement.[5]

Both short communications and case reports must be submitted with abstracts and keywords. The abstracts in short communications should not be structured and have a maximum of 100 words.

The São Paulo Medical Journal is interested in publishing rare or instructive case reports, accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[11] The search strategy for each database and the number of articles obtained from each database must be shown in a table. The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms are appropriate to be utilized for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT) the search dates should be indicated in the text or in the table.

Narrative reviews may be accepted by the São Paulo Medical Journal provided that a systematic search is made, and they should be structured as Original Articles. The search strategy and results should be presented as described above for case reports. By invitation from the Editor-in-Chief, narrative reviews addressing historical personal or collective experiences relating to clinical health sciences, epidemiology and public health may be accepted, but with no more than two authors.

Individual case reports should contain Introduction, Case Report, Discussion and Conclusion. Case reports should be structured in accordance with the norms of the CARE Statements.[7] Case reports published in São Paulo Medical Journal must be submitted with abstracts and keywords.

Letters to the editor

Letters to the editor may address articles published in the São Paulo Medical Journal publication or may deal with health issues of interest. Case reports must not be submitted as letters. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

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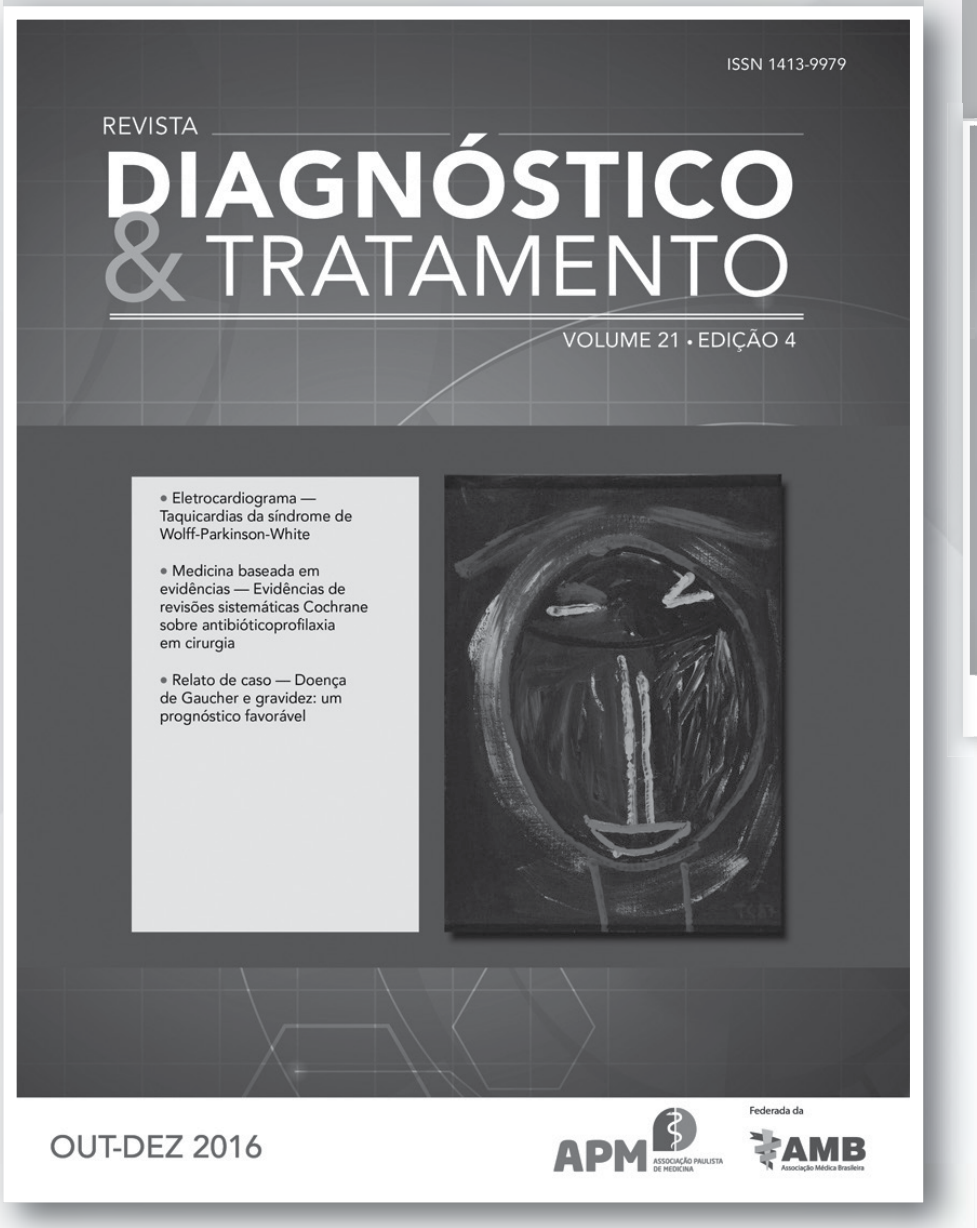

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