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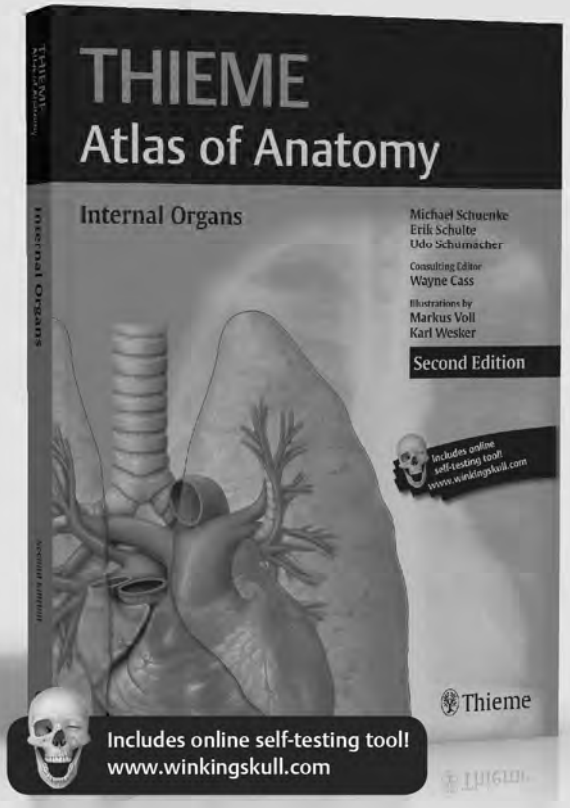

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Avenida das Américas, 8445 – sala 711
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Phone.: (+55 21) 2487-6336
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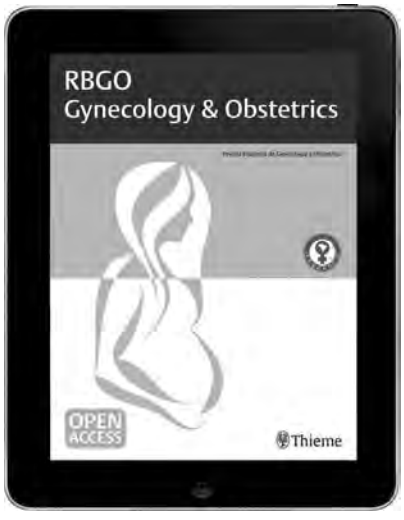

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Editorial

Biomarkers of Pelvic Endometriosis

Biomarcadores de endometriose pélvica

Fernando Marcos dos Reis[1] Cecília de Souza Monteiro[1] Márcia Mendonça Carneiro[1]

[1] Division of Human Reproduction, Hospital das Clínicas, Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

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Endometriosis is typically a symptomatic disease, and the symptoms often manifest as dysmenorrhea, dyspareunia, chronic pelvic pain, and/or infertility. Deep infiltrating endometriosis can also produce cyclic urinary or intestinal complaints. Nevertheless, the symptoms of endometriosis are not specific, and may be associated with many other different conditions. Severe dysmenorrhea in adolescent girls may be underestimated by health care providers and accepted as a physiological fate. Thus, patients can spend years without the accurate diagnosis and the proper treatment, with a tremendous negative impact on their quality of life.[1]

The use of a non-invasive biomarker would bring a remarkable advance to the management of endometriosis. A good test could shorten the time lapse between the onset of the symptoms and the beginning of the treatment, and allow monitoring of disease progression and recurrence.[2] Imaging methods such as transvaginal ultrasound and magnetic resonance allow the precise anatomical localization of some forms of endometriosis.[3,4] Although accurate for ovarian endometrioma and deep infiltrating lesions, these imaging methods are operator-dependent, require a highly specialized training, and fail to detect superficial peritoneal endometriotic implants.[5,6]

Decades of research have not led to a reliable biomarker for the non-invasive detection of endometriosis. The intriguing question is not "why have we failed," but "why should we have succeeded" in this endeavor. Do we have a good serum marker for breast cancer, coronary heart disease, or osteoporosis? Like these and many other prevalent and disabling conditions, endometriosis is a focal disease with some systemic features and biochemical signs that are too nonspecific to be accurately used as diagnostic biomarkers.

No endometriosis-specific antigen has been discovered so far, nor endometrium-specific molecules to be traced in search for ectopic endometrial implants. Endometriotic lesions are often small, have scarce cellular content, and usually do not release inflammatory mediators or growth factors in amounts that outweigh alternative sources of the same molecules. At best, some patients have increased serum levels of putative endometriosis products, but there is always a contingent of women with confirmed disease and normal serum marker levels, which confers low sensitivity (high false negative rate) to the method.

Currently, no serum biomarker is validated as a diagnostic test for endometriosis. A recent Cochrane review retrieved 141 studies that evaluated 122 serum or plasma biomarkers of endometriosis in more than 15,000 subjects. Meta-analysis was only possible for cancer antigen (CA)-125, CA-19.9, anti-endometrial antibodies and interleukin-6. The disappointing conclusion is that none of the evaluated biomarkers was accurate enough to be used in the daily practice.[7] The most studied of these proteins is CA-125, a glycoprotein produced by endometrial and mesothelial cells in response to inflammation.[8] The concentrations of CA-125 vary across the menstrual cycle, being higher during menstruation and lower in the follicular and ovulatory phases. The magnitude of the CA-125 increase during menstruation is amplified in women with endometriosis. However, CA-125 is not specific for endometriosis, and has low sensitivity for disease detection at any stage.[2] Therefore, it is not currently recommended as a diagnostic tool for endometriosis.

There are, however, some encouraging perspectives. High-throughput molecular studies have opened an avenue to the rational discovery of molecules that are overexpressed in endometriotic lesions and/or in the eutopic endometrium of patients with the disease.[9–11] This approach led to the discovery of five micro-RNAs with increased expression in peritoneal endometriotic lesions compared with healthy surrounding tissues.[9] Another study found 214 proteins differentially expressed in ovarian endometrioma versus eutopic endometrium from the same patients.[10] Performing

Address for correspondence Fernando M. Reis, MD, PhD, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil 14048-900 (e-mail: reis.ufmg@gmail.com).

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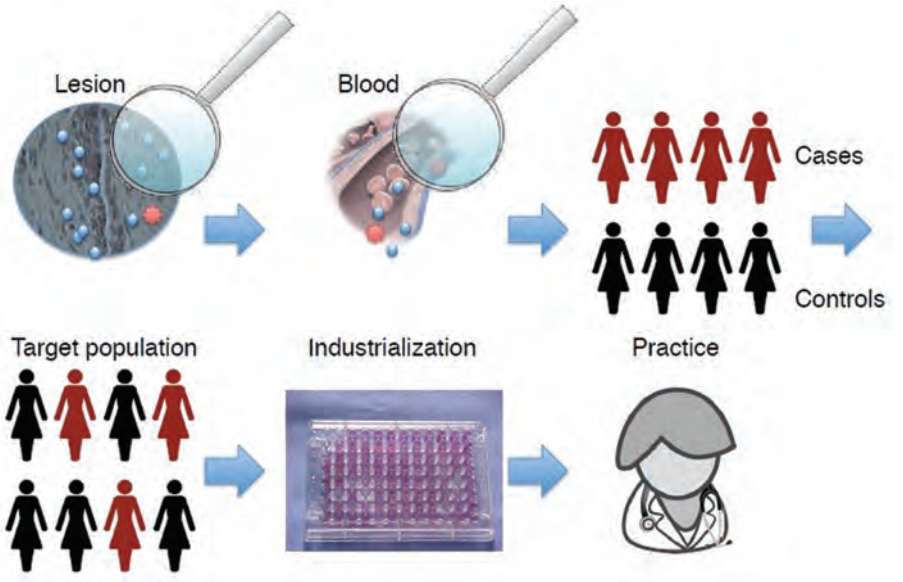

Fig. 1 Steps of endometriosis biomarker discover and validation before industrial production and routine use.

proteomic studies directly in the peripheral blood is an obvious shortcut to the discovery of new serum analytes that may be consistently altered in women with endometriosis.[12] However, this strategy is more vulnerable to noise from abundant serum proteins that mask the proteins of interest,[13] and has not yet revealed protein markers with diagnostic utility in endometriosis.

Crossing the bridge from bench to bedside remains a challenge for researchers in the field (►**Fig. 1**). First, experimental studies to select candidate markers based on unique pathophysiological mechanisms or large proteomic or metabolomic profiles should be performed. Second, test validation in the preclinical context, including the comparison between volunteers with an established diagnosis and a healthy control group should be made. Third, studies in the target population to assess the test performance and calculate its predictive value among individuals with unknown diagnoses, such as women with symptoms suggestive of endometriosis, must be performed. Last but not least, the industrial development of the test for clinical use must be set up.[14] Once these steps are completed, it will be time to define when and to whom the test will be applied.[15,16] Does it make sense to perform an endometriosis blood test in all infertile women, or in all cases of pelvic pain? Should the test be used for diagnosis, screening, or both?

To conclude, the saga of endometriosis biomarkers enters the year of 2017 still confined to the research territory, and it may take some additional time to cross the border of evidence-based clinical practice. Meanwhile, the constant improvement of imaging techniques and the recognition that endometriosis can be medically treated based on strong clinical suspicion[16] may allow earlier medical care and, if necessary, well planned, timely and thorough surgical intervention to relieve symptoms and improve the patients' quality of life.

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Salivary Iron (Fe) Ion Levels, Serum Markers of Anemia and Caries Activity in Pregnant Women

Níveis de íon ferro (Fe) salivar, marcadores séricos de anemia, e atividade de cárie em mulheres grávidas

Elisa Miranda Costa[1] Juliana Aires Paiva de Azevedo[2] Rafiza Félix Marão Martins[3] Vandilson Pereira Rodrigues[4] Cláudia Maria Coêlho Alves[1] Cecília Cláudia Costa Ribeiro[1] Erika Bárbara Abreu Fonseca Thomaz[1]

[1] Public Health Department, Universidade Federal do Maranhão, São Luís, Maranhão, Brazil
[2] Post-graduation Program in Odonthology, Universidade Federal do Maranhão, São Luís, Maranhão, Brazil
[3] Department of Odonthology, Universidade Ceuma, São Luís, Maranhão, Brazil
[4] Department of Morphology, Universidade Federal do Maranhão, São Luís, Maranhão, Brazil

Address for correspondence Elisa Miranda Costa, MSc, Departamento de Saúde Pública, Universidade Federal do Maranhão, Rua Barão de Itapary 155, 65020–070, São Luís, MA, Brazil (e-mail: elisamirandac@hotmail.com).

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Abstract

Introduction Anemia is a very frequent event among pregnant women. There are evidences of differences in the incidence of dental caries between pregnant and non-pregnant women, but the relationship between salivary iron (Fe) and serum markers of anemia and caries development has not been investigated.

Objective To evaluate the correlation between salivary (Fe) and serum iron (Fe, ferritin and hemoglobin) parameters in pregnant women with the development of dental caries.

Methods A prospective cohort was conducted with 59 women. The outcome of interest was represented by new dental caries lesions during pregnancy, using the Nyvad criteria. Pregnant women were evaluated at three clinical times: up to the 16th week of gestational age (GA) (T1), in the last trimester of pregnancy (T2), and postpartum (T3), at the Mother and Child Unit of University Hospital of the Universidade Federal do Maranhão. A stimulated saliva sample was collected for biochemical analysis of salivary Fe, and a blood sample was collected early in the morning. The correlation between salivary and serum Fe was evaluated through the Pearson correlation test. Analysis of variance (ANOVA) and Kruskal-Wallis were used to compare the means of anemia parameters at different times. The Student's *t* and Mann-Whitney tests were used to compare the anemia parameters between the groups of pregnant women (with and without new caries lesions).

Results Serum Fe concentrations were higher in the first trimester of pregnancy and lower after delivery ($p = 0.036$). It was also observed that the ferritin concentrations were higher in the first trimester and lower at the end of gestation ($p = 0.011$). There was no association between the expositions of salivary iron and anemia, and the development of dental caries. There was a positive correlation between serum Fe in T1 and salivary Fe in T2 ($p < 0.05$).

Conclusion The serum markers of anemia were more prevalent in the last trimester of pregnancy.

Keywords
- anemia
- saliva
- iron
- pregnancy
- tooth decay

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Resumo

Introdução A anemia é um evento muito frequente entre mulhres grávidas. Existem evidências de diferenças na incidência de cárie dentária entre mulheres grávidas e não grávidas, mas a relação entre o íon ferro (Fe) salivar, os marcadores séricos de anemia e o desenvolvimento de cárie não foi investigada.

Objetivo Avaliar a correlação entre os parâmetros salivares (Fe) e séricos (Fe, ferritina e hemoglobina) em gestantes e o desenvolvimento de cárie dentária.

Métodos Uma coorte prospectiva foi conduzida com 59 mulheres. O desfecho de interesse foi representado por novas lesões de cárie durante a gravidez, medido pelo critério Nyvad. Mulheres grávidas foram avaliadas em três tempos clínicos: até a 16ª semana de idade gestacional (IG) (T1), no último trimestre de gravidez (T2), e no puerpério (T3), na Unidade Materno-infantil do Hospital Universitário da Universidade Federal do Maranhão. A amostra de saliva estimulada foi coletada para análise bioquímica de Fe salivar, e a amostra de sangue foi coletada no início da manhã. A correlação entre o Fe salivar e o Fe sérico foi avaliada através do teste de correlação de Pearson. Os testes ANOVA e Kruskal-Wallis foram utilizados para comparar parâmetros de anemia em diferentes momentos. Os testes t de Student e Mann-Whitney foram utilizados para comparar os parâmetros da anemia entre os grupos de gestantes (com e sem lesões de cárie).

Resultados As concentrações séricas de Fe foram maiores no primeiro trimestre de gestação e menores após o parto ($p = 0,036$). Observou-se também que as concentrações de ferritina foram maiores no primeiro trimestre e menores no final da gestação ($p = 0,011$). Não houve associação entre as exposições e o desenvolvimento de cárie dentária. Houve correlação positiva entre o Fe sérico em T1 e o Fe salivar em T2 ($p < 0,05$).

Conclusão Os marcadores séricos de anemia foram mais prevalentes no último trimestre de gestação.

Palavras-chave

- anemia
- saliva
- ferro
- gravidez
- cárie dentária

Introduction

There is evidence of increased incidence of dental caries during pregnancy,[1,2] potentially due to significant differences in dental caries indicators between pregnant and non-pregnant women.[3] The state of activity of carious lesions during pregnancy can be influenced by behavioral factors (frequency of brushing and flossing, changes in dietary patterns, occurrence of vomiting)[4,5] and by systemic factors (immunological, hormonal and metabolic changes).[1]

It is suggested that during pregnancy, a period in which there is a strong mineral mobilization from maternal stores to generate fetal development,[6] there is a possibility that these minerals are displaced from the pregnant women's teeth, thus increasing the risk of dental caries.[6,7] This hypothesis is based on the "maternal depletion syndrome," whereby there can be maternal nutritional depletion, especially in the case of short interval between pregnancies.[6] But there is not enough evidence to establish this relationship.

In this period, local factors such as saliva may interfere with the development of dental caries. During pregnancy, decreased pH and buffering capacity are common due to episodes of vomiting and reduced salivary flow.[1,4] This may contribute to consider this population as a group more vulnerable to oral health disorders.

Inorganic components, such as sodium (Na), potassium (K), Calcium (Ca), chloride (Cl_2), bicarbonate (HCO_3-) and phosphate (PO43-) determine the relative fluid saturation respected to hydroxyapatite. Their concentrations vary in stimulated and unstimulated saliva.[8] Some of these biochemical markers present in saliva, such as Ca, phosphorus (P) and fluorine (F), play an important role in the process of enamel and dentin remineralization.[6,7,9–12] Although these elements have been more widely studied, it was also observed that iron (Fe) ion is a protective element against dental caries.[13,14]

The protective mechanism of Fe ion with respect to dental caries is not very clear.[13] It is possible that immunological and microbiological factors are involved.[14,15] However, there are some hypotheses about the role of Fe in modulating the activity of dental caries: it may increase enamel resistance to demineralization; it may have an antibacterial effect; and it may even have a specific effect on decreasing the activity of glycosyltransferases (GTFs) from *Streptococcus mutans*. However, studies on this subject are not conclusive.

The aim of this study was to investigate the association between Fe levels in saliva and serum, hemoglobin and ferritin levels, and the risk of dental caries development in pregnant women.

Methods

Design, Area, Study Population and Ethical Considerations

This prospective cohort study was conducted with a sample of pregnant women in the city of São Luís, Maranhão, in Brazil. This research was approved by the Ethics Research Committee of the Hospital Universitário of Universidade Federal do Maranhão in February of 2010, under the protocol 004 417/2010–20. Only pregnant women who agreed to participate and signed the informed consent form (ICF) were evaluated.

Sample Design, Inclusion and Exclusion Criteria

Using the Epi-Info software, version 6.0 (Center for Disease Control and Prevention, Atlanta, GA, USA), it was estimated that a sample of 39 pregnant women would allow us 80% of power to detect differences between exposed and non-exposed individuals, considering an incidence of the dental caries of 68.2% (estimated in a pilot study), and a 2:1 ratio between unexposed and exposed individuals, with a significance level of 5%. We added 10% to compensate for possible losses, and a minimum sample of 42 pregnant women was obtained (28 exposed and 14 unexposed to tooth decay).

Women were recruited at the Mother and Child Unit of Hospital Universitário of Universidade Federal do Maranhão during a prenatal visit. The recruitment period lasted 24 months, from July 2011 to June 2013. During this period, the maternity was visited daily by the researches, who registered all pregnant women in prenatal care.

Only women with a singleton pregnancy and gestational age (GA) up to 16 weeks were included. Individuals with endocrine-metabolic diseases, kidney, liver and cardiocirculatory dysfunctions, those with pregnancies considered of high risk, as well as those with premature births were excluded. Gestational age was assessed using ultrasonography performed in a routine service in the first trimester of pregnancy and, when not available, the last menstrual period or clinical estimate. Eligible patients were included in the study and followed until the postpartum period.

Definition of Variables and Data Collection

The pregnant women were interviewed, and medical and dental examinations for general health and to detect the presence of dental caries were performed. Blood and saliva were also collected for biochemical evaluations. For the interviews, a previously tested questionnaire was used. The data from the clinical examinations were recorded on a sheet made for this research. Medical data were also collected, such as the GA and the general health of the pregnant women.

The participants were evaluated at three clinical times: up to the 16th week of GA (T1), in the last trimester of pregnancy (T2), and in postpartum (T3). At all determined clinical times, the patients were submitted to an interview, to medical and dental examinations to evaluate the general health and detect the presence of dental caries, and blood and saliva collections for biochemical assessments. Preferably, T3 occurred at the hospital in the early days after delivery. If the mother had been discharged before the evaluation, she was invited to return to the hospital for na examination during the medical follow-up visit.

The choice of these gestational periods to carry out the biochemical assessments was due to the evidence that in the first trimester there is high absorption/low except of minerals to make up the maternal reserves. In the last trimester, there is a sudden increase in the fetus weight mainly at the expense of bone mass gain, with large mobilization of micronutrients from the maternal reserves for the formation of the baby, which can lead to maternal nutritional depletion if there is no sufficient intake/reserves to meet this demand.[16,17]

Dental examinations were performed by previously trained staff, under artificial light, after drying the teeth, with the pregnant woman sitting in the dental chair, using a millimeter probe (Hu-Friedy, Chicago, USA) and a mouth mirror recommended by the World Health Organization (WHO). All instruments were sterilized and individually packed. The clinical examinations were recorded on specific data sheets. The dependent variable was the incidence of dental caries in pregnant women. The presence of caries was assessed using the Nyvad criteria. The presence of caries-active white spot lesions, characterized as opaque spots, with soft and rough consistency, determining the current activity of caries, was observed.[18] With this criterion, therefore, we are capable of identifying the disease while still in its early stages, and it is more suitable for studies with a short follow-up period.[18]

The teeth were classified as healthy (codes 0 to 7), with inactive (codes 4, 5, 6 and 9) or active caries (codes 1, 2, 3 and 8).[18] The average decayed teeth (active + inactive lesions) were estimated for each individual. The sample was therefore classified into group 1 (G1), with no new caries lesions; and group 2 (G2), with the incidence of the disease.

The participants were told not to eat, drink or perform oral hygiene procedures in a time interval less than 1 hour prior to the saliva collection. Stimulated saliva samples were collected by asking the paeticipants to chew on a paraffin blade (Parafilm, Bemis, Neenah, USA) of 5 × 5 cm in size. The subjects were instructed to gently tilt the head forward and not to talk or swallow the saliva present in the mouth. Every minute, the participant spit the amount of accumulated saliva into a Falcon (Inlab, São Paulo, Brazil) collection tube graduate of 5mL to obtain a volume of 1 to 2 mL.

The samples were stored on ice and transported to processing in a time period shorter than two hours. The samples were centrifuged for 7 minutes at 7,000 rpm in a Falcon tube centrifuge. After this procedure, the supernatant volume was stored in 1.5 ml Eppendorf (Hamburg, Germany) tubes and frozen at - 80°C for subsequent biochemical analysis.

Salivary Fe quantification (mg/dL), K (mmol/L) was conducted in triplicate with the colorimetric method and specific reagents (kit produced by Doles, Goiânia, Brazil). The subtraction of the control solution (without sample),

followed by the multiplication by factor for the standard solution was used as a reference for calculating the converted measurement units. The reading of Fe absorbance in a 490 nm wavelength occurred after the homogenization of 10 µl of the sample with 1 ml of the color reagent (Ferrozine).

The other covariates included: a) sociodemographic data: schooling ($<$ 8 years of schooling, $\geq$8 years of schooling), head of the family (pregnant women, partner or other), maternal marital status (with or without partner), social class (A/B, C or D/E) according to the criteria of the Brazilian Market Reasearch Association (ABEP, 2008)[19], access to basic sanitation (yes or no); b) gestational health: history of vomiting in the current pregnancy (yes or no), visit to the dentist (yes or no), use of antianemic (yes or no); and c) practices related to oral health: daily tooth brushing frequency ($\geq$3 or $\leq$3 times), habit of brushing teeth after meals (yes or no), use of mouthwash (yes or no), use of dental floss (yes or no), dental appointment during pregnancy (yes or no), and access to fluoride (topical fluoride application, toothpaste, mouthwash, tablets) during pregnancy (yes or no).

Methodological Care for Measurement Bias

A manual with instructions on filling out the data collection instruments and the dental examination methods and criteria for the classification of variables was elaborated. Intra and interexaminer concordance (Kappa and intraclass correlation coefficient) was evaluated. Only evaluators who have obtained agreements $\geq$ 0.80 remained on the team. A pilot study was conducted at every stage of the research before starting data collection.

Data Analysis and Processing

Analyses were performed using the Stata software, version 14.0 (Stata Corp., College Station, Texas, USA). Descriptive statistics was processed using absolute frequency, relative frequency, average, standard deviation, median and interquartile interval. Chi-square or Fisher exact tests were used to analyze the difference in the frequencies of the categorical variables between the groups. For the numerical variables, the normality of the sample distribution was assessed through the Shapiro-Wilk test. The Student's *t*-test (between groups at the same time) was selected for the comparative analysis of the numerical variables. To analyze variations in serum hemoglobin, ferritin, erythrocyte, serum Fe and salivary Fe levels, the Tukey Test, Kruskal-Wallis, Newman-Keuls and analysis of variance (ANOVA) were used. The Pearson coefficient was calculated to assess the correlation between the salivary Fe ion concentration and the collected serum Fe. The significance level adopted was 5%.

Results

Total 539 pregnant women were screened at baseline. Of these, eighty-three (83) were eligible and agreed to participate in the study, and went through an interview, a clinical examination and a blood collection at T1, and 56 of them (67.5%) returned for evaluations in T2 and T3, constituting the final sample of this study.

►**Table 1** expresses the socioeconomic variables and the frequency of oral hygiene habits in pregnant women between the two caries activity groups. Most pregnant women assessed: had 8 or more years of schooling (79.6%); lived with partners (92.7%) who were the head of the family in 60% of cases; had no access to basic sanitation (64.2%); belonged to economic class C (58.9%); reported Fe supplement use during pregnancy (85.7%); reported no dental visits (89.3%); and had not underwent topical fluoride application in a dental office (91.1%). Regarding oral hygiene habits, 54.6% of the pregnant women reported. brushing their teeth 3 or more times per day, and 69.6% only after meals; The daily use of mouthwashes and dental floss was reported by 80.4% and 44% of the sample. There were no statistically significant differences for these variables among caries activity groups.

The comparative analysis of serum biomarkers among caries activity groups in the pregnant women is expressed in ►**Table 2**. We observed that the serum ferritin levels were statistically higher at postpartum than in the first and last trimesters of gestation ($p = 0.018$). We also observed that the serum Fe level was statistically higher ($p < 0.005$) in the first trimester of pregnancy (106.4 ± 27.5) compared with the last trimester (90.0 ± 40.5) and after delivery (86.3 ± 26.3). On the other hand, statistically significant differences in salivary Fe levels were not observed among the three time periods ($p = 0.170$). The hemoglobin and hematocrit levels showed no statistically significant variations in time periods analyzed ($p \geq 0.05$). The analysis also suggests that there are no statistically significant differences in the levels of these biomarkers among caries activity groups.

Furthermore, the linear correlation measures between serum and salivary Fe levels in the three clinical time periods were calculated (►**Table 3**). There was a direct correlation between serum Fe in T1 and salivary Fe in T2 ($r = 0.99$; $p = 0.045$). No other statistically significant correlations were observed.

Discussion

The findings of this study suggest that serum Fe concentrations were higher in the first trimester of pregnancy and lower after delivery, and that serum ferritin concentrations were lower in T2 and higher in T3. On the other hand, salivary concentrations were higher in the T2 and also lower in T3. There was a positive correlation between serum Fe in T1 and salivary Fe in T2.

Pregnant women with higher schooling, higher socioeconomic status and included in medical prenatal care from the first trimester of pregnancy were more likely to adopt preventive oral health measures and perform dental treatments,[19–21] therefore showing lower frequency of oral problems such as dental caries. However, in our sample, socioeconomic factors did not differ between groups with and without dental caries, showing that groups are homogeneous and comparable. This reduces potential confounding biases.

Serum Fe concentrations had significant changes during and after pregnancy, with higher values in T1 and lower in T3.

Table 1 Incidence of dental caries according to socioeconomic conditions and oral hygienic habits

Variables	Total		G1 New caries = 0		G2 New caries ≥1		*p*-value
Qualitative variables	n*	%	n	%	n	%	
Schooling							
< 8 years school	10	20.4	6	60.0	4	40.0	0.496[a]
≥ 8 years	39	79.6	18	46.1	21	53.9	
Marital status							
With partner	51	92.7	22	43.1	29	56.9	0.320[a]
Without partner	4	7.3	3	75.0	1	25.0	
Head of the family							
Pregnant woman	7	12.7	2	28.6	5	71.4	0.541[a]
Partner	33	60.0	17	51.5	16	48.5	
Other	15	27.3	6	40.0	9	60.0	
Basic sanitation							
Yes	19	35.8	6	31.6	13	68.4	0.194[b]
No	34	64.2	17	50	17	50.0	
BECC**							
A-B	14	25.0	9	64.3	5	35.7	0.278[a]
C	33	58.9	14	42.4	19	57.6	
D-E	9	16.1	3	33.3	6	66.7	
Use of anti-anemic							
Yes	48	85.7	21	47.3	27	56.3	0.451[a]
No	8	14.3	5	62.5	3	37.5	
Nausea							
Yes	47	85.5	23	48.9	24	51.1	0.708[a]
No	8	14.5	3	37.5	5	62.5	
Visit to the dentist							
Yes	6	10.7	3	50.0	3	50.0	0.509[a]
No	50	89.3	23	46.0	27	40.0	
TFA performed at the dental office							
Yes	5	8.9	2	40.0	3	60.0	0.569[a]
No	51	91.1	24	47.1	27	52.9	
Frequency of daily tooth brushing							
≥ 3	30	54.6	14	46.7	16	53.3	0.921[b]
≤ 3	25	45.4	12	48.0	13	52	
Brush after meals							
Yes	39	69.6	17	43.6	22	56.4	0.519[b]
No	17	30.4	9	52.9	8	47.1	
Use of dental floss							
Yes	22	44.0	11	50.0	11	50.0	0.449[b]
No	28	56.0	11	39.3	17	60.7	
Use of mouthwash							
Yes	41	80.4	19	46.3	22	53.7	0.835[b]
No	10	19.6	5	50.0	5	55	

Abbreviations: BECC, Brazilian Economic Classification Criteria; TFA, topical fluoride application.
*Total number is not equal to 56 due to loss of information for some variables.
**BECC is determined by the Brazilian Market Research Association (Associação Brasileira de Empresas de Pesquisa [ABEP]).
[a]Fisher exact Test.
[b]Chi-square Test.

Table 2 Levels of hemoglobin, erythrocyte, ferritin and Fe (serum and salivary) in pregnant women with and without new dental caries lesions

Variables	Total		*p*	Dental caries activity				*p*
				Yes (G1)		No (G2)		
	mean (SD)	average (Q1-Q3)		mean (SD)	average (Q1-Q3)	mean (SD)	average (Q1-Q3)	
Hemoglobin								
T1	12.0 (1.4)	12.5 (11.3–12.8)[a]	0.199	12.4 (0.3)	12.4 (12.0–12.7)	13.0 (1.0)	12.6 (12.5–13.6)	0.306
T2	12.4 (1.8)	12.3 (11.4–13.4)[a]		12.4 (0.9)	12.3 (11.5–13.4)	11.7 (1.9)	11.4 (9.9–13.3)	0.442
T3	14.2 (0.9)	14.2 (13.6–14.9)[a]		–	–	14.2 (0.9)	14.2 (13.6–14.9)	–
Erythrocyte								
T1	4.2 (0.5)	4.2 (4.1–4.3)[a]	0.419	4.4 (0.3)	4.3 (4.2–4.7)	4.5 (0.6)	4.3 (4.2–4.9)	0.727
T2	4.2 (0.7)	4.1 (3.8–4.7)[a]		4.1 (0.4)	3.9 (3.9–4.5)	4.0 (0.9)	3.8 (3.6–4.5)	0.556
T3	4.8 (0.1)	4.8 (4.7–4.9)[a]		–	–	4.8 (0.1)	4.8 (4.7–4.9)	–
Ferritin								
T1	52.5 (53.8)	36.3 (28.9–58.9)[a]	0.011	43.3 (19.9)	36.9 (29.9–61.6)	49.3 (45.7)	31.3 (28.3–49.7)	0.386
T2	44.5 (53.7)	22.4 (13.3–49.0)[a]		90.1 (95.6)	34.1 (23.5–153.90	32.3 (34.3)	17.8 (11.7–38.5)	0.147
T3	61.8 (43.5)	43.9 (35.6–83.3)[b]		68.9 (40.6)	49.1 (40.7–95.9)	49.9 (33.4)	41.9 (27.0–68.3)	0.129
Serum Fe								
T1	106.4 (27.5)	107.3 (93.0–116.4)[b]	0.036	97.0 (27.1)	94.3 (86.0–115.0)	104.9 (31.2)	111.7 (83.5–127.0)	0.582
T2	90.0 (40.5)	91.0 (61.0–123.0)[a]		103.5 (29.4)	97.5 (91.0–135.0)	84.4 (46.9)	76.0 (49.0–123.0)	0.363
T3	86.3 (26.3)	87.5 (63.0–101.0)[a]		93.1 (23.9)	95.0 (81.3–106.0)	81.1 (24.6)	86.2 (62.0–94.6)	0.156
Salivary Fe								
T1	57.8 (77.3)	9.9 (3.3–135.0)[a]	0.170	3.8 (5.4)	3.8 (0.0–7.6)	–	–	–
T2	93.1 (175.7)	25.0 (10.0–60.0)[a]		14.3 (11.4)	13.6 (5.8–22.8)	135.9(144.7)	87.6 (37.6–234.2)	0.145
T3	42.3 (124.3)	5.9 (1.7–19.9)[a]		4.4 (6.7)	0.0 (0.0–6.7)	16.0 (13.4)	13.5 (5.0–27.0)	0.130

Abbreviations: Av., average; Fe, iron; SD, standard deviation; T1, 1st trimester; T2, 3rd trimester; T3, postpartum.
Note: Analysis of variance (ANOVA) or Kruskal-Wallis tests were used in the comparative analysis of the biomarkers of the pregnant women at the three clinical times. Student's *t* or Mann-Whitney tests were used in the comparative analysis of the biomarkers among caries activity groups at the same time. Different superscript lowercase letters (a and b) indicate statistically significant differences in the biomarker at the different clinical times (Tukey and Student-Newman-Keuls test).

This result is in agreement with the literature. In addition, the maternal Fe reserves are formed in the first trimester of pregnancy, when larger amounts of this substance are required for the maturation and the biological functions of the fetus during the last trimester of pregnancy.[22–24] The lower serum Fe values observed in T3 may be associated with the presence of anemia before and during pregnancy and blood loss during delivery.[22–25]

Serum ferritin concentrations varied significantly over the three clinical times, being lower in T2 and higher in T3. Iron deficiency developed in three phases.[26,27] In the first phase, there is a decrease in serum ferritin, which is directly

Table 3 Pearson correlation coefficient between salivary and serum Fe at different evaluation times

Variables	Times	Serum Fe			Salivary Fe		
		T1	T2	T3	T1	T2	T3
Serum Fe	T1	1.0					
	T2	0.32	1.0				
	T3	−0.23	0.12	1.0			
Salivary Fe	T1	−0.39	−0.39	−0.55	1.0		
	T2	0.99	−0.16	−0.01	−0.06	1.0	
	T3	−0.40	0.12	−0.22	−0.64	−0.25	1.0

Abbreviations: Fe, iron; T1, 1st trimester; T2, 3rd trimester; T3, postpartum.

related to the available Fe reserves.[27] In the second phase, there is a decline in serum Fe concentration and increased Fe binding capacity.[27] In the third, there is a restriction in the synthesis of hemoglobin. Therefore, it seems that ferritin is the most sensitive serum parameter in the diagnosis of anemia.[28,29] Over the gestational periods, there is an increasing demand for Fe from the developing fetus; therefore, the decrease in ferritin in the last trimester of pregnancy is biologically plausible. It has been hypothesized that after delivery, the maternal reserves that are available to the body are partially replenished, explaining the higher serum ferritin levels found in T3.

There was no difference in serum and salivary levels according to the incidence of dental caries. However, a study performed in Northern India evaluated Fe, magnesium (Mg), copper (Cu) and F levels present in the saliva of children and found no consistent relationship between the concentration of these ions and dental caries.[30] Moreover, a study in Malaysia[31] analyzing variations in Cu, Mg, Fe, and zinc (Zn) concentrations found positive correlations between Cu and Zn concentrations in children with dental caries. The data obtained show diverges greatly from the literature; moreover, this interaction had not been investigated in pregnant women, which is an original feature of this of study in the analysis of the hypothesis of this association.

The Fe concentrations in the saliva were higher in the T2 and T3. Saliva has a protective effect up to the limit of pH 5.5. Lower values create conditions for increased solubility and dissolution of enamel crystals. When critical pH periods become more common, there may be a predominance of loss of tooth ions, forming caries lesions.[32] It is hypothesized that tooth demineralization occurs, and the concentration of Fe ions in the saliva is higher, explaining the higher values of this ion during T2 and the lower values in T3.

It was observed that the higher the serum Fe concentration in T1, the higher the salivary Fe concentration in T2. There is high absorption/low exception of minerals to compose a maternal reserve in the first trimester.[26] Thus, it is possible that the Fe levels are higher in early pregnancy compared with subsequent trimesters and soon after delivery.[22–24] In addition, the second and third trimesters are periods that require a greater metabolic use of minerals for the growth and maturation of the fetus.[16]

Regarding the highest salivary Fe concentration in T2, this data differs from the literature, in which the presence of the Fe ion acts in reducing the glycosyltransferases (GTFs)[33] and extracellular polysaccharide synthesis (EPS),[33] both virulence mechanisms of *S. mutans*. Furthermore, Fe supplementation led to the inhibition of the progression of dental caries,[34] and increased Fe concentrations decreased enamel demineralization.[13] However, it is suggested that differences in the salivary composition during pregnancy, such as reduced pH, higher concentration of mucins and physiological anemia may interfere with the biochemical composition of saliva, leading to the increased Fe ion concentration present in the sample and to the susceptibility to dental caries.

It is important to point out some limitations of the study, such as the small sample size, the losses, and the fact that it was selected for convenience. Furthermore, the microbiological analysis of the biofilm, the quantification of Fe ions present, salivary flow tests, and an analysis of the buffering capacity were not performed, which are interesting resources to monitor the susceptibility to local diseases like periodontitis and dental caries,[35,36] and systemic diseases such as infectious, malignant, hereditary, autoimmune and endocrine disorders. They are also interesting resources to monitor the evaluation of therapeutic drug levels.[35]

However, the study presents the following strengths: the criterion of choice for the diagnosis of dental caries (Nyvad criteria) allows for the identification of lesions in the early stages of the disease. The study design was a prospective cohort, in which pregnant women were evaluated at three clinical time periods. Moreover, the recruitment was of a homogeneous population, in which socioeconomic and behavioral factors did not differ, reducing the confounding bias, so the results are independent of these factors.

This study presented many innovative elements, as it aimed at evaluating Fe ions present in saliva, dental caries activity and the correlation with anemia in pregnant women longitudinally, something that had not been done so far. Thus, this topic should be further studied.

There was a direct correlation between serum Fe in the first trimester and salivary Fe in the last trimester, and the serum markers of anemia were more prevalent in the last trimester of pregnancy.

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Does the Access to Sun Exposure Ensure Adequate Levels of 25-Hydroxyvitamin D?

A exposição ao sol assegura níveis adequados de 25-hidroxivitamina D?

Ênio Luis Damaso[1] Francisco José Albuquerque de Paula[2] Silvio Antônio Franceschini[1] Carolina Sales Vieira[1] Rui Alberto Ferriani[1] Marcos Felipe Silva de Sá[1] Lucia Alves da Silva Lara[1]

[1] Department of Obstetrics and Gynecology, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil
[2] Department of Internal Medicine, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Address for correspondence Ênio Luis Damaso, Rua Romeu Engrácia de Faria, 325, Apartment 34, Nova Aliança, Ribeirão Preto, SP, Brazil (e-mail: eniodamaso@yahoo.com.br).

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Abstract

Objectives To assess the prevalence of hypovitaminosis D, altered arterial blood pressure, and serum levels of glucose and lipids in community-dwelling women in the city of Ribeirão Preto, in the southeast of Brazil.

Methods This was a cross-sectional study of women aged 40–70 years old. Calcium intake and level of sun exposure were assessed by means of a questionnaire. A blood sample was used to determine glucose, lipid profile and 25-hydroxyvitamin D (25[OH]D) concentration.

Results Ninety-one women were enrolled (age = 54.2 ± 7.1 years). The mean serum 25(OH)D concentration was 25.7 ± 8.9 ng/mL. A total of 24 (26.4%) women had 25 (OH)D levels < 20 ng/mL. Seventy women (76.9%) had 25(OH)D levels < 30 ng/mL. Seventy-five women (90.4%) had inadequate calcium intake, and 61 women (67%) had appropriate sun exposure, 49 of whom (80.3%) had serum 25(OH)D levels < 30 ng/mL.

Conclusion This study indicates that even in community-dwelling women, living in a city with high sun exposure, serum levels of 25(OH)D > 30 ng/ml are hardly reached. Thus, it is probable that other intrinsic factors besides sun exposure may regulate the levels of vitamin D.

Keywords
- Female Gender
- 25–hydroxyvitamin D
- vitamin D deficiency
- sun exposure

Resumo

Objetivos Estimar a prevalência de hipovitaminose D, hipertensão arterial, e níveis séricos de glicose e perfil lipídico em uma comunidade de mulheres de Ribeirão Preto, no Sudeste brasileiro.

Métodos Estudo transversal com mulheres de 40 a 70 anos de idade, submetidas a um questionário para determinar ingestão diária de cálcio e nível de exposição solar, e coleta de sangue para determinar glicose, perfil lipídico e concentração de 25-hidroxivitamina D (25[OH]D).

Resultados Noventa e uma mulheres foram incluídas (idade = 54,2 ± 7,1 anos). O nível sérico médio de 25(OH)D foi 25,7 ± 8,9 ng/mL. Um total de 24 (26,4%) mulheres

Palavras-chave
- sexo feminino
- 25–hidroxhvitamina D
- deficiência de vitamina D
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teve níveis de 25(OH)D < 20 ng/mL. Setenta mulheres (76.9%) tiveram níveis de 25 (OH)D < 30 ng/mL. Setenta e uma mulheres (90.4%) tiveram uma ingesta inadequada de cálcio e 61 mulheres (67%) tiveram exposição solar adequada; 49 das quais (80.3%) tiveram níveis séricos de 25(OH)D < 30 ng/mL.

Conclusão Este estudo indica que mesmo morando em uma cidade com exposição solar adequada, níveis séricos de 25(OH)D > 30 ng/mL dificilmente são atingidos por mulheres climatéricas. Logo, é provável que outros fatores intrínsecos podem regular o nível de vitamina D.

Introduction

Vitamin D, classically, was considered a hormone specialized in the control of bone and mineral metabolism. However, the role of vitamin D on human physiology has been completely re-dimensioned due to new evidences indicating that vitamin D is a pleiotropic hormone able to participate in the endocrine control of several systems, such as: cardiovascular, energy metabolism and immune.[1] Vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) are the two main forms of vitamin, and act as prohormones. The ultraviolet radiation in sunlight promotes vitamin D_3 synthesis in the skin, and this accounts for $\sim$ 60–80% of the required amount.[1] The non-enzymatic conversion of pro- to pre-, and subsequently to vitamin D formation in response to solar UVB radiation, leads thereafter to the production of the active hormone, through hydroxylation in the liver (forming 25-hydroxyvitamin D [25- OHD][1](and then 1 α hydroxylation in the kidney (synthesizing 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]). The active form of vitamin D, 1,25$(OH)_2$D, binds to nuclear receptors of cells in the intestine and kidney, and stimulates calcium and phosphorus absorption. Vitamin D also binds to osteoclasts and stimulates bone reabsorption.[2] However, the synthesis of this secosteroid in the skin is tightly controlled to avoid vitamin D intoxication, even during continuous and excessive sun exposure. For instance, melanin production in the skin is stimulated by the sunlight and compete for UVB rays. In addition, inert compounds are synthesized by a thermos-regulated process during the steps of pre- and post-vitamin D formation synthesis within the skin.[1]

There is controversy regarding the healthy range of serum vitamin D. The Institute of Medicine (IOM) proposed that circulatory levels of 25(OH)D above 20 ng/mL are adequate for the most part of the population. Moreover, the IOM indicated that severe vitamin D deficiency occurs only when the 25(OH)D levels are below 12 ng/mL.[3] On the other hand, the Endocrine Society dictates that vitamin D sufficiency is achieved at levels above 30 ng/mL, while the range from 20 to 30 ng/mL indicates vitamin D insufficiency and levels below 20 ng/mL indicate deficiency.[2,4] Thus, the prevalence of hypovitaminosis D depends on which recommendations are accepted.[5]

Vitamin D deficiency induces abnormal calcium, phosphorus, and bone metabolism. A reduced level of vitamin D decreases intestinal calcium absorption, thereby increasing the level of parathyroid hormone (PTH), which in turn stimulates the renal synthesis of 25(OH)D-1α-hydroxylase, the renal enzyme that converts 25(OH)D into 1,25$(OH)_2$D. The elevation of PTH also increases osteoclast activity, causing bone loss and, ultimately, leading to osteopenia and osteoporosis. Vitamin D deficiency has also been associated with muscle weakness, and increased fall risk in adults and elderly individuals,[2,6] as well as with metabolic diseases,[7,8] and insulin resistance.[9]

In most individuals, the serum level of vitamin D is higher in the summer than in the winter.[10] Previous research estimated that a large number of adult men and women suffer from hypovitaminosis D, and that the elderly are the most affected.[2,11,12] Even though Brazil is situated in the tropics and subtropics, previous study indicated a high prevalence of hypovitaminosis D in some subpopulations from certain regions.[13]

The metabolic consequences of the vitamin D status are shown in recent studies; hypertension and endothelial dysfunction,[14] as well as dyslipidemia[15] are associated with low levels of 25 (OH) D. Supplemental vitamin D and calcium are frequently recommended for preservation of bone mass,[16] although regular sun exposure is the simplest method to attain an adequate level of vitamin D. In particular, exposure to 5 to 15 minutes of sunlight between 10 AM and 3 PM in the spring, summer, and fall is sufficient for maintenance of adequate levels of vitamin D.[17] Although Brazil has a continental size, most of his territory has abundant incidence of sun light, supposedly, precluding a high incidence of D hypovitaminosis. The main objective of the present study was to assess the prevalence of hypovitaminosis D in community-dwelling women from Ribeirão Preto, a sunny city in the southeast of Brazil

Methods

Women aged 40 to 70 years old who came to the Climacteric Outpatient Clinic of the Centro de Saúde Escola Sumarezinho from January to December of 2013 for routine gynecological visits were eligible for participation in this cross-sectional study. The nursing staff informed all women about the study, and invited them to participate. Institutionalized women, and women who had limited movement without the help of devices (ortheses, prostheses, wheelchairs, etc.) or of other

people were excluded because such individuals are known to have low serum levels of vitamin D.[18] A total of 150 women were invited to participate in this study, 110 of whom agreed to it. Nineteen of these women were excluded because they did not undergo blood collection, and 91 women were ultimately enrolled.

In a private office, a doctor evaluated each participant using a semi-structured interview that asked about the use of medications (hormones, supplemental vitamin D and/or calcium), consumption of calcium-rich foods, duration of sun exposure, and duration of weekly physical activity.

Body mass index was estimated in all subjects and they were classified in accordance with the World Health Organization classification: underweight (BMI $<$ 18.5 kg/m^2), normal weight (BMI = 18.5–24.9 kg/m^2), grade I overweight (BMI = 25–29.9 kg/m^2), grade II overweight (BMI = 30–39.9 kg/m^2), or grade III overweight (BMI $\geq$ 40 kg/m^2).

A blood sample was collected between March and June of 2013 (fall and winter in Brazil) for measurement of serum 25(OH)D levels, ionized calcium, fast glucose, and lipid profile. Also, creatinine was measured as it is negatively correlated with vitamin D levels.[19] Renal function was assessed by measurement of serum creatinine by automated spectrophotometry (Wiener Laboratory, Rosario, Santa Fe, Argentina). Serum 25(OH)D was determined by a chemiluminescence immunoassay using the LIAISON 25 OH Vitamin D TOTAL assay (DiaSorin, Saluggia, Italy). Glucose was determined by automated spectrophotometry using the AA liquid enzymatic glycaemia kit (Wiener Laboratory, Rosario, Santa Fe, Argentina). Ionized calcium was determined by automated spectrophotometry using the Arsenazo III AA kit (Wiener Laboratory, Rosario, Santa Fe, Argentina). Serum total cholesterol, HDL, and triglycerides were determined by automated spectrophotometry (Wiener Laboratory, Rosário, Santa Fe, Argentina).

Consumption of calcium was estimated according to the milk and dairy products data in the food composition table suggested by the US Department of Agricultural Research Service.[20]

Sun exposure was estimated based on self-reported outdoor time and based on the professional and life habits of each subject. Exposure was considered adequate if at least 20% of the body surface (face, neck, arms, and legs) was exposed for at least 10 minutes, 3 or more times per week between 10 AM and 3 PM,[17] and inadequate when the exposure was below this mark. The duration of physical activity (walking) was classified based on the time recommended for the prevention of osteoporosis by the American College of Sports' Guidelines for Exercise Testing and Prescription[21]: 20 to 40 minutes of exercise, 2 to 4 times per week.

Based on indications of the Endocrine Society, serum 25 (OH)D concentration was classified as deficient ($<$ 20 ng/mL), insufficient (21–29 ng/mL), or sufficient (30–100 ng/mL).[2,4]

Statistical Analysis

All statistical procedures were performed using the PROC MEANS and PROC FREQ features of the SAS 9.0 (Cary, NC, USA) software. For the description of quantitative variables, mean and standard deviation were calculated. For qualitative variables, the absolute and relative frequencies were calculated. Fisher exact test was used to determine the association between two qualitative variables. Women with vitamin D deficiency ($<$ 20 ng/ml) and adequate vitamin D levels were compared in terms of qualitative variables (systemic hypertension, diabetes mellitus, hypothyroidism, alcoholism, smoking, physical activity and sun exposure) and quantitative variables (age, BMI, serum calcium, blood glucose, HDL, total cholesterol, triglycerides and creatinine). Exploratory data analysis was performed through central position measurements and dispersion. Data distribution was checked by normal graphics. A non-parametric Mann Whitney test was used to compare quantitative variables between groups. The chi-square test was used to compare the distributions of qualitative variables in the groups. The variable sun exposure was dichotomized with cutoff of three times a week as adequate exhibition. A model of logistic regression was performed to verify the influence of the variables age, creatinine and sun exposure in relation to vitamin D concentration. Significance was set at $p < 0.05$.

Results

Ninety-one women participated in the study. ►**Table 1** shows the demographic, laboratory and clinical characteristics of the enrolled women.

Mean serum 25(OH)D was 25.7 $\pm$ 8.9 ng/mL. A total of 24 (26.4%) women had 25(OH)D levels $<$ 20 ng/mL. Seventy women (76.9%) had 25(OH)D levels $<$ 30 ng/mL (18 of 20 black women [90%] and 54 of 71 white women [76%]) and 21 (23.1%) women had sufficient 25(OH)D levels ($\geq$ 30 ng/mL). Serum 25(OH)D concentration was dichotomized as $<$ 20 ng/ml and $\geq$ 20 ng/ml. ►**Table 2** shows the distribution of the studied qualitative variables and the comparison between groups regarding the incidence of these variables according to the vitamin D levels. There was no difference between women with vitamin D deficiency regarding the incidence of hypertension, diabetes mellitus, hypothyroidism, and habits of smoking and drinking. Also, there was no difference between women with adequate amount of physical activity and adequate sun exposure (►**Table 2**).

►**Table 3** shows the distribution of the studied quantitative variables and the comparison between groups of the incidence of such variables. There was no significant difference between women with vitamin D deficiency regarding age, BMI, and dosage of calcium, fast glucose, lipid profile, and creatinine.

Based on recall, average calcium consumption was 560.4 $\pm$ 435.8 mg/day, and 75 women (90.4%) had inadequate calcium intake ($<$ 1200 mg/day). Sixty-one women (67%) reported adequate sun exposure and 49 of these women (80.3%) had serum 25(OH)D levels $<$ 30 ng/mL.

There was no significant difference in the serum 25(OH)D concentration for women with adequate and inadequate sun exposure ($p = 0.88$, ►**Fig. 1A**). In addition to this, there was

Table 1 Anthropometric, laboratory, and clinical characteristics of the enrolled women ($n = 91$)

Variable	Mean ± SD or n (%)
Age (years)	54.2 ± 7.1
BMI (kg/m^2)	27.1 ± 4.8
25(OH)D (ng/mL)	25.7 ± 8.9
Calcium ion (mmol/L)	1.09 ± 0.05
Glucose (mg/dL)	90.4 ± 18.5
Total cholesterol (mg/dL)	187.1 ± 38.5
HDL-C (mg/dL)	46. 6 ± 11.8
Triglycerides (mg/dL)	115.2 ± 59.9
Creatinine (mg/dL)	0.80 ± 0.11
Diseases	
Arterial hypertension	30 (32.9)
Hypothyroidism	11 (12.0)
Diabetes mellitus	7 (7.6)
Dyslipidemia	15 (16.4)
Other	25 (27.4)
None	28 (30.7)
Race	
White	71 (78.0)
Mulatto/Black	20 (21.9)
Habits	
Smoking (Yes)	11 (12.0)
Alcohol ingestion (Yes)	20 (22)
Physical activity	
≤ 150 minute/week	76 (83.5)

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; n, number of subjects; SD, standard deviation

no significant difference in serum concentration of ionized calcium for women with adequate and inadequate calcium consumption ($p = 0.73$, ►**Fig. 1B**).

Finally, we used multiple linear regression analysis to determine the effect of sun exposure, age, and serum creatinine on serum 25(OH)D concentration (►**Table 4**). The results show that a normal level of serum creatinine was significantly and independently associated with a higher level of serum 25(OH)D. The other variables had no significant effect on the levels of serum 25(OH)D.

Discussion

The present study of women living in a sunny subtropical region indicated a high prevalence (76.9%) of vitamin D insufficiency (< 30 ng/mL), even in women whose self-reported sun exposure was considered to be adequate. Moreover, 26.4% of the women had a deficiency of vitamin D (serum 25(OH)D < 20 ng/mL). These findings confirm previous studies conducted in other Brazilian cities at different latitudes.[18,22] The frequency of arterial hypertension and dyslipidemia in our study were similar in women with sufficient and deficient 25(OH)D levels. In fact, vitamin D supplementation in hypertensive patients with low 25(OH)D had no significant effect on blood pressure (BP) and several cardiovascular risk factors.[23] Also, long-term vitamin D supplementation, which increased mean 25(OH)D concentration to 30 ng/mL or higher, had no effect on BP.[24] Furthermore, oral vitamin D supplementation to correct vitamin D deficiency does not improve the lipid profile.[25] It is worth to emphasize that the relationship between vitamin D and cardiovascular disease, as well as other clinical conditions, comes from observational studies, while further controlled studies do not show such a relationship

In the southeast region of Brazil, 43.7% of postmenopausal women have 25(OH)D levels < 20 ng/mL, and 25(OH)D deficiency increases significantly with age.[22] The São Paulo Aging & Health Study evaluated individuals older than 65 years old in the city of São Paulo and reported 25(OH)D deficiency in more than half of this population, and this deficiency was particularly notable in women, subjects with type 2 diabetes, and during the winter and spring,[26] seasons when ultraviolet radiation and 25(OH)D concentrations reach a nadir in the city of São Paulo.[27] World data demonstrate that 5 to 25% of the independent elderly population and 60 to 80% of institutionalized patients have vitamin D deficiencies.[28] Likewise, 20 to 100% of elderly subjects in North America, Canada, and Europe,[2,11] as well as postmenopausal women living in southeastern and central Europe,[29] are believed to have vitamin D deficiencies. We did not expect a high prevalence of 25(OH)D insufficiency/deficiency in the present study because these women live in a region that is sunny throughout the year, and wear warm clothing for only short periods of time. Furthermore, this region has high agricultural activity, and the population is frequently exposed to sunlight. Nevertheless, only a minority of these women had sufficient levels of 25(OH)D (> 30 ng/mL).[4] This is clinically significant because previous observational studies demonstrated that hypovitaminosis D is associated with some clinical conditions, such as cardiovascular diseases, secondary hyperparathyroidism, osteoporosis, and fractures.[18,30,31]

A previous population-based study of osteoporosis in Brazil (BRAZOS) indicated that vitamin D levels did not affect the risk for fractures,[32] possibly because fractures are associated with polymorphisms of the vitamin D receptors. However, several previous studies failed to establish this correlation in Brazilian and British populations.[33,34] Moreover, associations between 25(OH)D levels with other clinical conditions were reported in some but not all observational studies,[35] and the results from randomized and controlled clinical trials are not yet available. These observational trials were also limited due to small sample sizes and short duration, which affected the quality of the investigations.[36,37] On this basis, the current recommendation is to measure serum 25(OH)D levels only in situations in which there is a risk for hypovitaminosis D due to certain morbidities.[4] This implies that the information given by patients

Table 2 Distribution of qualitative variables according to vitamin D cutoff classification as deficient [< 20 ng/mL] and adequate [≥ 20 ng/mL]) vitamin D levels.

	Vitamin D levels		*p*
	< 20 ng/mL (*n* = 24)	≥ 20 ng/mL (*n* = 67)	
Arterial hypertension			
Yes	7 (29.1%)	23 (34.3%)	0.64
No	17 (70.8%)	44 (65.6%)	
Diabetes Mellitus			
Yes	2 (8.3%)	5 (7.4%)	0.91
No	22 (91.6%)	62(92.5%)	
Hypothyroidism			
Yes	2 (8.3%)	9 (13.4%)	0.51
No	22 (91.6%)	58 (86.5%)	
Alcohol ingestion			
Yes	6 (25.0%)	14 (20.9%)	0.68
No	18 (75.0%)	53 (79.1%)	
Smoking			
Yes	5 (20.8%)	6 (8.9%)	0.13
No	19 (7.1%)	61 (91.0%)	
Physical activity			
None	2 (8.3%)	3 (4.6%)	0.14
≤ 150 minute/week	21 (87.5%)	48 (73.8%)	
> 150 minute/week	1 (4.1%)	14 (21.5%)	
Sun exposure			
Adequate	17 (7.8%)	51 (7.2%)	0.53
Inadequate	7 (29.1%)	15 (2.7%)	

Abbreviations: n, number of subjects; p- value.

about their supply of vitamin D (from exposure to sunlight and use of supplements) and clinical observations are needed for estimation of vitamin D status.

The present study indicated no significant difference in serum vitamin D levels of women classified as having adequate or inadequate sun exposure and no correlation between serum 25(OH)D levels and reported duration of sun exposure. Information on sun exposure was self-reported, and may have been biased due to imprecise recall[38,39] and misinterpretation by the interviewer of the information

Table 3 Distribution of quantitative variables according to vitamin D cutoff classification as deficient [< 20 ng/mL] and adequate [≥ 20 ng/mL]) vitamin D levels.

	Vitamin D levels		*p*
	< 20 ng/mL (*n* = 24) Median (IQR)	≥ 20 ng/mL (*n* = 67) Median (IQR)	
Age (years)	54.5 (47.5–57.0)	54.0 (49.0–60.0)	0.36
BMI (kg/m^2)	28.9 (24.9–31.9)	26.4 (23.8–28.8)	0.15
Calcium ion (mmol/L)	1.09 (1.08–1.11)	1.1 (1.06–1.13)	0.97
Glucose (mg/dL)	93.5 (80.0–101.0)	89.3 (79.0–94.0)	0.55
Total cholesterol (mg/dL)	200.9 (176.5–218.0)	182.1 (158.0–203.0)	0.03
HDL-C (mg/dL)	44.3 (37.0–50.5)	47.5 (37.0–56.0)	0.30
Triglycerides (mg/dL)	129.5 (78.5–172.0)	110.1 (73.0–130.0)	0.25
Creatinine (mg/dL)	0.77 (0.70–0.83)	0.81 (0.70–0.90)	0.22

Abbreviations: IQR, interquartile range; n, number of subjects; p- value.

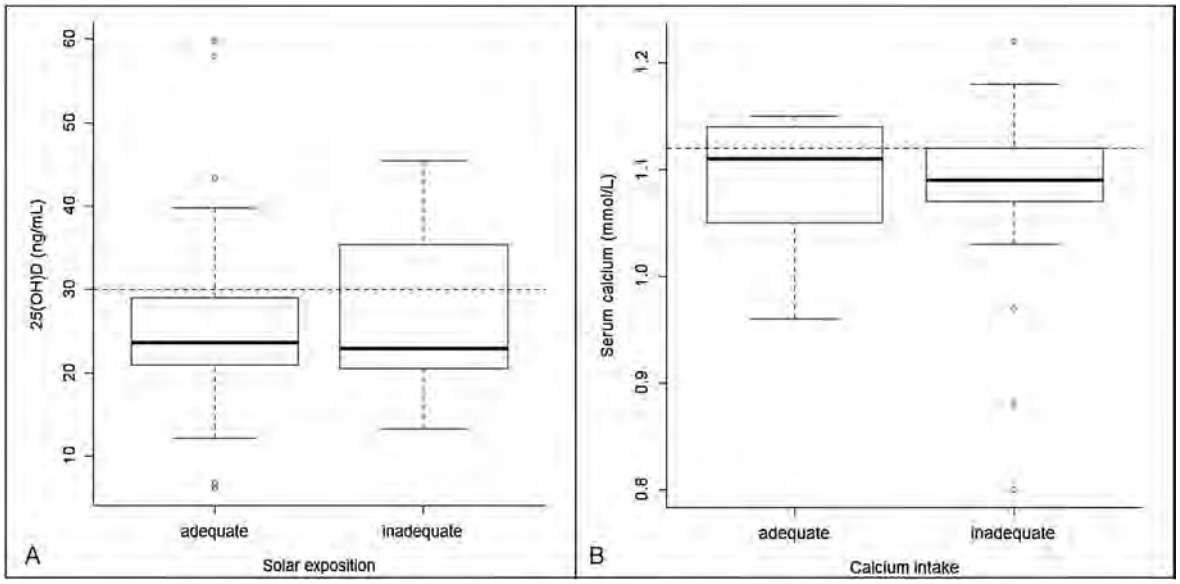

Fig. 1 Serum 25(OH)D concentrations in women with adequate and inadequate sun exposure (**A**) ($p = 0.88$), and serum ionized calcium concentrations (**B**) ($p = 0.73$) in women with adequate and inadequate calcium consumption ($n = 91$).

given by the interviewee. Consequently, the method of quantifying exposure to sunlight based on self-reporting has questionable accuracy, and more accurate methods are needed so that clinicians can provide guidelines to patients. However, it is also necessary to consider that other biological factors may interfere with 25(OH)D concentration, such as skin color.[40,41] We did not thoroughly evaluate skin color in the present study by use of phototyping.[42] It should be noted that dark skin interferes with vitamin D synthesis, and there is extensive racial mixing in the Brazilian population.[41] A previous study conducted in São Paulo reported a high prevalence of hypovitaminosis D and secondary hyperparathyroidism in the sample population after the end of winter, and skin color significantly and independently correlated with this condition.[43]

Most subjects in the present study had a self-reported calcium consumption of less than 1200 mg/day, the minimum recommended by the Institute of Medicine in the dietary reference intake of calcium.[44] A previous study had reported similar results (calcium comsumption less than the minimum) for people residing in the state of São Paulo: an average of only 448 mg/day of calcium; although reported intake did not correlate with blood level of calcium, although intake did not correlate with reported intake or blood level of calcium.[45] We also found no correlation between serum calcium and recall of calcium consumption or between serum calcium and the presence of 25(OH)D deficiency/insufficiency. It is known that calcium sensors in the parathyroid glands sense a change in circulating calcium, and this leads to increased PTH synthesis, and maintenance of blood calcium within the normal range.[46] This may partially explain the absence, in the present study, of a correlation between self-reported calcium consumption and serum level of calcium ion.

Table 4 Multiple linear regression analysis of the effects of sun exposure (less than three times per week versus three or more times per week), age, and serum creatinine on serum 25(OH)D concentration ($n = 91$)

Variable	Odds Ratio	95% CI	*p*
Intercept	5.63	−14.09, 25.34	0.57
Sun exposure	0.36	−4.16, 4.87	0.88
Age	−0.03	−0.32, 0.26	0.83
Serum creatinine	28.11	10.36, 45.86	<0.01

Abbreviations: CI, confidence interval p- value.

Our multiple linear regression analysis demonstrated that a serum creatinine level in the normal range had a significant and independent association with higher serum 25(OH)D concentration. Previous research indicated that in the absence of renal insufficiency, the renal production of 1,25 $(OH)_2D$, the hydroxylation product of 25(OH)D, is normal,[47] but that renal insufficiency, which is associated with abnormally high serum creatinine, is associated with a low level of serum 25(OH)D.[48,49]

Finally, the present findings support the need for a more objective evaluation of calcium and vitamin D supply, possibly by means of a 24-hour home diary.[50] Patient recall about the duration of sun exposure and calcium intake was not effective in assessing the actual levels of these substances in the present population. Although most of our study population had 25 (OH)D insufficiency, there was no higher prevalence of arterial hypertension in women with insufficient 25(OH)D levels. However, the present study was limited due to the sample allocation being restricted to a climacteric outpatient clinic, which can potentially mean that our outcomes may not be applicable to other populations.

Conclusion

This study indicates that even in community-dwelling women living in a city under high sun exposure, levels of serum

25(OH)D > 30 ng/ml are hardly reached, leading to the conclusion that other intrinsic factors besides sun exposure may regulate vitamin D levels.

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Frequency of Chromosomal Abnormalities in Products of Conception

Frequência de anomalias cromossômicas em material de aborto

Thaís Mesquita Alves Teles[1] Carolina Maria Marques de Paula[1] Mariana Gontijo Ramos[1] Helena B. B. L. Martins da Costa[2,3] Cyntia Roberta Almeida Andrade[2] Sarah Abreu Coxir[3] Maria Lectícia Firpe Penna[1]

[1] Department of Biomedical Sciences, College of Human, Social and Health Sciences, Universidade FUMEC, Belo Horizonte, MG, Brazil
[2] Department of Molecular Biology, Códon Biotechnology Laboratory, Belo Horizonte, MG, Brazil
[3] Department of Cytogenetics, Códon Biotechnology Laboratory, Belo Horizonte, MG, Brazil

Address for correspondence Maria Lectícia Firpe Penna, PhD, Rua Cobre, 200, Belo Horizonte, MG, Brazil (e-mail: mlpenna@fumec.br).

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Abstract

Purpose To describe the frequencies of chromosomal abnormalities found in abortion material, and to observe its correlation to maternal age.

Methods A retrospective study was conducted based on data obtained from the databank of a medical genetics laboratory in Belo Horizonte, MG, Brazil. A total of 884 results from products of conception analysis were included, 204 of which were analyzed by cytogenetics, and 680 by molecular biology based on quantitative fluorescence polymerase chain reaction (QF-PCR). The frequency of individual chromosomal aberrations and the relationship between the presence of anomalies and maternal age were also evaluated.

Results The conventional cytogenetics technique was able to detect 52% of normal and 48% of abnormal results in the analyzed material. Quantitative fluorescence polymerase chain reaction revealed 60% of normal and 40% of abnormal results from the samples evaluated by this method. The presence of trisomy 15 was detected only by cytogenetics, as it was not included in the QF-PCR routine investigation in the laboratory. A significant increase in abnormal results was observed among women aged 35 years or older compared with younger women ($p = 0.02$).

Conclusion Chromosomal aberrations are still a major cause of spontaneous abortion, and the conventional cytogenetics technique is efficient for miscarriage material analysis, but molecular methods such as QF-PCR are adequate complementary strategies to detect the major chromosomal anomalies, leading to technical reports with reliable results.

Keywords
- spontaneous abortion
- aneuploidies
- QF-PCR
- cytogenetics

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Resumo

Objetivos Descrever a frequência de anomalias cromossômicas encontradas em material de aborto, e observar se estas estão relacionadas com a idade materna.

Métodos Foi realizado um estudo retrospectivo no banco de dados de um laboratório de genética médica em Belo Horizonte, MG. O estudo incluiu 204 resultados avaliados por citogenética, e 680 resultados por biologia molecular baseada em reação em ensaio fluorescente da reação em cadeia da polimerase (QF-PCR), totalizando um número de 884 análises. A frequência de diferentes anomalias cromossômicas e a relação entre a presença de anomalias e a idade materna também foi avaliada.

Resultados A citogenética convencional foi capaz de detectar 52% de resultados normais e 48% de resultados anormais no material analisado. A QF-PCR revelou 60% de resultados normais e 40% de anormais nas amostras avaliadas por esta técnica. A presença da trissomia 15 foi detectada por citogenética, mas até então não era incluída na investigação por QF-PCR no laboratório. Um aumento significativo na quantidade de resultados anormais foi observado em mulheres com idade de 35 anos ou mais, quando comparado a mulheres mais jovens ($p = 0,02$).

Conclusão As aberrações cromossômicas são causas importantes de abortos espontâneos, e o estudo citogenético é eficaz para a análise das amostras de material de aborto, mas as técnicas moleculares, como a QF-PCR, representam métodos complementares adequados para detectar as principais anomalias cromossômicas, possibilitando a liberação de laudos com resultados confiáveis.

Palavras-chave

- aborto espontâneo
- aneuploidias
- QF-PCR
- citogenética

Introduction

Miscarriage is defined by the World Health Organization (WHO) as the premature loss of a fetus before the 20th week of pregnancy, or, if the gestational age is unknown, the loss of an embryo or fetus weighing less than 400 g.[1] Spontaneous pregnancy loss is the most common complication of pregnancy, and occurs in ~12–15% of clinically recognized pregnancies. The chance of a couple experiencing two consecutive losses is of 2 to 4%, but most women who have miscarriages can give birth to a healthy child later in life.[2]

The etiology of abortion is multifactorial, and may involve endocrine, anatomic, immunological, infectious, environmental and genetic factors.[3] Chromosomal abnormalities have been reported in 50% of spontaneously aborted fetuses of clinically recognized pregnancies, and can be divided in two basic groups: numerical and structural anomalies. These can involve one or more autosomal, sex or both chromosomes simultaneously.[4,5]

The most frequent autosomal anomaly observed in specimens from spontaneous losses is trisomy 16 (thought to be lethal and incompatible with full fetal development), followed by other autosomal aneuploidies and X monosomy.[6,7] As fetal chromosomal abnormalities are largely responsible for the inefficiency of human reproduction and its associated burdens, it is necessary to perform laboratory investigations of the products of conception (POC) using different diagnostic techniques to help to understand the possible causes of miscarriage and to provide adequate assistance for future pregnancies.[8–10]

The analysis of POC has been traditionally performed by cytogenetic karyotyping through the microscope examination of banded chromosomal preparations, detecting numerical and structural alterations. Molecular cytogenetic diagnostic tests are based on studying the fetal karyotype directly at the DNA level, and use DNA extracted from fetal cells, not requiring tissue culture and allowing the analysis of specimens fixed in ethanol, formaldehyde or included in paraffin.[11,12] Some of these techniques are fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and quantitative fluorescent polymerase chain reaction (QF-PCR).

All reproductive losses should be investigated by cytogenetics, considering that conventional karyotyping has been the gold standard for the chromosomal investigations of POC. This method allows the detection of structural (translocations, deletions and inversions) as well as numerical chromosomal aberrations. However, it is a laborious, time consuming procedure that can lead to a significant amount of cases with no results, as it depends on human cells in active process of replication. The rate of culture failure is of 10–40%, as POC tissues are frequently macerated, contaminated or fixated in alcohol or formaldehyde.[12,13] Molecular cytogenetic techniques can be used to study POC abnormalities, as they do not require cell culture; however, the results could be limited because only numerical chromosomal alterations can be identified on the analyzed chromosomes.[14]

The genetic studies of POC provide important information for the genetic counseling of couples who experience pregnancy failure, as they help to elucidate the possible causes of fetal losses, indicating if any chromosomal abnormality was responsible for the miscarriage. They can also indirectly suggest if one of the parents could be the carrier of any structural disorder.[12,14]

The aim of this study was to describe the frequencies of chromosomal abnormalities found in abortion material, and to determine if there is a correlation between the presence of aberrations and maternal age.

Methods

Study Type and Samples

An observational, retrospective study was conducted to describe the results obtained from miscarriage material analysis performed by a private medical genetics laboratory in Belo Horizonte, MG, Brazil. The laboratory performs tests in miscarriage material received from different regions of Brazil, covering the whole country. The results were obtained from the laboratory databank, and, besides maternal age, no personal information from the patients was included. As the laboratory receives material from different medical facilities with limited information, clinical data such as gestational age at abortion and clinical history of the parents was not available. A total of 884 results from miscarriage material analysis performed between January 2011 and December 2015 was included.

Sample Analysis

Miscarriage material analysis techniques were performed by professional staff, according to the laboratory routine, using conventional cytogenetics, or QF-PCR.

Conventional cytogenetics was performed in samples containing tissue that had fetal origin, using standard culture, harvesting and staining conditions, by an experienced cytogenetics technician. A total of 204 cytogenetics results were obtained and included in the study. The aberrations and karyotypes were classified according to the International System for Human Cytogenetic Nomenclature 2013 (ISCN 2013).[15]

In case of cell growth failure by the cytogenetics technique, and according to the laboratory demand, the QF-PCR molecular technique was performed, as previously described.[16] For detection of chromosome aberrations, markers for sexual X and Y, and for autosomal 13, 16, 18, 21 and 22 chromosomes were performed by experienced professionals, according to the laboratory routine. A total of 680 QF-PCR results were available. These included the samples with cell growth failure in cytogenetics that were investigated by molecular QF-PCR, and the miscarriage material samples unsuitable for, or in which cytogenetics was not demanded.

Statistical Analysis

The results were presented as frequency values according to the different technique performed (cytogenetics or molecular biology), and distributed according to the chromosomal alterations found by each method and total sample results. The relation between the frequency of chromosomal anomalies and maternal age was analyzed by logistic regression using the statistical software Minitab 17.3.1 (State College, PA, USA). Significance was set at $p < 0.05$.

The study was submitted and approved by the Ethics Committee of the institution under protocol CEP nº 1.346.235, on December 1, 2015.

Table 1 Normal and abnormal results observed by cytogenetics and molecular biology techniques

	Cytogenetics ($n = 204$)	Molecular biology ($n = 680$)	Total ($n = 884$)
Normal	106 (52%)	410 (60%)	**516 (58%)**
Abnormal	98 (48%)	270 (40%)	**368 (42%)**

Results

A total of 884 results from miscarriage material samples was included in the study. ►**Table 1** shows the frequency of normal and abnormal results obtained by conventional cytogenetics and molecular biology QF-PCR technique. From the total sample of 884, 368 (42%) cases of chromosome abnormalities were detected, while 516 (58%) cases had no detected alterations. Cytogenetics was able to identify 52% of normal results (106 out of 204 tested), and QF-PCR, 60% (410 out of 680 tested). Cytogenetics showed 48% (98 out of 204 tested) of abnormal results, and molecular biology detected 40% (270 out of 680 tested) of abnormal cases, considering the chromosomes analyzed by this technique.

The chromosomal abnormalities identified by each method are demonstrated in ►**Table 2**.

Cytogenetics results showed 18.4% X monosomy, 13.3% trisomy 16, and 10.2% trisomy 22. It also revealed that 8.2% of the abnormalities were trisomy15. Molecular biology results were 15.9% for X monosomy, 18.9% for trisomy 16, and 19.7% for trisomy 22. Chromosome 15 was not evaluated by QF-PCR in this study (►**Table 2**).

Considering all the samples, trisomy was the most common chromosome aberration, accounting for 63% (232 out of 368) of the abnormalities. The most frequent was trisomy 16 (17.4%), followed by trisomy 22 (17.1%). Monosomy accounted for 16.6% (61 out of 368) of the anomalies, and polyploidies, for 18.8% (69 out of 368) (►**Table 2** and ►**Table 3**).

The relationship between aneuploidy and maternal age is shown in ►**Fig. 1**. In the young maternal age group ($n = 452$), 62.2% (281) had normal results, while abnormalities were found in 171 cases (37.8%). For the advanced maternal age group ($n = 432$), 235 (54.4%) normal results were observed, compared with 197 (45.6%) abnormal findings. This difference was considered statistically significant ($p = 0.02$) (►**Fig. 1**).

Discussion

The analysis of chromosomal abnormalities in POC is useful to determine the possible causes of miscarriage, and to provide information and counseling for couples regarding future pregnancies.

This retrospective study showed the presence of aneuploidies in 42% of all the analyzed samples. When the different techniques were considered, the results were similar (40% by QF-PCR and 48% by cytogenetics). A recent study by Coelho et al[17] demonstrated the presence of aneuploidies in 54.6% of miscarriage material from Brazilian patients, analyzed by

Table 2 Frequency of chromosomal anomalies

Anomaly	Cytogenetics n (%)	Molecular biology n (%)	Total n (%)
X monosomy	18 (18.4%)	43 (15.9%)	61 (16.6%)
Trisomy 2	1 (1%)	NA	1 (0.3%)
Trisomy 4	2(2%)	NA	2 (0.5%)
Trisomy 7	1 (1%)	NA	1 (0.3%)
Trisomy 8	3 (3.1%)	NA	3 (0.8%)
Trisomy 10	3 (3.1%)	NA	3 (0.8%)
Trisomy 13	4 (4.1%)	25 (9.3%)	29 (7.9%)
Trisomy 14	2 (2%)	NA	2 (0.5%)
Trisomy 15	8 (8.2%)	NA	8 (2.2%)
Trisomy 16	13 (13.3%)	51 (18.9%)	64 (17.4%)
Trisomy 17	2 (2%)	NA	2 (0.5%)
Trisomy 18	5 (5.1%)	16 (5.9%)	21 (5.7%)
Trisomy 20	1 (1%)	NA	1 (0.3%)
Trisomy 21	9 (9.2%)	23 (8.5%)	32 (8.7%)
Trisomy 22	10 (10.2%)	53 (19.7%)	63 (17.1%)
47 XXY	1 (1%)	NA	1 (0.3%)
Triploidy	9 (9.2%)	56 (20.7%)	65 (17.7%)
Trisomies 16 and 22	0	2 (0.7%)	2 (0.5%)
Trisomies 16 and 21	0	1 (0.4%)	1 (0.3%)
Other aneuploidies	6 (6.1%)	NA	6 (1.6%)
Total	98 (100%)	270 (100%)	368 (100%)

Abbreviation: NA, Not analyzed by QF-PCR.

QF-PCR. Jenderny[15] reported a frequency of 61% abnormal results in POC analyzed by cytogenetics and QF-PCR in the German population. Other studies found similar (48%) or lower frequencies (36%) of total chromosomal aberrations.[18,19]

The present study revealed that the main chromosomal abnormality detected in abortion material was trisomy, followed by triploidy and monosomy X. These results corroborate with other studies that demonstrated similar results, showing that trisomies, especially those involving chromosomes 16 and 22, are implicated in spontaneous abortion.[17,20–25]

Table 3 Groups of chromosomal anomalies

Anomaly	Total n (%)
Trisomy	232 (63%)
Polyploidy	69 (18.8%)
X monosomy	61 (16.6%)
Others	6 (1.6%)
Total	368 (all)

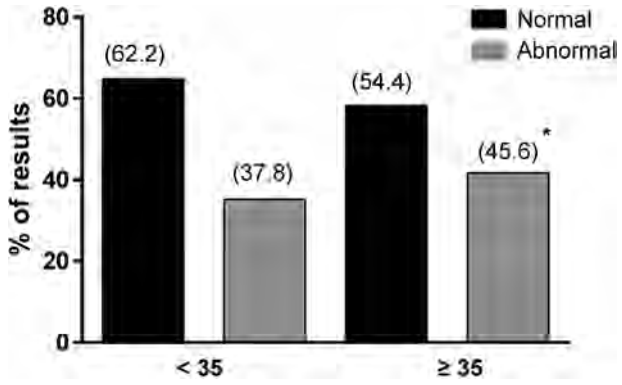

Fig. 1 Maternal age and chromosomal abnormality. Numbers between parentheses show the percentage of the results. The rates of abnormal results were significantly higher for the advanced maternal age group (n = 432) when compared to the younger maternal age group (n = 452) ($p = 0.02$).

An important point noted in the present study was that trisomy 15 was found in 8.2% of the samples analyzed by conventional cytogenetics. Similar values were observed by Coelho et al[17] (14.1%), Moraes et al[20] (9%), Subramaniyam et al[21] (13.5%), and Romero et al[25] (7,7%), suggesting that trisomy 15 is recurrent in POC samples. Until the present moment, chromosome 15 markers have not been included in routine QF-PCR analysis of abortion material at the laboratory. However, the results showed that a marker for chromosome 15 should be included in the molecular analysis, improving the quality of the released technical reports.

Conventional cytogenetics technique remains highly recommended for spontaneous miscarriage analysis. However, this method has certain disadvantages, such as a long laboratory cycle, labor intensity and culture failure, especially when the tissues obtained from patients are not well preserved.[17,26]

The QF-PCR technique is regarded as a highly accurate, low cost and rapid diagnostic method to facilitate the detection of clinically relevant chromosome aberrations. However, some limitations of the technique are the difficulty to offer a correct diagnosis for mosaicism, small deletions, translocations and duplications.[15,17]

Since our laboratory miscarriage material is received from different medical facilities, the QF-PCR method plays an important role as a reliable method for the detection of aneuploidies, when culture fails or when the karyotype is not possible due to inadequate material preservation.

Many reports have suggested that advanced maternal age is an important factor related to chromosomal aneuploidies.[22] Our study showed a significant increase in the rate of aneuploidy in the advanced maternal age group when compared with the young maternal age group ($p = 0.02$). Bastos et al[6] and Jia et al[22] also suggest a maternal age-related increase in chromosomal anomalies. Hormonal changes during the aging process, as decreased production of progesterone can lead to increased rates of miscarriage in women older than 35 years.[27]

In conclusion, chromosomal aberrations are still a major cause of miscarriage, and the conventional cytogenetics study

is highly recommended, as it can detect different types of chromosomal abnormalities. Molecular biology techniques, such as QF-PCR, are important complementary methods that can be effective to detect the main chromosomal anomalies, and may be used in combination with cytogenetics to allow the release of technical reports with reliable results.

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Upper Limb Functionality and Quality of Life in Women with Five-Year Survival after Breast Cancer Surgery

Funcionalidade do membro superior e qualidade de vida em pacientes com sobrevida de cinco anos após tratamento cirúrgico para câncer de mama

Thaís Lunardi Recchia[1] Amably Cristiny Prim[1] Clarissa Medeiros da Luz[1]

[1] Centro de Ciências da Saúde e do Esporte, Universidade do Estado de Santa Catarina (UDESC), Florianópolis, SC, Brasil

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Address for correspondence Clarissa Medeiros da Luz, PhD, Programa de Pós-Graduação em Fisioterapia da Universidade do Estado de Santa Catarina (UDESC), 88080-350–Florianópolis/SC, Brasil (e-mail: amablyfisio@gmail.com; lr_thais@yahoo.com.br).

Abstract

Objective To evaluate the correlation between upper limb functionality and quality of life in women with five-year survival following breast cancer surgical treatment. The secondary objective was to evaluate the function of the ipsilateral upper limb and the quality of life in relation to the type of surgery and the presence of pain.

Methods The Disabilities of Arm, Shoulder and Hand (DASH), and the Functional Assessment of Cancer Therapy – Breast plus Arm Morbidity (FACTB + 4) questionnaires were used to evaluate upper limb function and quality of life respectively. Data distribution was verified by the Shapiro-Wilk test. Pearson's correlation coefficient was used for the parametric variables, and Spearman's rank correlation coefficient was used for the distribution of non-parametric variables. The statistical significance was set at 5% ($p < 0.05$).

Results The study included 30 patients, with a mean age of 51.23 ($\pm$8.72) years. The most common complications were: pain (50%), adherence (33.3%), and nerve lesion (20.0%). There was a moderate negative correlation between the instruments DASH and FACTB + 4 (total score), r = -0.634, and a strong negative correlation between the DASH and the FACTB + 4 arm subscale, r = -0.829. The scores of both questionnaires showed significant difference on the manifestation of pain. However, there was no significant difference found when comparing the scores considering the type of surgery performed.

Conclusions Five years after surgery, the patients showed regular functionality levels on the ipsilateral upper limb and decreased quality of life, especially in the group manifesting pain.

Keywords

- mastectomy
- range of motion
- quality of life
- upper limb functionality

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Resumo

Objetivo Avaliar se há correlação entre a funcionalidade e a qualidade de vida em pacientes com sobrevida de cinco anos submetidas ao tratamento cirúrgico para câncer de mama e, secundariamente, avaliar a função do membro superior homolateral à cirurgia, e a qualidade de vida em função do tipo de cirurgia mamária e da presença de dor.

Métodos Foram utilizados os questionários DASH e FACTB + 4 para avaliar a função do membro superior e a qualidade de vida respectivamente. Os dados foram submetidos ao teste de normalidade de Shapiro-Wilk. O coeficiente de correlação de Pearson foi utilizado para as variáveis com distribuição paramétrica e, para as variáveis com distribuição não paramétrica, o coeficiente de correlação de Spearman. Adotou-se o nível de significância de 5% ($p < 0,05$).

Resultados Foram incluídas 30 pacientes, com média de idade de 51,23 (± 8,72) anos. As complicações mais incidentes foram: dor (50%), aderência cicatricial (33,3%), e lesão nervosa (20,0%). Foi observada correlação negativa de magnitude moderada entre os instrumentos DASH e FACTB + 4 (pontuação total), r = -0,634, e de magnitude forte entre o DASH e a subescala braço do FACTB + 4, r = -0,829. As pontuações dos questionários apresentaram diferença significativa em função da presença de dor. Entretanto, não foi observada diferença significativa quando comparadas as pontuações com relação ao tipo de cirurgia.

Conclusões Após cinco anos de cirurgia, as pacientes apresentaram grau regular de funcionalidade do membro homolateral à cirurgia e diminuição na qualidade de vida relacionada à saúde, principalmente no grupo que relatava presença de dor.

Palavras-chave

- mastectomia
- amplitude de movimento articular
- qualidade de vida
- funcionalidade de membro superior

Introduction

Breast cancer (BC) is currently the neoplasm with the highest incidence and is the most common cause of cancer-related deaths among women worldwide.[1,2] In Brazil, it represents the second leading cancer-related cause of death among women, after non-melanoma skin carcinoma.[3] Between the years 2014 and 2015, there was an estimated 57,120 new cases in Brazil, which represents an incidence of 56.1 cases per 100,000 women.[4]

The primary treatment choice for BC is surgery (radical or conservative), and the other options are radiation, chemotherapy, hormonal therapy and targeted therapy as adjuvant treatments.[5] Despite the advances in surgical techniques, the procedure is still associated with a high prevalence of complications on the ipsilateral upper limb, and more than half of the patients who undergo axillary clearance present post-surgery comorbidities.[6]

The main complications related to surgery are sensitivity impairment, seroma production, altered ventilatory capacity, lymphedema, axillary cording, reduced strength on the upper limb, reduced range of motion (ROM) of the arm and shoulder, and pain.[7,8] Pain itself is the main physical/functional impairment reported by patients,[9] with a prevalence of up to 60%.[9,10]

Functionality involves the interrelation of the personal aspects of an individual (physical and emotional) with the surroundings (the environment and the engagement on activities).[11] The morbidities resulting from BC treatment create a negative impact on upper limb functionality, affecting daily activities; when added to altered body image and emotional disturbances such as anxiety and depression, that can affect the quality of life of women who have undergone treatment.[12,13] Associated with the increasing survival rate in women with BC, this fact has led to a large number of studies about quality of life related to health.[14,15]

However, the majority of the studies focuses on the evaluation of comorbidities and their effects on functionality and quality of life up to two years after surgery.[16,17] These data should be complemented by longer studies of five and ten years following surgical treatment to obtain a better definition of the comorbidities and tof he impact on function and quality of life on this population.[18–20]

For the reasons presented before, this study has the primary objective of investigating a possible correlation between functionality and quality of life in patients with five-year survival who underwent surgical treatment for BC. The secondary goal is to evaluate surgical ipsilateral upper limb function and quality of life related to the type of surgery and pain.

Methods

This is a cross-sectional, observational analytical study with a quantitative approach, completed between February

and April 2015 at the Physical Therapy ambulatory of Maternidade Carmela Dutra (MCD), in the city of Florianópolis, Brazil.

The study population consisted of women living in Florianópolis submitted to surgery for BC treatment according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10), sectionC50 ('Malignant neoplasm of breast'), who were part of the Hospital Cancer Registry (HCR) at MCD between the years 2009 and 2010. The study excluded patients with other primary cancer types, metastasized cancer, and with an indication for nodulectomy surgery with no lymph node resection or sentinel lymph node biopsy. Subjects with cognitive impairments who were unable to complete the questionnaires were also excluded. The protocol was approved by the Ethics and Human Subject Research Committees at Universidade Estadual de Santa Catarina and MCD (CAAE 39722314.70000.0118). All participants signed an informed consent form as per the Helsinki Declaration and the Brazilian National Health Council Resolution n. 466, from December 2012. The Participants' identities and data confidentiality were protected.

Data Collection

All participants completed an initial survey containing: personal information, gynecological and obstetrics history, cancer history, daily habits and general questions related to the diagnosis and the physical therapy treatment for axillary cording. The participants then underwent a physical exam to investigate possible adherence, breast sensitivity through Semmes-Weinstein monofilament and ipsilateral arm lymphedema as per the Simplified Clinical Classification for Lymphedema.[21] The McGill questionnaire was used to evaluate pain.[22]

Stature and body mass were verified by Sanny® (American Medical do Brasil Ltda, São Bernardo do Campo, Brazil) stadiometer and digital bioimpedance scale Ironman Segmental Body Composition Monitor (Tanita Corporation, 14-2, 1-Chome, Maeno-Cho, Itabashi-Ku, Tokyo, Japan) model BC-558 respectively. All measurements were performed barefoot, standing erect with the head aligned. The body mass index (BMI) was calculated according to World Health Organization (WHO) guidelines.[23]

The participants completed the Disabilities of Arm, Shoulder and Hand (DASH) and the Functional Assessment of Cancer Therapy – Breast plus Arm Morbidity (FACTB + 4) questionnaires, which were applied by a single examiner, and translated and culturally adapted for the Brazilian population.[24,25]

The DASH questionnaire is an instrument used to evaluate physical function. It verifies the impact of disability and the symptoms of upper extremities as a functional unit, and measures the difficulty or inability to perform specific activities. It consists of 30 items, which are divided in physical function, symptoms and social function. Each item is rated on a scale from 1–5. The total score ranges from 0–100 points; the higher the score, the more severe the disability.[24] Scores between the 25–75th quartiles are indicative of some disability, while scores lower than the 25th quartile represent minimal or no disability, and those greater than the 75th quartile indicate a high disability level.[26]

The FACTB + 4 questionnaire was developed to assess quality of life in patients with breast cancer, and it was validated for the study population.[27] It consists of 36 questions: 27 of them refer to quality of life in general; 9 relate to specific problems faced by women with breast cancer; and 4 relate to upper limb morbidity. This instrument is divided in six scales, which may be scored separately: physical well-being; social/family well-being; emotional well-being; functional well-being; breast cancer subscale; and arm subscale. Each one has scores that range from 0–4. The total score ranges from 0 to 164 points, and high scores are associated with a better quality of life.[28]

Statistical Analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences (IBM-SPSS, Inc., Chicago, IL, USA – licensed for use at Universidade Estadual de Santa Catarina) software, version 20.0, and presented as descriptive statistics (mean and standard deviation) and frequencies. Data distribution was verified using the Shapiro-Wilk test, and the correlation was used to describe the degree and direction of the relationship between the variables. Pearson's correlation coefficient was used for the parametric variables, and Spearman's rank correlation coefficient for the non-parametric ones. Levene's test was used to analyze the homogeneity of variance. The comparison of the group means was done using the Student's *t*-test for parametric variables and the Wilcoxon–Mann–Whitney test for non-parametric variables. The statistical significance was set at 5% ($p < 0.05$).

Results

The mean time to complete both questionnaires was 30 minutes. Thirty patients were included; they had a mean age of 51.23 (±8.72) years, and a mean BMI of 28.18 (±5.06), characterizing an overweight population. The majority of the patients were married or on a stable union (73.3%), were caucasians (93.3%), and had less than 8 years of schooling (60.0%). Aside from that, 36.7% of them were ex-smokers, and 16.7% were smokers. Half of the participants did not practice any physical activity. ►**Table 1** shows the participants' demographics and habits.

The progression of the disease and the frequency of the complications are shown in ►**Table 2**. Early stage diagnosis occurred in 83.3% of the participants, and the predominant histological type was ductal infiltrating (66.7%). Conservative surgery was performed in 53.3% of the patients, and 70.0% of this group did not have either early or late breast reconstruction performed after surgery. The most frequent complications were: pain (50%), adherence (33.3%), nerve lesion (20.0%) and lymphedema (13.3%). Axillary cording was presented by 13.3% of the participants; half of them underwent treatment, and half progressed with spontaneous resolution with no specific interventions.

►**Table 3** illustrates the mean, minimal, maximal and standard deviation data for the scores on both questionnaires

Table 1 Participants' demographics and daily life activities

Variables	Mean (SD)
Age (years)	51.23 (8.72)
BMI (Kg/m)	28.18 (5.06)
Variables	**N (%)**
Marital status	
Single	1 (3.3)
Married/stable union	22 (73.3)
Widow	2 (6.7)
Separated	5 (16.7)
Race	
White	28 (93.3)
African American	1 (3.3)
Brown	1 (3.3)
Educational level	
< 8 years	18 (60.0)
> 8 years	12 (40.0)
Smoking status	
Non-smoker	14 (46.7)
Ex-smoker	11 (36.7)
Smoker	5 (16.7)
Physical Activity	
Inactive	15 (50.0)
Eventual	4 (13.3)
Regular	11 (36.7)
Unknown	3 (10.0)

Abbreviations: BMI, Body Mass Index; SD, Standard deviation.
Note: N = 30.

Table 2 Disease progression and frequency of post-surgery complications

Variables	N (%)
Stage	
Early	25 (83.3)
Advanced	2 (6.7)
Unknown	3 (10.0)
Histology	
Intraductal in situ	1 (3.3)
Invasive Lobular	5 (16.7)
Ductal infiltrative	20 (66.7)
Other	4 (13.3)
Surgery type	
Conservative	14 (46.7)
Non-conservative	16 (53.3)
Reconstructive surgery	
No	21 (70.0)
Yes	7 (23.3)
Unknown	2 (6.7)
Scar adherence	
No	20 (66.7)
Yes	10 (33.3)
Cording	
No	26 (86.7)
Yes	4 (13.3)
Cording treatment	
Treated	2 (50.0)
Spontaneous recovery	2 (50.0)
Nerve lesion	
No	24 (80.0)
Yes	6 (20.0)
Lymphedema	
No	26 (86.7)
Yes	4 (13.3)
Pain	
No	15 (50.0)
Yes	15 (50.0)

Note: N = 30.

and their individual domains. The mean score for the DASH was 41.03 (±22.27) points, and the total score for the FACTB + 4 was 92.40 (±17.27), with scores of 19.8 and 19.6 for the additional concerns and the physical domains respectively.

There was a negative correlation between the DASH and the total score of the FACTB + 4, between the physical domain of the FACTB + 4 and the DASH, and a high correlation coefficient between the DASH and the upper limb subscale of the FACTB + 4. Only the social/family domain of the FACTB + 4 did not show a significant statistical correlation with any other variables. The detailed correlation analysis and the more relevant data are presented in ►**Table 4** and ►**Fig. 1**.

The analysis of the scores of both questionnaires and their domains in reference to the type of surgery performed (conservative or non-conservative) showed no statistical significant difference. However, when considering the presence or absence of pain, there was an influence on the score for both questionnaires ($p < 0.05$) (►**Table 5**).

Discussion

The correlation found between the DASH and the total score of the FACTB + 4 was -0.634, and the one between the DASH and the physical domain of the FACTB + 4 was -0.720. These findings indicate a moderate correlation among the variables. Still, a strong correlation (r = -0.829) between the arm subscale of the FACTB + 4 and the DASH was observed, with

Table 3 Mean, minimum and maximum scores and standard deviation of the FACTB + 4 and DASH questionnaires

Questionnaire (variable)	Minimum	Maximum	Mean	SD
DASH	2.00	76.00	41.03	22.27
FACTB + 4 (physical)	9.00	28.00	19.60	5.49
FACTB + 4 (social/family)	10.00	27.00	18.23	4.61
FACTB + 4 (emotional)	8.00	26.00	17.43	4.93
FACTB + 4 (functional)	3.00	26.00	17.13	4.46
FACTB + 4 (AC)	6.00	29.00	19.80	5.52
FACTB + 4 (total)	54.00	124.00	92.40	17.27

Abbreviations: AC, additional concerns; DASH, Disabilities of Arm, Shoulder and Hand; FACTB + 4 Functional Assessment of Cancer Therapy – Breast plus Arm Morbidity; SD, standard deviation.
N = 30.

Table 4 Correlation between the DASH and the FACTB + 4 domains

DASH			
	R	R^2	*p*
FACTB + 4 (social/family)	0.003	0.000	0.988
FACTB + 4 (emotional)	−0.495	0.245	0.005
FACTB + 4 (physical)	−0.720	0.518	0.000
FACTB + 4 (functional)	−0.425	0.180	0.019
FACTB + 4 (AC)	−0.462	0.213	0.010
FACTB + 4 (total)	−0.634	0.401	0.000
FACTB + 4 (arm subscale)	−0.829	0.687	0.000

Abbreviations: AC, additional concerns; DASH, Disabilities of Arm, Shoulder and Hand; FACTB + 4 Functional Assessment of Cancer Therapy – Breast plus Arm Morbidity; r, correlation coefficient; R^2, coefficient of determination.

a coefficient of determination indicating that 68.7% of the score variability on the FACTB + 4 arm subscale is related to the variability of the DASH.

The strong correlation between the DASH and the FACTB + 4 arm subscale, and the moderate correlation between the DASH and the physical domain of the FACTB + 4 could be explained by the fact that both are related to physical aspects and symptoms such as pain, edema, reduced ROM, rigidity and paresthesia of the ipsilateral arm. According to a study conducted by Fernandes,[29] the DASH does not correlate with instruments of distinct concepts. Thus, when a correlation is performed between the DASH score and the total score of the FACTB + 4 (which presents domains not related to physical function, such as emotional well-being and family/social well-being), there is a decrease in the magnitude of the correlation.

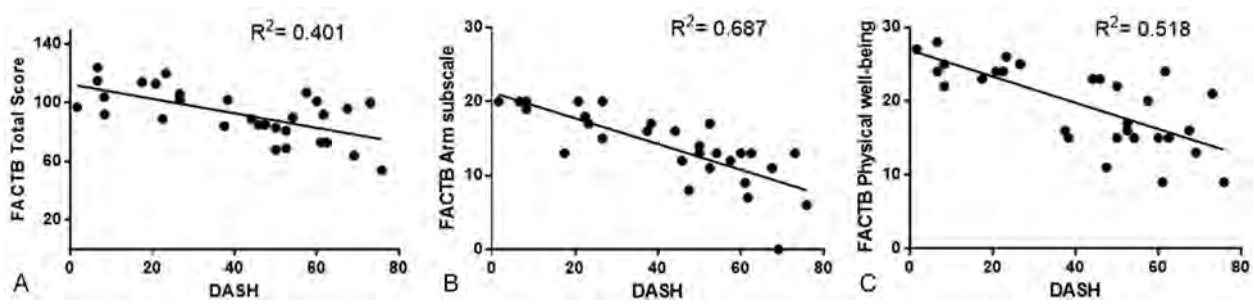

Fig. 1 Correlation between the DASH and FACTB + 4 scores. (**A**) Pearson's correlation analysis between the DASH and the total score of the FACTB + 4. Correlation coefficient = -0.634; coefficient of determination (R^2) = 0.401; p = 0.000. (**B**) Spearman's coefficient correlation between the DASH and the arm subscale of the FACTB + 4. Correlation coefficient = -0.829; R^2 = 0.687; p = 0.000. (**C**) Spearman's coefficient correlation between the DASH and the physical domain of the FACTB + 4. Correlation coefficient = -0.720; R^2= 0.518; p = 0.000.

Table 5 Mean score values and comparison tests between the mean values on the FACTB + 4 domains and on the DASH in reference to pain

Questionnaire (variable)	(SD)	(SD)	*p*
	No pain ($N = 15$)	With pain ($N = 15$)	
DASH	25.87 (19.42)	56.19 (12.07)	0.000*
FACTB + 4 (social/family)	18.53 (4.96)	17.93 (4.40)	0.728
FACTB + 4 (emotional)	19.60 (3.98)	15.27 (4.95)	0.014*
FACTB + 4 (physical)	22.87 (4.50)	16.33 (4.38)	0.001*
FACTB + 4 (functional)	18.87 (3.42)	15.40 (4.81)	0.032*
FACTB + 4 (AC)	21.80 (5.29)	17.80 (5.16)	0.045*
FACTB + 4 (total)	101.87 (14.94)	82.93 (14.23)	0.001*
FACTB + 4 (arm subscale)	16.33 (4.54)	12.06 (4.47)	0.015*

Abbreviations: AC, additional concerns; DASH, Disabilities of Arm, Shoulder and Hand; FACTB + 4 Functional Assessment of Cancer Therapy – Breast plus Arm Morbidity; SD, Standard deviation.
Note: *Significance level of 5% ($p < 0.05$).

The subjects presented, on average, moderate upper limb dysfunction (41.3 points) according to the DASH, and decreased quality of life (92.04 points) according to the FACTB + 4. These results are similar to those of other studies[17,30,31] that showed a significant correlation between the morbidity of the arm and reduced quality of life after BC treatment. The present study also confirms the findings of the study conducted by Fangel et al,[12] which showed a moderate correlation between the physical domain on the 30-item European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC-QLQ-C30) and function.

In agreement with other study findings,[32] but diverging from other authors[20] as well, when the questionnaire scores were analyzed according to the type of surgery (conservative or non-conservative), there was no significant difference. This could be explained by the fact that the extent of the procedure (with or without lymph node dissection) could have more influence on comorbidities than the type of surgery itself.[32,33] The presence of positive lymph nodes indicates more aggressive surgical procedures, systemic therapy and longer radiation treatment, which contributes to increased functional impairment.[34]

On the other hand, when the comparison is performed considering the presence or absence of pain on the ipsilateral arm, there was a significant difference on the total score of the FACTB + 4 (101.87 no pain; 82.93 with pain) and, above all, on the DASH (25.87 no pain; 56.19 with pain). Some studies mention pain as the most incident comorbidity directly related to the worsening of upper extremity function and/or a worse quality of life.[5,16,33,34] The onset of pain may occur immediately after surgery, or it may occur as a consequence of radiation,[5] and the pain can endure for a long period of time. The intercostobrachial nerve lesion is considered the main cause of pain,[33] and some other causes may be myofascial pain syndrome and axillary cord.[8,35] Milder symptoms, such as pain and paresthesia, are common between two and five years after the axillary lymphadenectomy.[36]

The present study showed a lower percentage of comorbidities (pain, adherence, nerve lesion and lymphedema) related to BC treatment compared with those found in the literature.[25] One of the main reasons for this finding is that most studies focus on comorbidities up to two years post-surgery,[9,17,34,37] and rarely on the data about the prevalence of comorbidities after five or more years.[25,36]

Apart from the surgical treatment, another important factor associated with upper limb morbidities is radiation. Radiation causes adverse effects such as pain, fatigue, fibrosis, sensitive changes and cutaneous impairment, like radiodermitis.[5,19] Sensitivity changes are associated with the intercostobrachial nerve lesion caused during the surgical procedure, or caused by other therapies, such as radiation.[32]

Sensorial damage and pain may influence the accomplishment of functional tasks due to the inhibitory muscle effect.[38] Levy et al[34] suggested that pain, sensitivity changes, fatigue and weakness may coexist and have a significant cumulative effect, contributing to long-term functional morbidity. Furthermore, changes on the axillary region due to the dissection of the lymph nodes, as well as scar retraction, tissue fibrosis, stiffness, thoracic muscle hypotrophy and hypomobility contribute to decreased shoulder ROM and function.[38,39] According to Fangel et al,[12] the dysfunctions resulting from BC treatment lead to changes in the routines of the patients, at the family and professional levels, which may affect their self-esteem and, consequently, their quality of life.

Many symptoms and dysfunctions are not measured by the conventional clinical methods. The use of questionnaires completes this gap, for they contribute to the recognition of functional and emotional problems, aid in the description of a group and in the evaluation of the results of an intervention, and that refines the clinical results.[14,40] Moreover, questionnaires are low-cost, convenient and easy tools that allow the identification of the needs of the patients; they also assist the physical therapist in terms of rehabilitation planning and selection of the adequate therapies.[12]

Even though the sample size was not calculated in advance, the power of all correlations with $\alpha = 0.05$ was 95%.[41]

The main limitation of the present study was the lack of follow-up during the five-year survival, which would enable a yearly report of the quality of life and function in this group. Also, the results refer to a group treated at a highly recognized facility, which may not reflect the general population.

Conclusion

There was a moderate negative correlation between the total scores of the DASH and FACTB + 4 questionnaires and a strong correlation between the DASH and the arm subscale of the FACT + 4.

The participants presented on average regular function based on the DASH, and decreased quality of life based on the FACTB + 4. Those who presented pain had worse ipsilateral upper limb function and worse quality of life compared with the participants with no pain. There was no significant difference between groups when considering the type of surgery performed.

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Underdiagnosis of cervical intraepithelial neoplasia (CIN) 2 or Worse Lesion in Women with a Previous Colposcopy-Guided Biopsy Showing CIN 1

Subdiagnóstico de neoplasia intraepitelial cervical (NIC) 2 ou lesão mais grave em mulheres com biópsia dirigida por colposcopia prévia mostrando NIC 1

Carlos André Scheler de Souza[1] Michelle Garcia Discacciati[2] Maria Gabriela d'Otavianno[1] Silvia Maria Bergo[3] Markus Traue[3] Liliana Aparecida Lucci de Angelo Andrade[4] Luiz Carlos Zeferino[1]

[1] Department of Obstetrics and Gynecology, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil
[2] Laboratory of Cytopathology, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil
[3] Policlínica II, Campinas, São Paulo, Brazil
[4] Department of Pathology, Faculdade de Ciências Médicas, UNICAMP, Campinas, São Paulo, Brazil

Address for correspondence Luiz Carlos Zeferino, MD, PhD, Rua Alexander Fleming, 101, Cidade Universitária Zeferino Vaz, 13083-881 Campinas, SP, Brazil (e-mail: zeferino@fcm.unicamp.br).

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Abstract

Objective Expectant follow-up for biopsy-proven cervical intraepithelial neoplasia (CIN) 1 is the current recommendation for the management of this lesion. Nevertheless, the performance of the biopsy guided by colposcopy might not be optimal. Therefore, this study aimed to calculate the rate of underdiagnoses of more severe lesions in women with CIN 1 diagnosis and to evaluate whether age, lesion extent and biopsy site are factors associated with diagnostic failure.

Methods Eighty women with a diagnosis of CIN 1 obtained by colposcopy-guided biopsy were selected for this study. These women were herein submitted to large loop excision of the transformation zone (LLETZ). The prevalence of lesions more severe than CIN 1 was calculated, and the histological diagnoses of the LLETZ specimens were grouped into two categories: "CIN 1 or less" and "CIN 2 or worse."

Results The prevalence of lesions diagnosed as CIN 2 or worse in the LLETZ specimens was of 19% (15/80). Three women revealed CIN 3, and 1 woman revealed a sclerosing adenocarcinoma stage I-a, a rare type of malignant neoplasia of low proliferation, which was not detected by either colposcopy or previous biopsy. The underdiagnosis of CIN 2 was not associated with the women's age, lesion extension and biopsy site.

Conclusions The standard methods used for the diagnosis of CIN 1 may underestimate the severity of the true lesion and, therefore, women undergoing expectant management must have an adequate follow-up.

Keywords
- colposcopy
- cervical intraepithelial neoplasia
- colposcopic surgical procedures
- uterine cervical neoplasms
- biopsy

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Resumo

Objetivo O seguimento de mulheres com neoplasia intraepitelial cervical (NIC) 1 comprovada por biópsia é atualmente a recomendação de conduta para esta lesão. Entretanto, o desempenho da biópsia guiada por colposcopia pode falhar. Assim, este estudo teve como objetivo estimar a taxa de subdiagnóstico de lesões mais graves em mulheres com diagnóstico de NIC 1 e avaliar se a idade, a extensão da lesão e o local da biópsia são fatores associados à falha do diagnóstico.

Métodos Foram selecionadas 80 mulheres com diagnóstico de NIC 1 obtido por biópsia dirigida por colposcopia. Estas mulheres foram submetidas a excisão da zona de transformação por alça diatérmica (EZTAD). A prevalência de lesões mais graves do que NIC 1 foi calculada, e os diagnósticos histológicos feitos nas amostras obtidas por EZTAD foram agrupados em duas categorias: "NIC 1 ou menos grave" e "NIC 2 ou mais grave".

Resultados A prevalência de lesões diagnosticadas como NIC 2 ou mais grave nas amostras de EZTAD foi de 19% (15/80). Três mulheres apresentaram NIC 3, e uma mulher revelou adenocarcinoma esclerosante estágio I-a, um tipo raro de neoplasia maligna de baixa proliferação, que não foi detectado por qualquer exame de colposcopia ou biópsia anterior. O subdiagnóstico de NIC 2 não foi associado à idade, à extensão da lesão ou ao local da biópsia.

Conclusão Os métodos de referência utilizados para o diagnóstico da NIC 1 podem subestimar a gravidade da lesão verdadeira e, portanto, as mulheres submetidas a conduta expectante devem ter um seguimento adequado.

Palavras chaves

- colposcopia
- neoplasia intraepitelial cervical
- procedimentos cirúrgicos colposcópicos
- neoplasias do colo do útero
- biópsia

Introduction

Cervical intraepithelial neoplasia (CIN) 1 is a highly prevalent lesion in young women, and its prevalence is decreasing with age.[1] Around 90% of the cases are associated with high-risk human papillomavirus (HPV); however, the regression rates of these lesions may reach up to 80%.[2,3] Expectant management with colposcopy and/or cytology follow-up has been proposed for CIN 1, since the probability of this lesion progressing to invasive carcinoma is low.[3–6] The objective of this clinical approach is to reduce the rate of unnecessary surgical procedures that may involve morbidities, such as risk of impaired fertility and obstetric outcome.[7–10] Meta-analysis studies showed that cold knife conization and large loop excision of the transformation zone (LLETZ) are associated with poorer obstetric outcomes related to preterm delivery and low birthweight.[10,11]

Nevertheless, there is a concern with respect to the precision of the histological diagnosis of CIN 1, since biopsy, even if directed to the most suspected areas identified by the colposcopist, consists of the analysis of a relatively small tissue sample that depends on several factors, such as the colposcopist's skills and the interobserver variability intrinsic to this procedure.[12] A possible error resulting from conservative management is to diagnose CIN 1 when the woman actually has a more severe lesion. Therefore, the objective of this study was to re-evaluate cervical lesions in women with a previous histological diagnosis of CIN 1 established by colposcopy-guided biopsy. The same patients were thus submitted to LLETZ to investigate if CIN 2, CIN 3 or cervical cancer lesions were underdiagnosed. We also evaluated whether age, lesion extent and biopsy site are factors associated with diagnostic failure.

Methods

This analysis is derived from a Brazilian casuistic of randomized trial to evaluate expectant management versus immediate treatment for low-grade CIN performed between January 2003 and March 2006.[3] The trial included women with previous cytology of low-grade squamous intraepithelial lesion (LSIL) or atypical squamous cells of undetermined significance (ASC-US), with a CIN 1 diagnosis revealed by colposcopy-guided biopsy. Eighty women consecutively randomized to undergo immediate treatment by LLETZ were included in this analysis. The LLETZ was performed up to 45 days after the CIN 1 diagnosis.[3]

Women were selected based on their cervical smear test routinely performed as part of a cervical cancer screening program. Patients were excluded for any of the following: unsatisfactory colposcopy; current pregnancy; prior therapy for dysplasia, including medical (5-FLUROURACIL), surgical (laser, loop electrosurgical excision procedure [LEEP]), or cryotherapy; prior gynecologic cancer; prior pelvic radiation therapy; other malignancies; immunosuppression due to diseases such as AIDS, organ transplantation, or use of immunosuppressive medications; cognitive impairment or inability to provide written informed consent.

For the colposcopic examination, the cervix was divided into four quadrants; the two anterior quadrants were

considered as the anterior lip of the cervix, and the other two as the posterior lip. The extension of the lesion was recorded according to the number of compromised quadrants of the cervix. The site selected for the biopsy was the one identified by the colposcopist as being the most suspicious. The excision of the transformation zone was performed using a diathermic loop to a depth of $\sim$ 5 mm to include the entire lesion identified. The histopathology of the biopsy and the conization were analyzed by the same pathologist.

For the purpose of analysis, the LLETZ histological diagnoses were allocated into two groups: "no neoplasia/CIN 1" and "CIN 2 or worse (CIN 2 +)". The associations between the LLETZ histological diagnosis and the age of the woman, the extension of the lesion and the site of biopsy were analyzed. The median age was 24 years; therefore, the women were also grouped into "younger than 24 years" and "24 or older". The magnitude of the associations was tested by odds ratios (ORs) and their respective 95% confidence intervals (95%CIs). This study was approved by the Internal Review Board of Faculdade de Ciências Médicas of Universidade Estadual de Campinas (number 023/2003).

Results

The LLETZ confirmed that 54% (43/80) of the patients had CIN 1, and no neoplasia was found in 28% (22/80) of the specimens (►**Table 1**). Cervical intraepithelial neoplasia 2 or worse in the LLETZ specimens was detected in 19% (15/80) of the women. Within this group, 3 women, aged 19, 22 and 40 (data not shown in tables), had a diagnosis of CIN 3 in the LLETZ specimen. There was one woman for whom the LLETZ specimen revealed a "sclerosing adenocarcinoma" stage I-a, a rare type of malignant neoplasia of low proliferation, which was not detected by either colposcopy or previous biopsy. This cancer was located in the cervical canal, and the LLETZ specimen revealed a positive endocervical margin, and the radical hysterectomy showed no residual disease.

Table 1 Distribution of histological diagnoses obtained from LLETZ specimens for women with CIN 1 established by colposcopy-guided biopsy

Histological diagnosis	n (%)
No neoplasia	22 (27.5)
CIN 1	43 (53.8)
CIN 2	11 (13.8)
CIN 3	3 (3.8)
Malignant neoplasia*	1 (1.3)
Total	80 (100)

Abbreviations: CIN, cervical intraepithelial neoplasia; LLETZ, large loop excision of the transformation zone.
Note: *Sclerosing adenocarcinoma stage I-a located at the cervical canal.

►**Table 2** shows that lesions extending to two or more quadrants were present in 34% of the women, but this finding was not significantly associated with the presence of CIN 2+ in the LLETZ specimens. Moreover, no significant association was found between the severity of the lesions and the age group or the biopsy site. Biopsies were performed in the anterior cervical lip in most cases ($n = 43$).

Discussion

This study found 19% of CIN 2 or worse in women with previous diagnosis of CIN 1 in colposcopy-guided biopsies. This result suggested that the colposcopy-guided biopsy samples are not always representative of the severity of the lesions, and that has been observed for the colposcopic

Table 2 Association between the histological diagnosis of the LLETZ specimens with age, lesion extension and biopsy site for women with CIN 1 established by colposcopy-guided biopsy

	Histological diagnosis		
	No neoplasia/CIN 1 n (%)	CIN 2+ n (%)	OR (95%CI)
Age group			
< 24 years	33 (51)	10 (67)	1.93 (0.59–6.30)
≥ 24 years	32 (49)	5 (33)	
Extent of the lesion[a]			
One quadrant	42 (66)	9 (70)	0.85 (0.19–3.52)
Two or more quadrants	22 (34)	4 (30)	
Site of the biopsy[b]			
Anterior lip	38 (60)	5 (33)	2.92 (0.79–11.29)
Posterior lip	26 (40)	10 (66)	

Abbreviations: 95%CI, 95% confidence interval; CIN, cervical intraepithelial neoplasia; LLETZ, large loop excision of the transformation zone; OR, odds ratio.
Notes: [a]Information was missing for one woman with "No neoplasia/CIN 1" and two women with "CIN 2 + ".
[b]Information was missing for one woman with "No neoplasia/CIN 1".

examination.[13] Another issue that should be considered is the observer variability regarding the CIN 1 histological diagnosis, as well as the interlaboratory variability.[12,14] Following a prospective two-year follow-up, the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) reported 13% of CIN 2 and CIN 3 in patients with an initial histological diagnosis of CIN 1; 11.3% had a previous normal colposcopy, and 11.7% had a previous negative biopsy.[15] Boonlikit et al[16] reported an agreement rate between biopsy and LLETZ of 66% (Kappa = 0.24; fair agreement) in women under 50 years of age. Nevertheless, the biopsy failure rate seems to decrease with the increasing severity of the histological diagnosis.[17]

In our study, we found 53.8% of agreement for CIN 1 diagnosis, and 18.7% of underdiagnosis. The remaining 27.5% of the cases showed no neoplasia, which could be a consequence of the total removal of the lesion by the colposcopy-guided biopsy, or a regression of the lesion due to the clearance of the HPV infection.

No association was found between age and the presence of CIN 2 or worse in the LLETZ specimens. Studies have shown that the CIN 3 prevalence is higher in older women not enrolled in the cervical cancer screening, while age is not a determinant factor in women with previous screening tests.[1] Indeed, the women in this study were previously subjected to cervical cancer screening and, therefore, we did not find a high prevalence rate of more severe lesions in older women, as expected.

The extension of the lesion at colposcopy and the site of the biopsy were not associated with CIN 2 or worse at the LLETZ, that is, these factors were not associated with diagnostic failure. This finding suggests that, in the case of more extensive lesions, the efficacy of colposcopy in selecting the biopsy site was similar to that found when the lesions were confined to one quadrant. Studies have shown that the number of biopsies can increase the performance of the colposcopy-guided biopsy. Pretorius et al[18] reported 43% of undetected high-grade lesions when performing only one colposcopy-guided biopsy, and they suggested that diagnosis would be more precise if random biopsies or endocervical canal curettage were performed. Moss et al[19] concluded that single colposcopically directed punch biopsy appears to be insufficient to exclude underlying CIN 2 or 3 in women with an ASC-US or LSIL cytological result and minor colposcopic findings. Increasing the number of biopsies increases the detection rate of CIN 3, and random biopsies from apparently normal cervical tissue increase the chance of finding hidden lesions.[20,21]

Our study diagnosed 19% of CIN 2 or worse in women with previous diagnosis of CIN 1 in colposcopy-guided biopsies, and these findings are relevant mainly for younger women, for whom a more conservative approach must be considered. However, such an approach does not seem to affect the clinical success of the expectant management of CIN 1, as shown by a previous clinical trial.[3] Considering that the current recommendation for CIN 1 management is follow-up without treatment, the colposcopic examination should reach high performance to offer a reasonable guarantee that the woman does not have worse lesions. If the patient is adequately followed-up, a more severe lesion might be detected at the control visits, which could minimize the occurrence of underdiagnosis of CIN lesions.

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Are There Changes in the Fatty Acid Profile of Breast Milk with Supplementation of Omega-3 Sources? A Systematic Review

Existem mudanças no perfil de ácidos graxos no leite materno com a suplementação de fontes de ômega 3? Uma revisão sistemática

Yasmin Notarbartolo di Villarosa do Amaral[1] Daniele Marano[1] Leila Maria Lopes da Silva[1] Aline Carnevale Lia Dias Guimarães[1] Maria Elisabeth Lopes Moreira[1]

[1] Clinical Research Unit, Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

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Address for correspondence Yasmin Notarbartolo di Villarosa do Amaral, MSc, Fundação Oswaldo Cruz, Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira, Unidade de Pesquisa Clínica, Av. Rui Barbosa, 716, Flamengo, 22250-020, Rio de Janeiro, RJ, Brasil (e-mail: yasminamaral@hotmail.com).

Abstract

Purpose To evaluate the effect of supplementation with omega-3 sources on the fatty acid composition of human milk.

Methods The review consisted of the search for articles published in PubMed, Biblioteca Virtual de Saúde (Virtual Health Library[VHL]) and Web of Science databases using the following keywords: *fatty acids*, *omega-3*, *human milk* and *supplementation*; for this purpose, we have used the program of research to integrate the services for the maintenance of autonomy (PRISMA) checklist. The following selection criteria were used: articles in English, Portuguese, Spanish or Italian, published between 2000 and 2015, and about studies performed in humans. We found 710 articles that met the established criteria; however, only 22 of them were selected to be part of this study.

Results All studies found a positive relationship between the consumption of omega-3 sources and their concentration in human milk. The differences in the findings are due to the distinct methods used, such as the specific time of the omega-3 supplementation, the type of omega-3 source offered, as well as the sample size.

Conclusion Although the studies were different in several methodological aspects, it was possible to observe the importance of omega-3 supplementation during gestation and/or the puerperium.

Keywords
- pregnant women
- breastfeeding
- human milk
- omega-3 fatty acids
- systematic review

Resumo

Objetivo Avaliar o efeito da suplementação com fontes de ômega 3 sobre a composição de ácidos graxos do leite humano.

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Métodos A revisão consistiu na busca de artigos publicados nas bases de dados PubMed, Biblioteca Virtual de Saúde e *Web of Science* utilizando-se as palavras-chave: *ácidos graxos*, *ômega-3*, *leite materno* e *suplementação*; para isso, foi utilizado o checklist PRISMA. Foram utilizados os seguintes critérios para a seleção: artigos publicados em inglês, português, espanhol ou italiano, entre os anos de 2000 a 2015, sobre estudos realizados em humanos. A busca bibliográfica, segundo a estratégia estabelecida, resultou em 710 artigos. Entretanto, apenas 22 destes foram selecionados para compor a presente revisão.

Resultados Todos os estudos encontraram relação positiva entre o consumo de fontes de ômega 3 e sua concentração no leite humano. As diferenças nos achados se devem aos métodos empregados como, por exemplo, o momento da suplementação do ômega 3, o tipo de fonte de ômega 3 ofertado, e o tamanho amostral.

Conclusão Apesar de os estudos serem díspares em inúmeros aspectos metodológicos, observou-se a importância da suplementação do ômega 3 na gestação e/ou no puerpério.

Palavras-chave
- gestantes
- aleitamento materno
- leite humano
- ácidos graxos ômega-3
- revisão sistemática

Introduction

The importance of polyunsaturated fatty acids of the omega-3 (ω-3) series (docosahexaenoic acid [22:6 ω3, DHA] and eicosapentaenoic acid [20:5 ω3, EPA]) in the development of the fetal brain, as well as in the cognitive and visual acuity of the child, is widely recognized. These fatty acids are part of the composition of the cell membranes and the nervous system, especially DHA, which is preferentially transported by the placenta to the fetus and provides important components to the phospholipid membrane.[1,2]

The amount of fatty acids in human milk (HM) depends on maternal stocks, dietary intake and synthesis thereof in the mammary glands.[3] The concentration of DHA varies specifically, probably due to the woman's feeding habits, since its synthesis in the mammary gland is minimal.[1,2,4] During gestation and lactation, this synthesis is limited by the fetus. For this reason, numerous studies have been conducted to evaluate the effects of the supplementation of this fatty acid on the composition of HM .[4,5] Other facts to be taken into consideration are that the concentration of DHA in HM decreases as lactation progresses, and that supplementation during lactation raises DHA concentrations in breast milk.[6]

The study conducted by Bortolozo et al (2013)[2] aimed to evaluate the impact of omega-3 fatty acid supplementation between the third trimester of pregnancy and the third month after delivery, and its influence on the composition of HM. Although no statistical difference was found in the total lipid values between the studied groups, the milk of mothers supplemented with fish oil had higher concentrations of DHA and EPA, demonstrating that a higher consumption of omega-3 may influence its concentration in HM.[2] However, the results on the effects of omega-3 supplementation during gestation are still contradictory.[4]

Due to the controversies between the studies, as well as to the importance of the theme for the health of the newborn, this systematic review aims to evaluate the studies that verified the effects of omega-3 supplementation during pregnancy and/or the puerperium on the composition of HM. The bibliographical survey of this theme aims to assist the maternal and infant populations, together with health professionals, to determine the importance of supplementation, offering subsidies for its practice.

Methods

A systematic review of the available literature consisted of a retrospective search of scientific articles that aimed to evaluate the composition of HM after supplementation with omega-3 fatty acids.

The following bibliographic databases have been used: PubMed, Biblioteca Virtual da Saúde (Virtual Health Library [VHL]) and Web of Science. The search for the articles was performed independently by two researchers, and it began in August and ended in October of 2015. The selected studies were published during the period comprised between 2000 and 2015. The following keywords were used in the search strategy: *fatty acids*, *omega-3*, *human milk* and *supplementation*.

The bibliographic search was performed according to the established strategy, and resulted in 710 articles. A total of 163 articles were found in the VHL database; however, after reading the abstracts, we have selected 11 thereof; 239 articles were found in the PubMed database; however, we have only selected 21of these; and 308 articles were found in the Web of Science database, from which we have selected 2 articles. Thus, a total of 22 articles have been selected to compose the present study, reiterating that there were 12 articles replicated in the analyzed databases. The others were suppressed for the following reasons: discussion of different associations between omega-3 and HM, such as allergy, visual acuity and growth; literature reviews; studies replicated across different databases; studies published in other languages and/or that were were not available in their entirety. We used a checklist with 27 items and a 4-step flowchart, advocated by PRISMA,[7] which aims to help authors improve the reporting of systematic reviews.

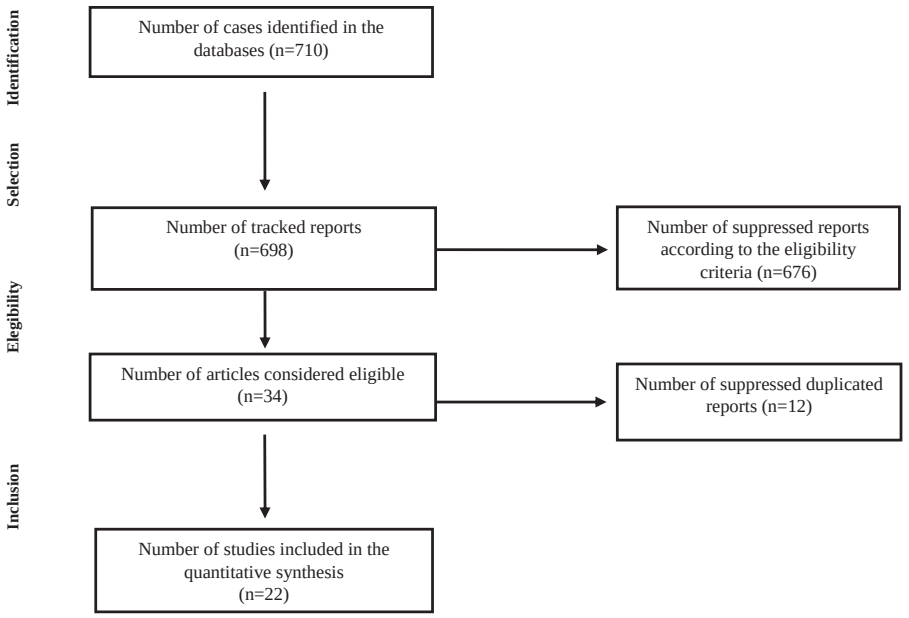

Fig. 1 Flowchart of the selection process for selected articles – PRISMA.

Therefore, a summary of each stage of the selection process of articles that composed this systematic review was arranged in the Flowchart (►**Fig. 1**).

The criteria used in the selection of articles for the review included language (Portuguese, English, Spanish and Italian) and year of publication (2000 to 2015).

The selected articles were compared in relation to the following parameters: year of publication, country of origin, sample size, average age of participants, type of design, rate of follow-up losses, period in which the woman and the milk have been evaluated, omega-3 supplementation period, type of supplement, amount offered, omega-3 evaluation methods, confounding factors controlled in the analysis, estimators used in the statistical analysis, and main results observed.

Results

Once the established strategy was put into practice, 22 articles were selected by bibliographic search to compose the present revision. Four were originated in the United States, one in Canada, one in Denmark, three in Brazil, three in Australia, three in Chile, one in Israel, one in Iceland, one in Mexico, two in Germany, and two in the Netherlands. Regarding the age groups, the majority of the articles (18) only reported the average age of the participants. Regarding language, two articles were written in Spanish, one in Portuguese and the remaining ones in English (►**Table 1**).

As for the design employed, intervention studies were used in most cases, and there was only one observational study. Information on follow-up losses were obtained from 21 studies. The losses ranged from 0 to 78%. The results among the studies, regarding the period of evaluation of the woman and the milk, were quite dissimilar. Regarding the period of supplementation, it was observed that 12 studies evaluated supplementation in infants, 7 in pregnant women, and only 3 evaluated supplementation both in pregnancy and in the puerperium (►**Table 2**).

In regards to the type of supplement or food offered to the participants, 3 studies used dairy supplements, 16 provided DHA-rich oils (such as, tuna, single-celled algae, cod, flaxseed), 2 supplied fish (sardine and jure) and 1 study performed food education to increase the consumption of fish sources. As for the method to evaluate the amount of omega 3 in HM, it was observed that all the studies used chromatography. The DHA value ranged from 170 mg to 2,200 mg per day (►**Table 3**).

Among the 22 selected articles, 9 did not evaluate the consumption of omega-3 food sources. Among those who reported it, the majority (32%) used the food frequency questionnaire (FFQ). Regarding the estimators, it was observed that nine studies used the average, seven used the correlation index, five the median and only one study used the combination of correlation index with the average. Regarding the exclusion criteria, nine articles did not mention them in the methods (►**Table 4**).

Regarding supplementation, all studies found a positive relationship between the consumption of omega-3 sources and their concentration in HM, therefore, highlighting the importance of supplementation of these fatty acids during pregnancy and/or puerperium, which translates into positive results in both cognitive development and visual acuity.

Discussion

The omega-3 and omega-6 polyunsaturated fatty acids consumed through dietary triglycerides are digested in the small intestines and can then be absorbed, transported into the bloodstream and taken up between tissues throughout the body (including brain, retina and heart).[8] Essential dietary

Table 1 Year of publication, origin, sample size, and age of the participants of the selected studies on the fatty acid profile of breast milk with the supplementation of omega-3 sources, 2000–2015

Authors	Pub year	Country	Sample (n)	Age (years)
Atalah et al[5]	2009	Chile	352 pregnant women	Intervention group 26.7 and control group 25.0 (average)
Bergmann et al[38]	2008	Germany	144 pregnant women	30.7 (average)
Boris et al[27]	2004	Denmark	44 pregnant/ puerperal women	NI
Bortolozo et al[2]	2013	Brazil	80 pregnant/ puerperal women	25.0 (average)
Dunstan et al[20]	2007	Australia	98 pregnant women	NI
Fidler et al[17]	2000	Germany	10 puerperal women	30.6 (average)
Francois et al[36]	2003	United States	9 puerperal women	28.0 to 39.0
Gaete and Atalah[1]	2003	Chile	26 puerperal women	26.9 (average)
Gaete, Atalah and Araya[31]	2002	Chile	28 puerperal women	Intervention group 25.6 and control group 26.4 (average)
van Goor et al[39]	2009	Netherlands	182 pregnant/ puerperal women	32.4 (average)
Hawkes et al[40]	2001	Australia	120 puerperal women	30.2 (average)
Imhoff-Kunsch et al[13]	2011	Mexico	1,094 pregnant women	26.0 (average)
Jensen et al[18]	2000	United States	26 puerperal women	29.2 (average)
Marc et al[32]	2011	Canada	32 puerperal women	Intervention group 27.2 and control group 26.9 (average)
Olafsdottir et al[41]	2006	Iceland	77 puerperal women	31.0 (average)
Patin et al[35]	2006	Brazil	31 puerperal women	27.9 (average)
Ribeiro et al[30]	2012	Brazil	51 pregnant women	20.0 to 30.0
Sherry et al[42]	2015	United States	89 pregnant women	29.0 (average)
Smit et al[37]	2000	Israel	26 puerperal women	23.5 (average)
Smithers et al[43]	2010	Australia	121 puerperal women	30.0 (average)
Valentine et al[44]	2013	United States	21 puerperal women (donors)	31.0 (average)
Weseler et al[45]	2008	Netherlands	52 pregnant/ puerperal women	31.7 (average)

Abbreviation: NI, Not informed.

Table 2 Type of design, losses, period of evaluation and supplementation of sources of omega-3, 2000–2015

Authors	Design	Losses (%)	Period in which the women were evaluated	Period in which the milk was evaluated	Period of supplementation of omega-3 fatty acids
Atalah et al[5]	Clinical trial	70.0	Three times during gestation and once in the second month after delivery	2nd month after childbirth	During pregnancy
Bergmann et al[38]	Randomized double blind clinical trial	45.8	21st and 37th weeks of gestation, at delivery, and 1 and 3 months after delivery	3rd month after childbirth	21st to 37th week, continuation was optional
Boris et al[27]	Randomized clinical trial	18.2	30th gestational week and the 1st month after delivery	4th, 16th and 30th days after childbirth	From the 30th gestational week until delivery (group 1) or until the 30th day after delivery
Bortolozo et al[2]	Randomized controlled clinical trial	25.0	Last trimester of pregnancy until the 3rd month of lactation	30th, 60th, 90th days after childbirth	Last trimester of pregnancy (baseline) until the 3rd month of lactation
Dunstan et al[20]	Randomized double blind clinical trial	25.0	3rd and 6th days, and 6th month after childbirth	3rd and 6th days, and 6th month after childbirth	20th week of gestation until delivery
Fidler et al[17]	Randomized clinical trial	0.0	From the 4th week until the 6th after delivery	In the 4th week after delivery (before starting supplementation), at the 6th week after delivery (after supplementation), and at 6, 12, 24, 36 and 48 hours after intake of the supplement	From 4 to 6 weeks after delivery (14 days)
Francois et al[36]	Clinical trial	22.0	10 weeks: 2 weeks of washout* at baseline (to stabilize the omega-6 and 3 intakes); 4 weeks of linseed oil supplementation and 4 weeks after supplementation	1 sample at baseline, 1 sample after washout* (2 weeks after the start of study), 4 samples at weekly intervals during 4 weeks of supplementation, and 4 samples at weekly intervals during the post-supplementation period (4 weeks).	4 weeks
Gaete and Atalah[1]	Cohort	7.69	From entry into the study until the 2nd week after food education	2 weeks after food education	Food education on the day of study entry
Gaete et al[31]	Randomized clinical trial	17.2	From the study entry to the 15th day after intervention	15 days after intervention	15 days
van Goor et al[39]	Randomized double blind clinical trial	51.6	Registration day up to 12 weeks after delivery	2nd to 12th weeks after delivery	17th week of gestation until the 12th week after childbirth
Hawkes et al[40]	Randomized double blind clinical trial	31.7	3rd day after birth until the end of the 12th week after delivery	In the 4th week after delivery	12 weeks

Table 2 (*Continued*)

Authors	Design	Losses (%)	Period in which the women were evaluated	Period in which the milk was evaluated	Period of supplementation of omega-3 fatty acids
Imhoff-Kunsch et al[13]	Randomized double blind clinical trial	11.0	Gestation (18th to 22nd weeks) until 1 month after delivery	1 month after birth	From the 18th to the 22nd gestational week until delivery
Jensen et al[18]	Clinical trial	7.7	2nd to 8th weeks after delivery	At the 2nd, 5th and 8th weeks after delivery	6 weeks
Marc et al[32]	Clinical trial	25.0	1 postnatal week (between 3 and 7 days) before starting DHA supplementation and at follow-up at 15 days (3 weeks) and 49 days (7 weeks)	First postnatal week (between 3rd and 7th days) before starting supplementation and at follow-up on days 15 and 49	1 week after delivery until term (36 weeks) - > 8–12 weeks of supplementation
Olafsdottir et al[41]	Randomized clinical trial	48.0	2nd and 4th months after delivery	2nd and 4th months after delivery	Registration day up to 4 months after delivery
Patin et al[35]	Clinical trial	NI	0, 15 and 30 days after delivery	0, 15 and 30 days after delivery	1st and 15th days after delivery
Ribeiro et al[30]	Randomized clinical trial	78.4	30th gestational week up to 15 days after delivery	15th day after delivery	15 days (from the 30th gestational week)
Sherry et al[42]	Clinical trial	7.9	From enrollment (4th to 6th weeks after delivery) up to 6 weeks after supplementation	Baseline and 6th week aftersupplementation	6 weeks
Smit et al[37]	Clinical trial	11.0	For one week	Baseline shortly after ingestion of the supplement. On day 1 and day 7 after the intake of the supplement	1 week
Smithers et al[43]	Randomized double blind clinical trial	19.0	During all hospitalization of the preterm newborn	At intervals of 2 weeks during the hospitalization of the newborn	From study entry (delivery < 33 weeks) until the expected date of delivery
Valentine et al[44]	Randomized clinical trial	38.0	3 days before supplementation until 12 weeks post supplementation	0, 7th, 14th, 21st, 28th and 84th days after the supplementation	During all the time they donated milk to the milk bank (from 7–90 days)
Weseler et al[45]	Randomized double blind clinical trial	34.6	Pregnancy (36 weeks) up to the 11th week after delivery	3rd, 5th and 11th weeks after delivery	Gestation (36th week) up to 11th week postpartum

Abbreviation: NI, not informed.
Note: *Washout: time necessary for the concentration of a medicinal product to be negligible after cessation of therapy.

Table 3 Characteristics of the selected studies on the profile of fatty acids in breast milk with omega-3 sources supplementation, 2000–2015

Authors	Type of supplement/food used as source of omega-3	Amount offered	Placebo/control	Method used to evaluate omega-3 intake	Method to evaluate the amount of omega-3 present in HM
Atalah et al[5]	Milk drink made from powdered milk and hydrolyzed cereals enriched with microencapsulated vitamins, minerals and omega-3 fatty acids	60 mg DHA + 14 mg EPA in 200 mL (2 kg/month)	Powdered milk	Food survey	Chromatography
Bergmann et al[38]	Supplement based on acidified and flavored milk	Group 1: Basic supplement plus 4.5 g FOS Group 2: Basic supplement with FOS + 200 mg of DHA	Basic supplement enriched with vitamins and minerals	NI	Chromatography
Boris et al[27]	Fish oil	900 mg DHA + 1300 mg EPA/day	Olive oil	NI	Chromatography
Bortolozo et al[2]	Fish oil	315 mg DHA + 80 mg EPA/day	Maize starch	24h reminder on alternate days of the week, including a weekend day	Chromatography
Dunstan et al[20]	Fish oil	2200 mg DHA + 1100 mg EPA / day	Olive oil	NI	Chromatography
Fidler et al[17]	Oil rich in DHA (DHASCO)[1]	200 mg DHA / day	Mixture of soybean and corn oils	7-day food record	Chromatography
Francois et al[36]	Linseed oil	10.7 g ALA	NI	Food survey	Chromatography
Gaete and Atalah[1]	Food education	NI	NI	Food survey	Chromatography
Gaete et al[31]	Canned fish(horse mackerel)	160 g fish 2 times a week	Regular food	Food survey	Chromatography
van Goor et al[39]	DHA + ARA capsules, DHA capsules	Group 1 = 220 mg DHA + 36 mg EPA + 220 mg ARA + 7 mg ALA + 46 LA Group 2 = 220 mg DHA, 34 mg EPA + 15 mg ARA + 32 mg ALA + 274 LA	Soybean oil	NI	Chromatography
Hawkes et al[40]	Tuna oil	Group 1 = 300 mg DHA + 70 mg EPA/day Group 2 = 600 mg DHA + 140 mg EPA/day	Sunflower seed oil	NI	Chromatography
Imhoff-Kunsch et al[13]	Seaweed oil	400 mg DHA	Olive oil	Food survey	Chromatography

Table 3 (*Continued*)

Authors	Type of supplement/food used as source of omega-3	Amount offered	Placebo/control	Method used to evaluate omega-3 intake	Method to evaluate the amount of omega-3 present in HM
Jensen et al[18]	Group 1: supplement made from algae with a high content of DHA Group 2: eggs with a high content of DHA Group 3: fish oil	Group 1: 230 mg DHA/day Group 2: 170 mg DHA/day Group 3: 260 mg DHA/day	Eggs	NI	Chromatography
Marc et al[32]	Oil rich in DHA (DHASCO)*	1200 mg DHA/day	No intervention	Food survey	Chromatography
Olafsdottir et al[41]	Cod liver oil	1107 mg DHA + 783 mg EPA/day	No intervention	24h reminder + additional questions about fish consumption	Chromatography
Patin et al[35]	Fried sardines	4 kg of sardines (2 kg on day 0 and 2 kg on the 15th day)	The study had no control group	24h reminder	Chromatography
Authors	Type of supplement/food used as source ofomega-3	Value offered	Placebo/control	Method used to evaluate omega-3 intake	Method to evaluate the amount of omega-3 present in HM
Ribeiro et al[30]	Fish oil	0.72 g ω3/day	Primroseoil	24h reminder	Chromatography
Sherry et al[42]	Oil rich in DHA	Group 1: 200 mg DHA/day Group 2: 400 mg DHA/day	NI	Food survey and 3-day food records	Chromatography
Smit et al[37]	Oil rich in ARA and DHA	Group 1: 300 mg ARA/day Group 2: 300 mg ARA + 110 mg EPA and 400 mg DHA/day	No intervention	NI	Chromatography
Smithers et al[43]	Tuna oil	900 mg DHA +195 mg EPA + 54 mg ARA / day	Soybean oil	NI	Chromatography
Valentine et al[44]	Seaweed oil	1000 mg DHA	Soybean oil	3-day foodrecords	Chromatography
Weseler et al[45]	Milk drink made from powdered milk enriched with LCPUFAs	Group 1: 200 mg ARA/day Group 2: 400 mg ARA/day Group 3: 320 mg DHA + 80 mg EPA	Powdered milk	NI	Chromatography

Abbreviation: ALA, α − linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FOS, fructooligosaccharide; HM, human milk; LA, linoleic acid; LCPUFAs, long chain polyunsaturated fatty acids; NI, Not informed.
*DHASCO is an oil derived from a single-celled alga, mainly containing DHA, myristic, palmitic and oleic acids.

fatty acids in the form of linoleic acids (LAs) and α − linolenic acids (ALAs) are activated in the forms known as ketoacyl-CoA, and then used for the conversion of long chain polyunsaturated fatty acids and other polyunsaturated products, such as those derived from the series of desaturation and elongation reactions that are particularly active in the liver and, to a lesser extent, in other tissues.[8]

Linoleic and ALA fatty acids need to be ingested through food, since the human body does not have enzymes to synthesize them. Some vegetables synthesize them and are, therefore, an abundant source of these fatty acids, as well as the products derived from these vegetables. Omega-3 fatty acids (DHA and EPA) can be synthesized by the human body to a certain level, albeit a very limited one. The consumption of omega-3 sources

through diet can be done by ingesting fish or fish oils, and foods enriched or fortified with these important fatty acids.[9]

Although ALA in humans is converted to EPA and DHA, the exact percentage of this conversion is unknown, but it is estimated to be low (5% EPA and 0.5% DHA).[10,11] Due to their enzymatic immaturity, children and especially neonates cannot convert all the DHA required for their development from ALA.[12] Therefore, feeding in the gestational period is of great importance as it determines the type of fatty acid that will accumulate in the fetal tissue. The essential fatty acids are transferred through the placenta, and in the third gestational trimester are deposited in the brain and retina of the fetus. It should be noted that the fetus withdraws a total of 50 to 75mg of polyunsaturated fatty acids from the mother, most of them being DHA.[13–16]

Numerous studies have been conducted to evaluate the effects of the supplementation of omega-3 and its metabolites in pregnancy and puerperium on the composition of HM. This is due to the synthesis of DHA probably occurring minimally in the mammary gland[6,17,18] as well as due to the role that this polyunsaturated fatty acid plays on visual acuity, cognition and in the formation of the nervous tissue of the newborn.[19]

Although supplementation appears to be the most reliable medium for increasing omega-3 levels in HM, there are numerous differences among the studies evaluated in relation to the following parameters: sample size, study design, timing of omega-3 supplementation (gestation and/or lactation), type of supplementation (fish oil, in natura fish consumption), and amount and type of omega-3 offered (EPA and/or DHA).

Regarding the diversity of the countries where the studies selected for this systematic review were performed, it is worth noting that the consumption of omega-3 rich foods in Western countries is well below that of other countries.[20] In the United States, the intake of omega-3 and its metabolites (DHA and EPA) was estimated at 1.6 and 0.1–0.2 g/day respectively, and the dietary ratio between omega-6 and omega-3 was $\approx$ 9.8:1.[21] A study with Canadian pregnant women showed that the average daily intake of omega-3 and DHA was 1.45 and 0.082 g/day respectively.[22] Populations living in coastal countries, such as Japan and Norway, where fish are widely consumed, have a higher dietary intake of omega-3 ($>$1 g/day), and consequently, high concentrations of DHA in their breast milk.[20,23,24] Although there is no official dietary recommendation for EPA and DHA in the US, several expert groups suggest a DHA intake of at least 200 mg/day, which may reach 1,000 mg DHA/day for pregnant and lactating women, and 1.4–2.7 g of omega-3, and suggest the omega-6/omega-3 ratio of $\approx$ 2–5:1.[21,25]

Corroborating the above recommendations, the consensus published by Koletzko et al[26] states that an average intake of at least 200 mg of DHA per day is advisable; it also states that consumption of up to 1 g of DHA or 2 to 7 g of omega-3 per day is safe. This amount can be achieved by consuming one to two servings of fish per week, including fatty fish such as herring, mackerel and salmon. However, it is known that the consumption of fish can contribute significantly to the exposure to contaminants such as methylmercury, which is particularly toxic to the developing brain and possibly harmful to infant growth. To decrease the amounts of methylmercury in the body, one should reduce the intake of contaminated foods during the pregestational and gestational periods. The fish with the highest levels of methylmercury are predatory fish such as marlin, pike, swordfish and shark. However, after an extensive literature review, the consensus points out that the beneficial effects of regular consumption of fish sources of DHA during pregnancy appear to overcome the potential drawbacks of the increased intake of contaminants.

Regarding the period of supplementation, the selected studies presented different time periods (pregnant and/or nursing) when omega-3 supplementation was performed and measured, which may partially justify the differences in the results we found. On this issue, in their randomized clinical trial, Boris et al[27] evaluated two hypotheses, namely: 1) whether omega-3 supplementation during pregnancy increased omega-3 levels at the beginning of breastfeeding; and 2) whether the continuation of supplementation after delivery was necessary to sustain the long-term increase in omega-3 levels. There was a marked drop in omega-3 levels in the group that stopped supplementation during the puerperium. Such a decrease in the concentration of DHA in breast milk as lactation progresses is corroborated by numerous studies.[4,28,29] On the other hand, the group that received fish oil during gestation and lactation showed levels of omega-3 three times higher, and double the levels of DHA.[27] It is worth mentioning that polyunsaturated fatty acids are deposited in the brain during the last gestational trimester, and that this process continues after delivery. Furthermore, the neurological development continues during the first years of life.[27] The results found by Ribeiro et al[30] also demonstrated that supplementation with fish oil limited to pregnancy was not as effective as supplementation during pregnancy and lactation. Therefore, supplementation during pregnancy and lactation is recommended by numerous studies.[20,30,31]

Important issues to take into account in these studies are the type of omega-3 source and the quantity that was supplied. It was observed that most of the selected studies used fish oil to increase the consumption of omega-3; however, some studies have used the supply of fresh food, fortified drinks and food education techniques. The use of fish oil has benefits, but it can lead to low compliance due to its adverse effects, such as fish flavor eructation, digestive discomfort and night sweats.[5,32] The randomized double blind clinical trial conducted by Dunstan et al[20] aimed to evaluate the effects of fish oil supplementation during pregnancy on the composition of HM and on the development of the infant in the first year of life. The concentration of fatty acids in the milk was analyzed on the third day, sixth week and sixth month after delivery. It was observed that women who received fish oil had a higher concentration of EPA and DHA in the milk on the third day and the sixth week after delivery.

Regarding the consumption of fish, the study by Henderson et al[33] demonstrated that ingesting 100–120 g of sardines 2 to 3 times a week resulted in increased levels of fatty acids without the need for fish oil. Harris et al[34] disagreed

Table 4 Controlled confounding factors, eligibility and exclusion criteria and main results found between supplementation of omega-3 sources on the fatty acid composition of human milk, 2000–2015

Authors	Estimator	Confounding factors controlled in the analysis	ligibility criteria	Exclusion criteria	Results
Atalah et al[5]	Median	Age, schooling, parity, initial weight, height, GA at baseline	GA $<$ 14 weeks, age $\geq$18 years, primiparous, absence of chronic pathologies	NI	50% increase in omega-3 concentration in total fatty acids in HM, a non-statistically significant value ($p = 0.06$), probably due to the small sample size and insufficient adhesion level
Bergmann et al[38]	Average	Age, pregestational BMI, gestational weight gain, gestational age, parity type, parity, marital status, nationality, work, education, female gender, Apgar $\leq$ 7 in 10 minutes, umbilical cord pH $\leq$ 7.2, weight, length and head circumference	Pregnant, caucasian, healthy women, aged $>$ 18 years and intending to breastfeed for at least 3 months	Severe illness, age $<$ 18 years, non-Caucasian, increased risk of preterm or multiple pregnancy, allergy to cow milk protein, lactose intolerance, diabetes, smoking, alcohol consumption, participation in another study, consumption of other supplements, prematurity malformations, hospitalization $>$ 1 week	The percentage of DHA in the breast milk was twice as high in the DHA-FOS group (0.50%) ($p < 0.001$), and the ratio of ARA to DHA in the DHA-FOS group compared with the other two groups was significantly reduced from 2.1 $\pm$ 0.76 to 1.0 $\pm$ 0.43 ($p < 0.001$). The Authors concluded that 200mg/day of DHA from mid-pregnancy to lactation appears to be adequate to improve the state of DHA in mothers and infants
Boris et al[27]	Average	NI	NI	NI	Comparing the two intervention groups, it was observed that women who received fish oil during gestation and lactation had increased levels of omega-3 compared with those who received until gestation only
Bortolozo et al[2]	Average	Age, schooling and income	Healthy, pregnant women aged 18–38 years, in the last trimester of pregnancy, non-smokers, no high-risk pregnancies, and adequate dietary patterns	NI	The milk of the mothers of the intervention group presented high levels of DHA and EPA at the 30th and 60th days, demonstrating that higher consumption of omega-3 could influence their concentration in HM, and there was no change between omega-3 and omega-6
Dunstan et al[20]	Correlation coefficient (R^2)	Parity, pre-gestational BMI, age and maternal allergy (allergic rhinitis or asthma)	Pregnant women between the 16th to the 20th gestational weeks and who had delivered after the 36th gestational week, with presence of allergic rhinitis, asthma or positive test in the Prick test	Pregnant smokers, with health problems and with fish consumption above two meals per week	In the intervention group, colostrum presented a high proportion of DHA and EPA when compared with the control group ($p < 0.001$). During the three moments, the drop was higher in the intervention group when compared with the control group ($p < 0.001$). However, the amount of DHA and EPA remained higher in the intervention group at 6 weeks postpartum when compared with the control group ($p< 0.001$). At 6 months, no differences were found between groups
Fidler et al[17]	Correlation coefficient (R^2)	Maternal age, height, weight (day 0), weight (day 14), BMI (day 14), milk secretion (mL/day), TL in HM (g/100 mL)	Healthy lactating mother, with omnivorous diet, with single, full-term, healthy newborns	NI	At baseline, there was no difference in fatty acid composition between the intervention and the placebo group. After two weeks of supplementation with 200mg of DHA/day, the milk from the intervention group contained a significantly higher percentage of DHA relative to milk from the placebo group ($p = 0.003$), a content almost 1.8 times higher of DHA. There was no significant difference in the content of any other fatty acids at any time point after supplementation.

(*Continues*)

Table 4 (*Continued*)

Authors	Estimator	Confounding factors controlled in the analysis	ligibility criteria	Exclusion criteria	Results
Francois et al[36]	Average	NI	Healthy women aged 28–39 years	NI	The omega-3 content in HM increased significantly over time, from 1.0% of total lipids (TL) at baseline to 6.8% of TL after one week of linseed oil supplementation. The omega-3 content remained high at 2 and 4 weeks. After 4 weeks of supplementation, the omega-3 concentration reached a peak of 7.7% of TL, and then returned to baseline (≈1.9% of TL) at 1 week after supplementation
Gaete and Atalah[1]	Median	Weight, height and BMI	GA > 37 weeks and exclusive breastfeeding	Women with diabetes, altered lipid metabolism and alcohol and drug dependence	After food education, the consumption of fish increased three times in relation to the initial consumption. The increased intake of DHA did not significantly modify the DHA content of the milk. However, in mothers with an intake of DHA > 200 mg/day there was a positive correlation between intake and milk content ($r = 0.71$, $p < 0.05$)
Gaete et al[31]	Median	Weight, height, BMI, parity, age of the newborn	GA > 37 weeks and exclusive breastfeeding	Women with diabetes, altered lipid metabolism and alcohol and drug dependence	After supplementation, an increase in the amount of EPA and DHA in HM was observed. DHA consumption in the intervention group increased significantly from 64 mg to 335.9 mg daily
van Goor et al[39]	Median	Maternal age, pregestational BMI, weight, gestational weight gain, GA at birth, birth weight and parity	Women with low risk, first or second pregnancy, and with single gestation	Vegetarian/vegan women and/or with diabetes mellitus	Compared with placebo, supplementation of ARA + DHA or DHA alone significantly increased the concentration of DHA in milk in both the second (59% and 43%, respectively) and in the 12th (56% and 52%, respectively) week after delivery
Hawkes et al[40]	Average	Maternal age, fish consumption, smoking, alcoholic beverages and side effects	Healthy women, age ≥18 years, single full-term infants, and breast-feeding for ≥ 12 weeks	Inflammatory diseases, use of anti-inflammatory drugs or fish oil supplements	The concentration of DHA in HM increased linearly in response to the diet with DHA. The authors concluded that the consumption of ≤ 600 mg/day of DHA and 140 mg/day of EPA for 4 weeks increased the concentrations of omega-3 and its metabolites in relevant tissues but did not cause changes in the concentrations of cytokines in human milk
Imhoff-Kunsch et al[13]	Correlation coefficient (R^2)	Age, GA, parity, BMI, schooling, GA at birth, prematurity and birth weight of the newborn	Women of gestational age between 18–22 weeks, who planned to breast-feed for at least 3 months, aged 18–35 years	High risk pregnancy, lipid absorption or metabolism disorder, regular intake of fish oil or supplements rich in DHA, and frequent use of certain medications	The concentration of DHA in breast milk in the intervention group was higher than for the placebo group ($p < 0.01$)
Jensen et al[18]	Correlation coefficient (R^2)	Age, parity, weight, height, GA, birth weight of the newborn	Pregnant women with the intention to exclusively breastfeed	Maternal age at birth < 19 or > 35 years, diabetes, egg allergy, gestational age < 37 weeks, and birth weight < 2,500 g or > 4,200 g	DHA supplementation increased plasma DHA concentrations in lactating women and in breast milk, resulting in a higher plasma concentration of DHA in children. A positive correlation was found between the DHA, EPA and ARA contents in maternal plasma and breast milk ($r^2 = 66.2\%$, $p < 0.001$)
Marc et al[32]	Average	Age, BMI, parity, schooling, work, marital status, use of vitamins, race and income	Childbirth with GA ≤29 weeks and intending to breastfeed	Age < 18 years or > 40 years, > 3 fish servings/week, use of omega-3 supplements, fish allergy, coagulation disorder, drug or alcohol use	The dietary DHA supplement provided during lactation increased the concentration of DHA in breast milk in mothers of preterm infants (GA ≤29 weeks) and in the plasma of these infants

Table 4 (*Continued*)

Authors	Estimator	Confounding factors controlled in the analysis	ligibility criteria	Exclusion criteria	Results
Olafsdottir et al[41]	Correlation coefficient (R^2)	Food intake, smoking, alcohol, drug use and schooling	Irish or having lived in the country for at least 15 years, single birth and breastfeeding	NI	EPA and DHA were significantly different between the groups, being 1.3–2.3 times higher in the milk of the intervention group when compared with the control group, without any negative effect on another fatty acids
Patin et al[35]	Correlation coefficient (R^2)	Age, BMI, Parity, gestational weight gain, weight and length of the newborn at birth	Exclusive breastfeeding, no smoking, no allergy/intolerance to sardines, birth weight $\geq$2500 g, GA between 37 and 42 weeks	NI	Consumption of 300 g of sardines per week increased DHA levels in HM
Ribeiro et al[30]	Averageand correlation coefficient (R^2)	NI	Age between 20–30 years, 30th week of gestation, no use of medication, no intolerance/allergy to fish, no use of dietary supplements with omega-3 and omega-6, and with intention to breastfeed exclusively	NI	The data confirmed that the omega-3 content in HM, DHA in particular, is influenced by the consumption of omega-3 by the pregnant woman. A positive correlation was found between omega-3 content in the phospholipids of erythrocytes of pregnant women and the content of these fatty acids in breast milk
Sherry et al[42]	Average	Age, BMI and skin color	Age $\geq$18 years, with full term infants, 4–6 weeks postpartum and who planned to breastfeed for $\geq$ 6 weeks	NI	Lactating women consumed $\sim$ 25% of the recommended amount of DHA/day. The data found demonstrated that supplementation significantly increased DHA in HM, as well as decreased the ratio of omega-6/omega-3
Smit et al[37]	Average	Maternal age, number of children and duration of lactation	Infants between the third and tenth months of breastfeeding	NI	The administration of 300 mg ARA + 110 mg EPA + 400 mg DHA increased the LCPUFAs content in HM, with no significant result
Smithers et al[43]	Average	Age, smoking, schooling, human milk production, breastfeeding at the end of the intervention, single gestation, gestational age at birth, gender and birth weight of the newborn	GA < 33 weeks	Coagulation disorders, congenital or chromosomal anomalies, and multiple births in which not all live births were eligible	Mothers in the intervention group had 3-fold higher levels of DHA in HM compared with women in the placebo group but also had slightly lower linoleic acid content
Valentine et al[44]	Median	Age and stage of lactation	Donor to the human milk bank	Women who did not have enough milk to donate	The DHA content of the milk increased in the group supplemented with DHA capsules ($p < 0.05$)
Weseler et al[45]	Correlation coefficient (R^2)	Age, pregestational BMI, blood pressure, number of gestations, total fatty acids in milk and erythrocyte	GA (34–35 weeks), intention to breastfeed, pregestational BMI (18–27 kg/m^2), consumption of fish < 2x per week, without use of omega-3 supplements, alcohol, cigarette, drugs or supplements	GA < 37 or > 43 weeks, allergy/intolerance to supplements and vegetarian components	It was observed that the concentrations of DHA in HM increased significantly after 2 weeks of supplement intake (320 mg DHA + 80 mg EPA)

Abbreviations: BMI, body mass index; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GA, gestational age; HM, human milk; LCPUFAs, long chain polyunsaturated fatty acids; NI, not informed; TL, total lipids.

with this, and have observed that in order to increase 0.5 to 1 g of DHA in breast milk, it is necessary to consume 350–750 g of 1% fat or 75–150 g of 10% fish fat. In the study by Patin et al,[35] it was observed that the levels of DHA in HM increased with the ingestion of 300 g of sardines per week, with 5% fat, without the need to use fish oil supplementation. This study recommended the consumption of fish two to three times per week during gestation.

Gaete and Atalah[1] conducted a prospective study with 26 pregnant women, which consisted of an educational feeding strategy to recommend individual consumption of different preparations based on marine foods. A guide with information on the importance of maternal lactation to the newborn, and on the importance of fish consumption by the mother to increase DHA levels was also distributed. The strategy of food education is considered an important intervention to raise awareness about the need for fish consumption during the gestational and puerperal period.

The study by Atalah et al[5] aimed to evaluate the effects of the introduction of omega-3 fortified milk beverages (DHA and EPA) during gestation on the composition of HM and red blood cells. One-hundred and seventy-five women from the intervention group and 177 from the control group were evaluated in the clinical trial. The pregnant women were evaluated at three moments of the pregnancy and once after delivery to evaluate the consumption near the date of the interview. The evaluation of milk composition was performed in only 16 women, and a 50% increase in omega-3 in breast milk was observed. However, there was no statistical difference between the evaluated groups in relation to the amount of EPA and DHA, probably due to the small sample size.

Regarding the type of omega-3 offered, seven studies offered DHA and EPA, seven offered DHA only, and three offered DHA, arachidonic acid (ARA) and EPA. It was observed that the amount of DHA was always higher than that of EPA, probably because of its important role on the nervous system, cognition and vision. It is worth noting that there is no consensus regarding the optimal levels of DHA consumption at different stages of life. However, most technical groups recommend around 200 to 500 mg/day in the adult population, and, during gestation, it is recommended to consume fish between two to three times per week.[5]

There are numerous factors that contribute to the variability of EPA and DHA content in breast milk, such as lactation stage, gestational age, and maternal nutritional status. What is verified is that certain selected studies[20,27,30,36] did not control the analyses for important confounding factors, such as food consumption. Therefore, estimates of association may be compromised by the fact that certain studies did not quantify follow-up losses, but also because they did not control important confounding factors.

All selected articles showed the importance of supplementation of omega-3 in different forms (capsules, dairy drinks, strategy for feeding education, consumption of fish) on the nutritional composition of HM in the gestational and/or puerperal periods. However, four studies[1,5,27,37] did not reach statistical significance. This can be partially explained by the sample size, which can reduce the strength of the study to elucidate possible associations, possible adhesion reduction in relation to the intake of supplements and the food education practices employed, as well as the follow-up losses, which may cause a decrease in the validity of the results.

Conclusion

Although the studies were disparate in several methodological aspects, the importance of omega-3 supplementation in pregnancy and/or the puerperium, especially DHA, as well as the safety of its supplementation were observed with the data from the studies that composed this systematic review. However, it is of great importance that further studies be conducted to establish the adequate amount of omega-3s and their metabolites during gestation and lactation that will bring benefit to newborns.

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Limb Body Wall Complex Associated with Placenta Accreta: A Mere Coincidence or a Sign of an Etiopathogenic Link?

Limb body wall complex associada à placenta acreta: uma mera coincidência ou um indício de um elo etiopatogênico?

Marcos Masaru Okido[1] Aderson Tadeu Berezowski[1] Sandra Regina Marques Carvalho[1] Geraldo Duarte[1] Ricardo de Carvalho Cavalli[1] Alessandra Cristina Marcolin[1]

[1] Department of Gynecology and Obstetrics, Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo – USP, Ribeirão Preto, SP, Brazil

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Address for correspondence Marcos Masaru Okido, MD, PhD, Department of Gynecology and Obstetrics, Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Avenida dos Bandeirantes, 3.900–Monte Alegre, 14049-900–Ribeirão Preto (SP), Brazil (e-mail: mokido@usp.br).

Abstract

A case was reported of a fetus with the anomaly of limb body wall complex associated with placenta accreta. To date, only one account of this condition has been published in the world literature. Due to the low frequency of both complications, the hypothesis has been raised that this association may have happened not by mere coincidence, but rather by a possible common etiopathogenic mechanism. For the first time, a study proposes the existence of a possible etiopathogenic connection between the anomaly of limb body wall complex and hypoxic disorders caused by inadequate placentation in previous uterine scarring.

Keywords

- ultrasonography
- prenatal diagnosis
- limb body wall complex
- placenta accreta
- etiology

Resumo

Foi relatado um caso de feto com anomalia de limb body wall complex associada a uma placenta acreta. Até o presente, apenas uma descrição com essa condição foi publicada na literatura mundial. Devido à baixa frequência das duas complicações, foi levantada a hipótese de que essa associação possa ter ocorrido não por uma mera coincidência, mas por um possível mecanismo etiopatogênico comum. Pela primeira vez, um estudo propõe a existência de uma possível possível ligação etiopatogênica entre a anomalia de limb body wall complex e os transtornos hipóxicos causados pela placentação inadequada em cicatriz uterina prévia.

Palavras-chave

- ultrassonografia
- diagnóstico pré-natal
- limb body wall complex
- placenta acreta
- etiologia

Introduction

Limb body wall complex (LBWC) is a lethal congenital anomaly, which is characterized by a spectrum of multiple defects. The most common issues include an extensive thoracoabdominal wall defect associated with deformity of limbs, kyphoscoliosis, and short or absent umbilical cord. Craniofacial lesions, single umbilical artery, intestinal atresia, and spina bifida have also been observed.[1,2]

Prevalence is variable among studies because of the different criteria used for diagnosis and the high rates of fetal loss during pregnancy.[3,4] In the first trimester, the diagnosis is

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confirmed in 1:7,500 pregnancies;[5] however, at birth, only 0.12 cases in 10,000[6] are identified.

The origin of the LBWC anomaly is not known. The endogenous theory ascribes the defects to an intrinsic disorder of the ectodermal placodes.[7] Placodes are differentiated areas of the ectoderm whose cells migrate to the neighboring mesoderm during the differentiation process of the embryonic tissues. Failure in this mechanism would lead to dysfunction of the mesoderm, which plays a fundamental role in the lateral folding process of the embryonic disk during the gastrulation period. According to the early amniotic rupture theory, we assumed that this phenomenon would cause the entrapment of the embryo to the mesodermal bands of the coelomic cavity. This would prevent the formation of the umbilical pedicle, and the embryo would be subjected to physical forces responsible for constricting malformations and mutilations.[8] Finally, according to the vascular disruption theory, fetal defects would be a result of hypoxic-ischemic attacks to susceptible tissues during embryogenesis because of disturbances in the blood supply to the embryo.[9–12]

Placenta accreta is the abnormal invasion of chorionic villi in the basal layer of the decidua and in the underlying myometrium, and it may reach the uterine serosa and contiguous organs.[13] It is clinically manifested by settings of massive hemorrhage during the attempt to remove the placenta. Its prevalence is of 1:533 pregnancies,[14] and its main risk factor is a history of cesarean section.[15]

The pathogenesis of placenta accreta is not completely understood either, but it is believed that there is a strong association with the remaining vascularization and decidualization defects in the uterine scarring areas. In subsequent pregnancies, there could be failures in the interaction between maternal tissues and trophoblastic cells. This would cause an exaggerated remodeling of the arcuate and radial arteries, an inadequate expression of some cell receptors, and the excessive release of trophoblast-modulating molecules, such as the vascular endothelial growth factor, due to the low oxygen tension.[15–17] Therefore, hypoxia would stimulate trophoblast proliferation, whereas normoxia would have an inhibitory effect. With the myometrium devoid of decidua, the trophoblastic advance is even more aggressive, because the inhibitory properties fostered by the metalloproteinases of the deciduous extracellular matrix are lost.

In this report, an anomaly case was presented associating the LBWC with placenta accreta. To date, only one account of this association has been published in the world literature.[18] Judging from the low frequency of both complications, the hypothesis has been raised that this association may have occurred due to a possible etiopathogenic mechanism, and not by mere coincidence. For the first time, a study proposes the existence of a possible etiopathogenic connection between placenta accreta and the LBWC anomaly.

Case

A pregnant woman, 27 years old, with a history of four cesarean sections, was followed-up at the high risk prenatal clinic of the Clinics Hospital of Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, at 31 weeks and 2 days, having been referred due to a fetus with multiple malformations. The 32-week ultrasonography showed a fetus weighing 1,300 g and an extensive defect of the anterior abdominal wall, severe kyphoscoliosis, and lower limbs offset from the fetal trunk axis. Liver and intestinal loops were observed outside the abdominal cavity in close contact with the placenta (►**Figs. 1A** and **1B**) and short umbilical cord (►**Fig. 2**). A fetal echocardiography showed the heart displaced downward, toward the abdomen, double output from the right ventricle, and intraventricular muscle communication.

The placental evaluation revealed central (total) placenta previa, with predominant insertion in the anterior segment of the uterus. At this location, the placenta was thick, full of vascular voids, and no myometrial layer was observed

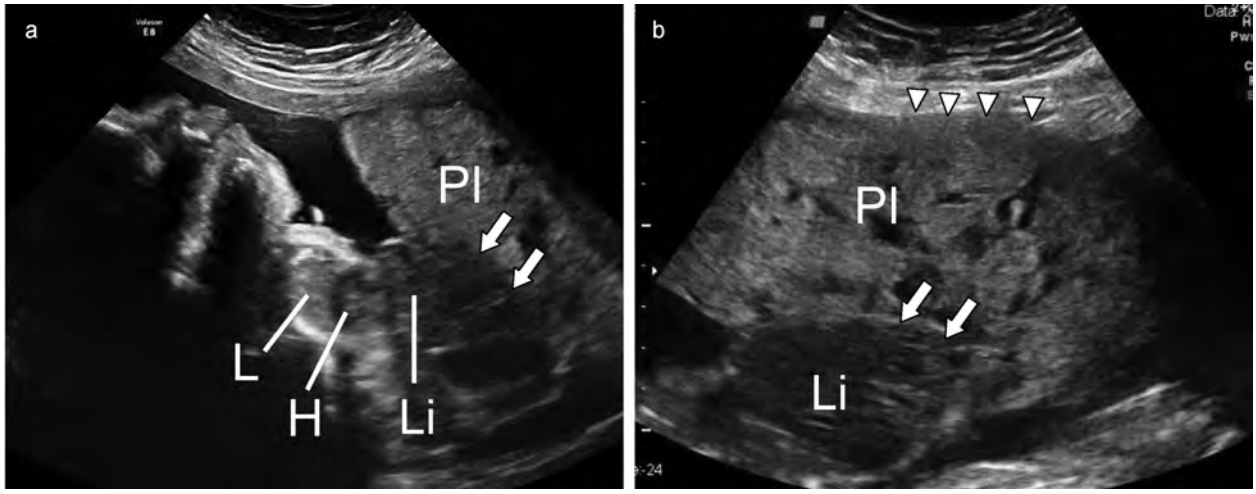

Fig. 1 Ultrasound image at 32 weeks. (a) Fetus in longitudinal view showing extensive abdominal wall defect, heart moved to the caudal position, and liver in contact with the placenta (arrows). (b) Placenta in the segmental region showing several vascular voids and loss of the myometrial layer (arrowheads). One can also see the fetal liver near the placenta (arrows). Abbreviations: PL, placenta; L, Lung; H, heart; Li, liver.

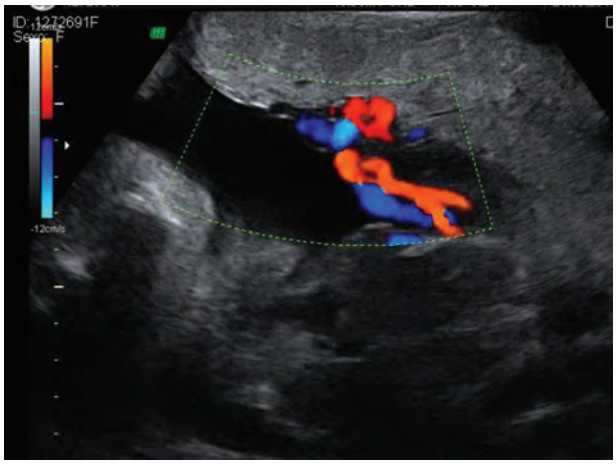

Fig. 2 Ultrasound image at 32 weeks. Observe short umbilical cord.

(►**Fig. 1B**). The conclusion of this exam was: 32-week topical gestation, limb body wall complex anomaly, total central placenta previa, and placenta accreta. Magnetic resonance imaging (MRI) confirmed the ultrasonography findings (►**Fig. 3**).

The pregnancy was interrupted at 33 weeks and 6 days. A cesarean delivery was performed followed by a hysterectomy with placenta in situ. Previous to the hysterectomy, an embolization of the internal iliac arteries was performed. The newborn weighed 1,900 g, with undefined sex. It died within a few minutes of life. A large thoracoabdominal defect on the right side and amelia of the ipsilateral upper limb was observed (►**Fig. 4**). The eventration of the abdominal organs with the placenta confirmed the non-obliteration of

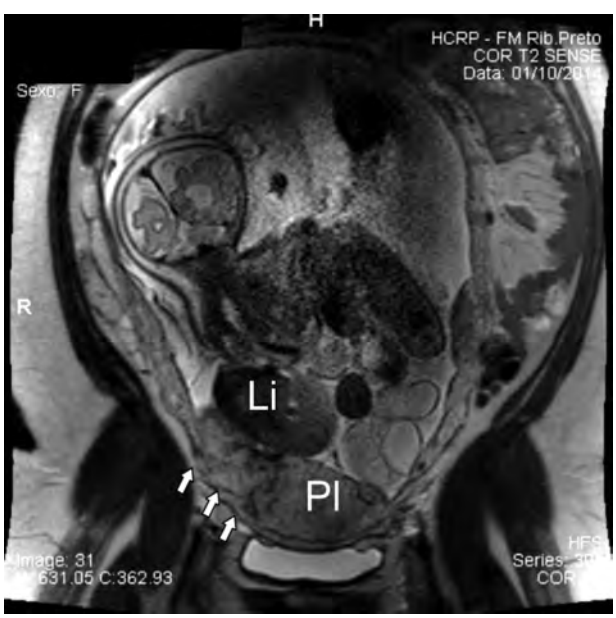

Fig. 3 Fetal MRI image showing severe kyphoscoliosis, liver and intestinal loops out of the abdominal cavity and placenta previa with irregular boundaries with the underlying myometrium (arrows). Abbreviations: PL, placenta; Li, liver.

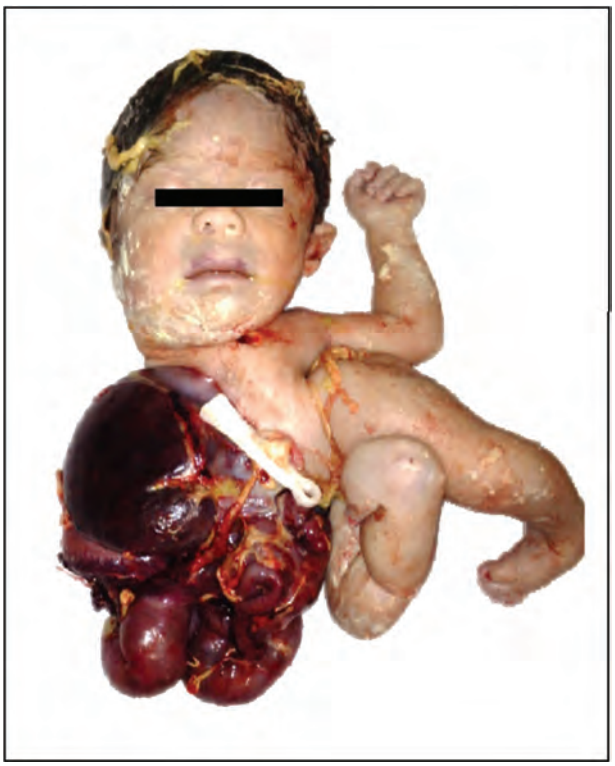

Fig. 4 Newborn photo showing extensive thoracoabdominal defect, serious spine and lower limb tortuosity. Amelia of right upper limb was also observed.

the extraembryonic coelomic cavity. The umbilical cord was observed, albeit short. The amniotic bands were not observed. The genetic examination ruled out other malformations such as the omphalocele-exstrophy-imperforate anus-spinal defects (OEIS) complex associated with meningomyelocele. In the anatomopathological exam of the surgical specimen, a third-trimester gestational placenta was observed infiltrating the myometrium by two-thirds of its thickness, with a final report diagnosing placenta increta. The histopathological examination of the placenta showed no mosaicism that could be a cause of placental insufficiency.

Discussion

The cause of the LBWC malformation is unknown, and although some theories attempt to explain its origin, little has been proven. We believe that the association observed in this case could create a new perspective for the vascular disruption theory. It has been proposed that fetal malformations may have been caused by hypoxic-ischemic mechanisms arising from the failure of the trophoblast to supply the embryo's oxygen demands. Since the abnormal trophoblast invasion of placenta accreta has an essentially hypoxic nature, the poor perfusion of the decidua underlying the trophoblast implantation bed was thought to have led not only to the process of improper invasion, but also to ischemia of the embryo's tissues at critical moments of its development.

The descriptive character of this study does not allow us to affirm that there is a causal relationship between poor

decidua perfusion and the LBWC malformation; however, some aspects discussed below are worth highlighting.

A key point in the discussion lies in the well-known association between placenta accreta and the presence of prior uterine scarring. If the hypothesis that proposes a pathogenic link between the LBWC and placenta accreta is true, a higher incidence of LBWC cases in women with uterine scarring would have to be proven, since placenta accreta is more frequent in women with this risk factor. So far, this association cannot be proven or ruled out because studies addressing this question have not been published. Another similar type of reasoning could assume that the prevalence of LBWC should be much higher, since cesarean section is a very common surgery. However, it is very likely that other factors must act synergistically, in addition to the poor perfusion of the uterine scar, for embryonic lesions to occur. Probably, in the vast majority of cases, control mechanisms would act on trophoblastic circulation to promptly restore the gas exchange balance and ensure the embryo's normal development. However, in some situations of serious vascular disruption, where the reduction in blood flow is abrupt and intense, there would be no time for such balance to take place, and hypoxia, in these cases, would be severe enough to cause damages to the embryo.

Some literature data show evidence that the association between prior uterine scarring and the LBWC may be true.

An epidemiological aspect to be considered relates to the prevalence of this complication in countries with high rates of caesarean section because, if the association between the LBWC and uterine scarring is true, the prevalence of this malformation should be greater in those populations, as is the case with placenta accreta. In this respect, a study performed in Brazil, where the rates of cesarean section are the world's highest, shows a relevant result. In one reference center, the LBWC cases were evaluated through established diagnostic criteria such as abdominal defect, kyphoscoliosis, and rudimentary umbilical cord. With an average of 2,800 births a year, 21 cases were found in 11 years, representing a prevalence of 1 case for every 1,810 births.[4] There is no data about the type of prior childbirth that women in the study had undergone; however, this prevalence, even considering the fact that the study was conducted at a reference center, is much higher than that observed in any other study ever published.

Another epidemiological fact that could suggest the association of LBWC and previous cesarean scarring would be an increased incidence over the years, since there has been an increase in cesarean sections in almost all countries. There are no studies seeking to specifically address this issue; however, a study conducted in Denmark shows that this fact may be true. In that country's birth records in 20 years, from 1970 to 1974, only 1 case was reported; from 1975 to 1979 and from 1980 to 1984, 4 cases; and from 1985 to 1989, 7 cases, without an increase in birth rates.[3]

It is also important to mention that some publications have associated LBWC malformations with other conditions of potential hypoxic risk to the embryo, such as the use of cocaine and multiple pregnancies. Cocaine, for its abrupt and severe vasoconstrictive action, was considered responsible for the LBWC anomaly in women who used the substance in the first trimester in two case reports.[19,20] On the other hand, the LBWC anomaly in twin pregnancies has been reported in various publications, including cases of a single twin affected in monochorionic pregnancies, which contradicts the hypothesis of the endogenous origin.[1,2,21–25] It is possible that the rapid growth of embryos can be accompanied by episodes of inadequate perfusion of parts of the trophoblast.

We concluded that multiple factors must be involved in the pathogenesis of the LBWC malformation; however, the association with placenta accreta, observed in this case, shows that the vascular mechanism can be considered one of its main causes. The complex interaction of factors such as the extension of the poorly perfused trophoblast, the timing of onset, and the duration and intensity of the exposure to hypoxia can be determining factors for the genesis of placenta accreta, LBWC malformation or both. The association with placenta accreta points to a possible role of uterine scarring in the etiopathogenesis of the LBWC malformation. This hypothesis is of extreme importance, because it ascribes a risk factor for some fetal malformations to cesarean section birth. Although low, this risk could not be disregarded in a worldwide scenario of increasing rates of cesarean sections. Other studies should be performed to confirm this association.

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Instructions to Authors

Scope and policy

The material submitted for analysis cannot be simultaneously submitted for publication in other journals or previously published. In the selection of manuscripts for publication, are evaluated the originality, relevance of the theme, quality of the methodology used, and adequacy to the editorial standards adopted by the journal. The published material becomes intellectual property of the Brazilian Journal of Gynecology and Obstetrics and Febrasgo.

Manuscripts evaluation

The manuscripts submitted to the journal are received by the Editorial Office that checks the mandatory documentation and examines if the editorial norms contained in the Instructions to Authors have been fulfilled. If the process is in compliance, the manuscript is sent to the Editor-in-Chief, who will make a merit evaluation of the material. If the Editor-in-Chief concludes the work is in favorable scientific and technical conditions, the manuscript is forwarded to the Associate Editors, who will designate reviewers (double mind process) to evaluate it. Then, the reviewers' opinions and editor's instructions are sent to authors to inform them about changes to be made. Then, the authors resubmit the text with the suggested changes within the requested deadline. When resubmitting the manuscript, the requested corrections should be highlighted in yellow. In cases of disagreement with the suggestions, observations should be included in the comments balloons. Be assertive and punctual with the inquiry, and support the hypothesis with references.

IMPORTANT! Authors must comply with the deadlines, since non-attendance will result in delay of manuscript publication or even archiving of the process. At any point in the process of analysis and editing of the text, the authors may request the process suspension and withdrawal of the manuscript, except when it is accepted for publication. The concepts and statements contained in the articles are of the authors' responsibility.

Preparing a manuscript for submission

Mandatory submission documents

When submitting a manuscript to RBGO, attach the documents listed below on the ScholarOne submission platform. Note that not attaching the documents will result in cancellation of the submitted process. Mandatory documentation for online submission:

- Authorization of copyright transfer signed by all authors (scanned and attached as supplementary document) **Model;**
- In accordance with chapter XII.2 of Res. CNS 466/2012, in Brazil, research involving human subjects needs to inform the registration number referring to the Certificate of Ethical Assessment (CAAE) or the approval number of the research (CEP/CONEP) in the Ethics Committee. International manuscripts must present local ethical documentation to proceed with the submission process;
- Cover Letter: written to justify the publication. The authors should be identified, together with the title of the team that intends to publish, origin institution of the authors and intention of publication;
- Title page;
- Manuscript.

Title Page

- Title of the manuscript in English with a maximum of 18 words;
- Authors' full name without abbreviations (maximum six);
- Corresponding author (full name, professional mailing address and contact email);
- Institutional affiliation of each author. Example: Faculty of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil;
- **Conflicts of interest:** authors should report any potential conflicts of interest whether political, economic, of resources for research execution or intellectual property;
- **Acknowledgements:** restricted to people and institutions that contributed to research development in a relevant way. Any financial support provided by development agencies or private companies should be mentioned in the section Acknowledgments. For Brazilian authors, RBGO requests the citation of CNPq, Capes, FAPESP and other financing agencies, together with the number of research process or granted scholarships.
- **Contributions:** according to the criteria for scientific authorship of the International Committee of Medical Journal Editors (ICMJE), authorship credit must be based on three conditions met in full: 1. Substantial contributions to conception and design, data collection or analysis, and interpretation of data; 2. Writing of the article or critical review of the intellectual content; and 3. Final approval of the version to be published.

Manuscript

Instructions to Authors

The Brazilian Journal of Gynecology and Obstetrics publishes the following categories of manuscripts:

Original Articles, complete prospective, experimental or retrospective studies. Manuscripts containing original clinical or experimental research results have priority for publication.

Case Reports, of great interest and well documented from the clinical and laboratorial point of view. In the letter of referral, authors should indicate new or unexpected aspects in relation to already published cases. The text of Introduction and Discussion sections should be based on an updated bibliographic review.

Review Articles, including comprehensive reviews, meta-analysis or systematic reviews. Spontaneous contributions are accepted. The methods and procedures adopted for obtaining the text should be described, and based on recent references, including the current year. As this subject is still subject to controversy, the review should discuss the trends and lines of research under way. In addition to the text of the review, there should be an abstract and conclusions. See the 'Instructions to Authors' section for information on the text body and title page;

Letters to the Editor, dealing with editorial matters or not, but presenting relevant information to readers. Letters can be summarized by the editor, but maintaining the main points. In case of criticism to published works, the letter is sent to the authors so their reply can be published simultaneously;

Editorial, only at the publisher's invitation.

Title

When writing a scientific article, the researcher should focus on the manuscript title, which is the business card of any publication. It should be elaborated very carefully, and preferably written only after the article finalization. A good title adequately describes the manuscript content. Generally it is not a phrase, because it does not contain the subject, only verbs and arranged objects. Titles rarely contain abbreviations, chemical formulas, adjectives, names of cities, among others. The title of manuscripts submitted to RBGO must contain a maximum of 18 words.

Abstract

The abstract should provide the context or basis for the study, establish the objectives, basic procedures, main outcomes and key findings. It should emphasize new and important aspects of the study or observations. Since the abstract is the only substantive part of the article indexed in many electronic databases, authors should ensure it reflects the article content in an accurate and highlighted manner. Do not use abbreviations, symbols and references in the abstract. In case of original articles from clinical trials, authors must inform the registration number at the end of the text.

Informational abstract of structured type of original articles

Abstracts of original articles submitted to RBGO must be structured in four sections and contain a maximum of 250 words:

Objective: What was done; the question posed by the investigator.

Methods: How it was done; the method, including the material used to achieve the objective.

Results: What was found, the main findings and, if necessary, the secondary findings.

Conclusion: The conclusions; the answer to the question asked.

Informational abstract of structured type of systematic review articles

Among the included items are the review objective to the question asked, data source, procedures for selecting the studies and data collection, the results and conclusions. The abstracts of systematic review articles submitted to RBGO must be structured in six sections and contain a maximum of 250 words:

Objective: Declare the main purpose of the article.

Data sources: Describe the data sources examined, including the date, indexing terms, and limitations.

Selection of studies: Specify the number of studies reviewed and the criteria used in their selection.

Data collection: Summarize the conduct used for data extraction and how it was used.

Data synthesis: State the main results of the review and the methods used to obtain them.

Conclusions: Indicate the main conclusions and their clinical usefulness.

Informational abstract of unstructured type of review articles, except systematic reviews and case studies

It shall contain the substance of the article, covering the purpose, method, results and conclusions or recommendations. It exposes enough details so readers can decide on the convenience of reading the full text (Limit of words: 150).

Keywords

The keywords of a scientific paper indicate the thematic content of the text they represent. The main objectives of the aforementioned terms are the thematic content identification, indexing of the work in databases, and rapid location and retrieval of contents. The keyword systems used by RBGO are DeCS (Health Sciences Descriptors - Lilacs Indexer) and MeSH (Medical Subject Headings - MEDLINE-PubMed Indexer). Please choose five descriptors that represent your work on these platforms.

Manuscript body (Manuscripts submitted to RBGO must have a maximum of 4000 words. Note that tables, charts and figures in the Results section and References are not counted).

Introduction

The **Introduction** section of a scientific article has the purpose of informing what was researched and the reason for the investigation. This part of the article prepares the reader to understand the investigation and justification of its realization. The content informed in this section should provide context or basis for the study (i.e. the nature of the problem and its importance); state the specific purpose, research objective, or hypothesis tested in the study or observation. The study objective usually has a more precise focus when formulated as a question. Both the primary and secondary objectives should be clear, and any analyzes in a pre-specified subgroup should be described; provide strictly relevant references only and do not include data or conclusions of the work being reported.

Methods

According to the Houaiss dictionary, **Methods** "is an organized, logical and systematic process of research". The method comprises the material and procedures adopted in the research in order to respond to the central research question. Structure the Methods section of RBGO starting with the study design; research scenario (place and period in which it was performed); sample of participants; data collection; intervention to be evaluated (if any) and the alternative intervention; statistical methods used and the ethical aspects of the study. When thinking about the writing of the study design, reflect if it is appropriate to achieve the research objective, if the data analysis reflects the design, and if what was expected with use of the design was achieved to research the theme. Following, the guidelines used in clinical or epidemiological research that should be included in the section Methods of manuscripts sent to RBGO:

Types of study (adapted from Pereira, 2014*):

Case Report (Case study): In-depth investigation of a situation in which one or a few people are included (usually up to ten);

Case series: A set of patients (for example, more than ten people) with the same diagnosis or undergoing the same intervention. In general, these are consecutive series of patients seen in a hospital or other health institution for a certain period. There is no internal control group formed simultaneously. The comparison is made with external controls. The name of external or historical control is given to the group used to compare the results, but that was not constituted at the same time within the study: for example, the case series is compared with patients from previous years.

Transversal (or Cross-sectional) study: Investigation to determine prevalence; examine the relationship between events (exposure, disease, and other variables of interest) at any given time. Cause and effect data are collected simultaneously: for example, the case series is compared with patients from previous years.

Case-control study: Particular form of etiological investigation of retrospective approach in which the search of causes starts from the effects. Groups of individuals, respectively with and without a particular health problem are compared in relation to past exposures in order to test the hypothesis that exposure to certain risk factors is the contributing cause of the disease. For example, individuals afflicted with low back pain are compared with an equal number of individuals (control group) of the same sex and age, but without low back pain.

Cohort study: Particular form of investigation of etiological factors in which the search of effects starts from the cause; therefore, the opposite of case-control studies. A group of people is identified, and pertinent information on the exposure of interest is collected, so the group can be monitored over time, checking those who do not develop the disease in focus, and if the prior exposure is related to occurrence of disease. For example, smokers are compared to nonsmoker controls; the incidence of bladder cancer is determined for each group.

Randomized study: This has the connotation of an experimental study to evaluate an intervention hence the synonym of *intervention study*. Can be performed in a clinical setting; sometimes referred to simply as clinical trial or clinical study. It is also conducted at the community level. In clinical trials, participants are randomly assigned to form groups called study (experimental) and control (or testimony), whether submitted or not to an intervention (for example, a drug or vaccine). Participants are monitored to verify the occurrence of outcome of interest. This way, the relationship between intervention and effect is examined under controlled observation conditions, usually with double-blind evaluation. In the case of a **randomized study**, inform the number of the Brazilian Registry of Clinical Trials (REBEC) and/or the number of the International Clinical Trials Registration Platform (ICTRP/OMS) on the title page.

Ecological study: Research performed with statistics: the unit of observation and analysis is not constituted of individuals, but of groups of individuals hence the synonyms: study of groups, aggregates, clusters, statistics or community. For example, research on the variation of mortality coefficients for diseases of the vascular system and per capita consumption of wine among European countries.

Systematic Review and Meta-analysis: Type of review in which there is a clearly formulated question, explicit methods are used to critically identify, select and evaluate relevant research, and also to collect and analyze data from the studies included in the review. There is use of strategies to

limit bias in the localization, selection, critical evaluation and synthesis of relevant studies on a given topic. Meta-analysis may or may not be part of the systematic review. Meta-analysis is the review of two or more studies to obtain a global, quantitative estimate of the question or hypothesis investigated; and employs statistical methods to combine the results of the studies used in the review.

Source: *Pereira MG. Artigos Científicos – Como redigir, publicar e avaliar. Rio de Janeiro: Guanabara-Koogan; 2014.

Script for statistical review of original scientific papers

Study objective: Is the study objective sufficiently described, including pre-established hypotheses?

Design: Is the design appropriate to achieve the proposed objective?

Characteristics of the sample: Is there a satisfactory report on the selection of people for inclusion in the study? Has a satisfactory rate of responses (valid cases) been achieved? If participants were followed up, was it long and complete enough? If there was a pairing (eg. of cases and controls), is it appropriate? How did you deal with missing data?

Data Collection (measurement of results): Were the measurement methods detailed for each variable of interest? Is there a description of comparability of the measurement methods used in the groups? Was there consideration of the validity and reproducibility of the methods used?

Sample size: Has adequate information on sample size calculation been provided? Is the logic used to determine the study size described, including practical and statistical considerations?

Statistical Methods: Was the statistical test used for each comparison informed? Indicate if the assumptions for use of the test were followed. Was there information about the methods used for any other analysis? For example, subgroup analysis and sensitivity analysis. Are the main results accompanied by accuracy of the estimate? Inform the p value and confidence interval. Was the alpha level informed? Indicate the alpha level below which the results are statistically significant. Was the beta error informed? Or indicate the statistical power of the sample. Has the adjustment been made to the main confounding factors? Were the reasons that explained the inclusion of some and the exclusion of others described? Is the difference found statistically significant? Make sure there are sufficient analyzes to show the statistically significant difference is not due to any bias (eg. lack of comparability between groups or distortion in data collection). If the difference found is significant, is it also relevant? Specify the clinically important minimal difference. Make clear the distinction between statistically relevant difference and relevant clinical difference. Is it a one- or two-tailed test? Provide this information if appropriate. What statistical program is used? Inform the reference where to find it, and the version used.

Abstract: Does the abstract contain the proper article synthesis?

Recommendation on the article: Is the article in acceptable statistical standard for publication? If not, can the article be accepted after proper review?

Source: *Pereira MG. Artigos Científicos – Como redigir, publicar e avaliar. Rio de Janeiro: Guanabara-Koogan; 2014.

IMPORTANT!

RBGO joined the initiative of the International Committee of Medical Journal Editors (ICMJE) and the EQUATOR Network, which are aimed to improve the presentation of research results. Check the following international guides:

Randomized clinical trial:
http://www.consort-statement.org/downloads/consort-statement

Systematic reviews and meta-analysis: http://www.scielo.br/pdf/ress/v24n2/2237-9622-ress-24-02-00335.pdf

Observational studies in epidemiology: strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_combined.pdf

Qualitative studies: http://intqhc.oxfordjournals.org/content/19/6/349.long

Results

The purpose of the Results section is to show the study findings. It is the original data obtained and synthesized by the author with the aim to answer the question that motivated the investigation. For the writing of the section, present the results in logical sequence in the text, tables and illustrations, first mentioning the most important findings. Do not repeat all information of the tables or illustrations in the text. Emphasize or summarize only important observations. Additional or supplementary materials and technical details may be placed in an appendix where they will be accessible without interrupting the flow of the text. Alternatively, this information may be published only in the electronic version of the Journal. When data are summarized in the results section, provide numerical results not only in derived values (eg. percentages), but also in absolute values from which the derivatives were calculated, and specify the statistical methods used for their analysis. Use only the tables and figures necessary to explain the argument of the work and evaluate its foundation. When scientifically appropriate, include data analysis with variables such as age and sex. Do not exceed the maximum limit of five tables, five charts or five figures. Tables, charts and/or figures should be included in the body of the manuscript and do not count the requested limit of 4000 words.

ATTENTION!

In Case Studies, the Methods and Results sections should be replaced by the term Case Description.

Discussion

In the **Discussion** section, emphasize the new and important aspects of the study and the conclusions derived therefrom. Do not repeat details of data or other information presented in the introduction or results sections. For experimental studies, it is useful to begin the discussion by briefly summarizing the main findings, comparing and contrasting the results with other relevant studies, stating the limitations of the study, and exploring the implications of the findings for future research and clinical practice. Avoid claiming precedence and referring to incomplete studies. Do not discuss data not directly related to the results of the presented study. Propose new hypotheses when justifiable, but qualify them clearly as such. In the last paragraph of the Discussion section, cite which information of your work contributes relatively to advancement of knowledge.

Conclusion

The **Conclusion** section has the function of relating the conclusions to the objectives of the study, but authors should avoid unfounded statements and conclusions not adequately supported by data. In particular, authors should avoid making statements about economic benefits and costs unless their original includes economic analysis and appropriate data.

References

A study is based on the results of other research that preceded it. Once published, it becomes support for future work on the subject. In the report of their research, authors state the references of prior works consulted that they deem pertinent to inform readers, hence the importance of choosing good References. Properly chosen references lend credibility to the report. They are a source for convincing readers of the validity of facts and arguments presented.

Attention! For manuscripts submitted to RBGO, authors should number the references in order of entry into the manuscript and use those numbers for text citations. Avoid excessive references by selecting the most relevant for each statement and giving preference to the most recent work. Do not use hard-to-reach quotations, such as abstracts of papers presented at congresses, theses or restricted publications (non-indexed). Seek to cite the primary and conventional references (articles in scientific journals and textbooks). Do not use references such as 'unpublished observations' and 'personal communication'. Authors' publications (self-citation) should be used only if there is a clear need and relationship with the topic. In this case, include in bibliographical references only original works published in regular journals (do not cite chapters or revisions). The number of references should be 35, in exception review articles. Authors are responsible for the accuracy of data contained in the references.

Please check the American Medical Association (AMA) Citation Style to format your references.

*The Instructions to Authors of this journal were elaborated based on the Vancouver guidelines and the literary work ***Artigos Científicos: Como redigir, publicar e avaliar de Maurício Gomes Pereira, Editora Guanabara Koogan, 2014.***

Submission of papers

The articles must, necessarily, be submitted electronically, according to the instructions posted on the site: http://mc04.manuscriptcentral.com/rbgo-scielo

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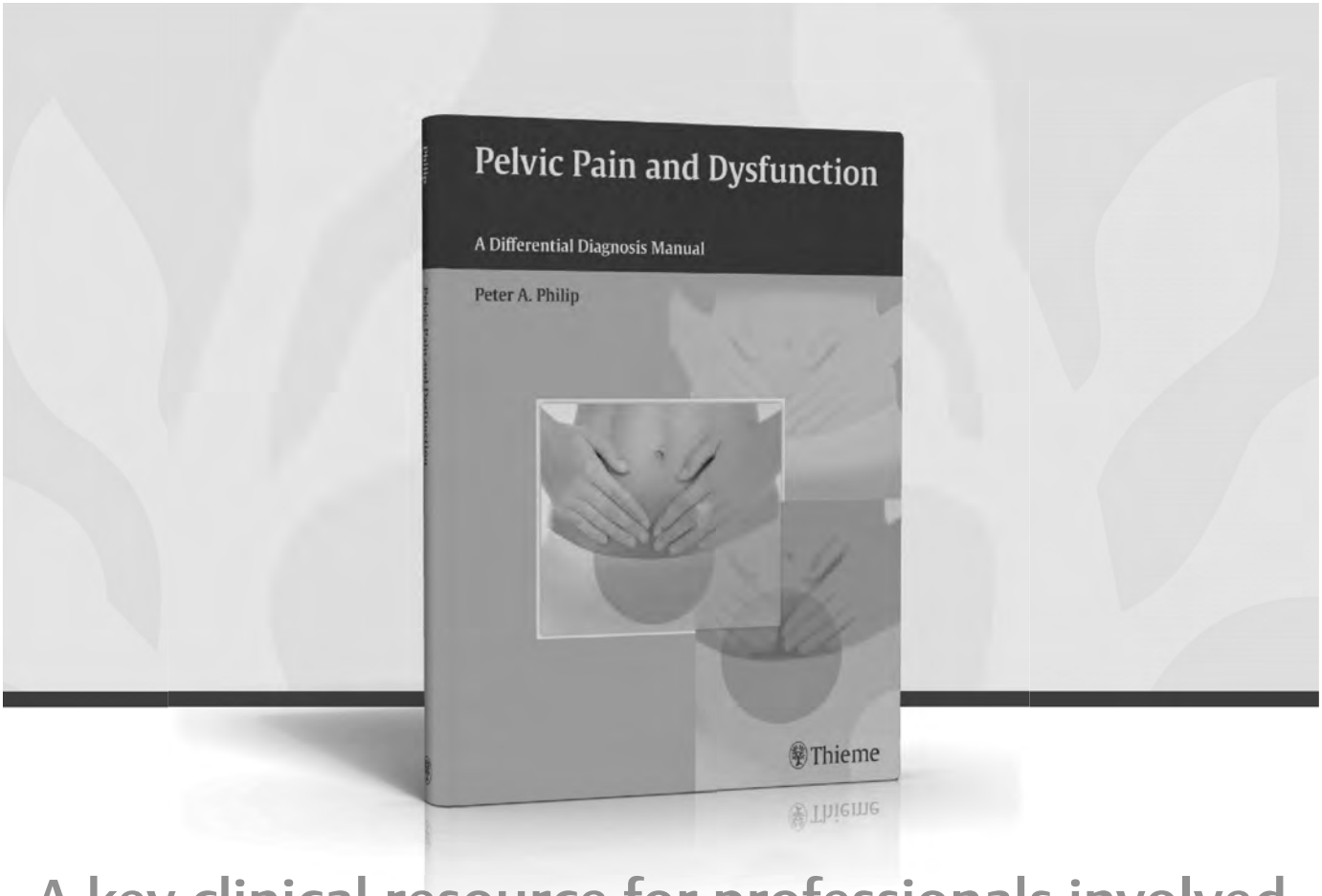

A key clinical resource for professionals involved in the treatment of pelvic pain and dysfunction

Pelvic Pain and Dysfunction
A Differential Diagnosis Manual

Peter A. Philip

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Junte-se à luta

FORÇA AMIGA

contra o câncer de colo do útero

Dê o próximo passo e lute por esta paciente.

Há muito a ser feito por ela.

DOUTORES, CONVIDAMOS TODOS VOCÊS
A FAZEREM PARTE DA CAMPANHA **#FORÇAAMIGA.**

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