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

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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

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, ≥ 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 (≥ 3 or ≤ 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 ≥ 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	0.496ᵃ
≥ 8 years	39	79.6	18	46.1	21	53.9	
Marital status							
With partner	51	92.7	22	43.1	29	56.9	0.320ᵃ
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	0.541ᵃ
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	0.194ᵇ
No	34	64.2	17	50	17	50.0	
BECC**							
A-B	14	25.0	9	64.3	5	35.7	0.278ᵃ
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	0.451ᵃ
No	8	14.3	5	62.5	3	37.5	
Nausea							
Yes	47	85.5	23	48.9	24	51.1	0.708ᵃ
No	8	14.5	3	37.5	5	62.5	
Visit to the dentist							
Yes	6	10.7	3	50.0	3	50.0	0.509ᵃ
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	0.569ᵃ
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	0.921ᵇ
≤ 3	25	45.4	12	48.0	13	52	
Brush after meals							
Yes	39	69.6	17	43.6	22	56.4	0.519ᵇ
No	17	30.4	9	52.9	8	47.1	
Use of dental floss							
Yes	22	44.0	11	50.0	11	50.0	0.449ᵇ
No	28	56.0	11	39.3	17	60.7	
Use of mouthwash							
Yes	41	80.4	19	46.3	22	53.7	0.835ᵇ
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]).
ᵃFisher exact Test.
ᵇChi-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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