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ARTICLE

CORRELATION BETWEEN SALIVARY BIOMARKERS AND PLAQUE-INDUCED GINGIVITIS EXACERBATED BY PREGNANCY.

Asociación entre el nivel de indiferencia al tratamiento dental y los hábitos de higiene bucal.

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ABSTRACT:

Objetive: To evaluate the correlation between salivary biomarkers (the salivary antioxidant ability, salivary level of polyphenols, and other antioxidants) with plaque-induced gingivitis exacerbated by pregnancy in pregnant and nonpregnant women.

Material and Methods: For this observational study, medical records, dental examinations, and analyses of saliva samples were carried out in pregnant and nonpregnant women. A *p*-value of <0.05 was considered significant.

Results: The pregnant women (n =17) exhibited a lower antioxidant capacity (*p*-value=0.0041), higher levels of polyphenols, gingival index, bleeding on probing, and subjects consuming mineral-enriched products (*p*-value from <0.0001 to 0.0466), and unchanged levels of phosphotungstic acid reactive substances, proteins, oral hygienic habits, plaque index and probing depth (*p*-value from 0.0683 to 0.8358), in comparison with the nonpregnant women (n=9). Also, a positive correlation between the gingival index and salivary polyphenol content was observed (*r*-value = 0.4087, *p*-value = 0.0202).

Conclusion: The salivary polyphenols correlate with plaque-induced gingivitis exacerbated by pregnancy, suggesting a deficiency of salivary antioxidant protection.

KEYWORDS:

*Saliva; biomarkers; antioxidants; polyphenols; *pregnancy; plaque-induced gingivitis.*

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RESUMEN:

Objetivo: Evaluar la correlación entre los biomarcadores salivales (la capacidad antioxidante salival, el nivel salival de polifenoles y otros antioxidantes) con la gingivitis inducida por placa exacerbada por el embarazo en mujeres embarazadas y no embarazadas.

Material y Métodos: Para este estudio observacional, se realizaron registros médicos, exámenes dentales y análisis de muestras de saliva en mujeres embarazadas y no embarazadas. Se consideró significativo un valor de $p<0,05$.

Resultados: Las gestantes (n=17) presentaron menor capacidad antioxidante ($p=0,0041$), mayores niveles de polifenoles, índice gingival, sangrado al sondaje y los sujetos que consumían productos enriquecidos con minerales ($p<0,0001$ a $p<0,0466$) , y no hubo diferencias en los niveles de sustancias reactivas al ácido fosfotúngstico, proteínas, hábitos de higiene bucal, índice de placa y profundidad de sondaje ($p=0,0683$ a $0,8358$), en comparación con las mujeres no embarazadas (n=19). Además, se observó una correlación positiva entre el índice gingival y el contenido de polifenoles salivales ($r = 0,4087$, $p = 0,0202$).

Conclusión: Los polifenoles salivales se correlacionan con la gingivitis inducida por placa y exacerbada por el embarazo, lo que sugiere una deficiencia de protección antioxidante salival.

PALABRAS CLAVE:

Saliva; Biomarcadores; Antioxidantes; Polifenoles; Embarazo; Gingivitis inducida por placa.

INTRODUCTION.

The pregnancy-associated gingivitis has prevalence rates ranging from 30 to 100% of pregnant women.[1] During the pregnancy, the onset and progression of gingivitis is produced by an altered host response to the bacterial plaque, dilated microvasculature, and high permeability of oral blood vessels, where all these physiological changes in the women are attributed to their fluctuations in serum levels of estrogen, progesterone and a reduction in salivary antioxidant capacity.[2,3]

Saliva contains unique information about the oral physiological changes. Thus, this biofluid is a promising prognostic biomarker for the periodontal tissue condition.[4] Uric acid, albumin, ascorbic acid, and glutathione are antioxidant compounds, which strongly contribute to the salivary antioxidant capacity.[5,6] To date, only one study has described the changes caused by pregnancy on the periodontal status, salivary antioxidant ability, and endogenous salivary antioxidants, since that study included a comparison between pregnant and nonpregnant women.[7] The remainder of studies have evaluated the relationship between the periodontal parameters and some salivary antioxidant markers in pregnant women with different comorbidities or pregnancy-related complications.[8-12]

Moreover, no previous studies have determined the salivary levels of exogenous antioxidant compounds, like polyphenols and minerals, alongside the antioxidant ability and periodontal status in pregnant and nonpregnant females.[7-12] The importance of measuring the polyphenol compounds and minerals is due to their potential role to promote a prooxidative oral environment, which can produce gingival damage.[13-15]

Therefore, our study aimed to evaluate the correlation between salivary biomarkers (the salivary antioxidant ability, salivary level of polyphenols, and other antioxidants) with plaque-induced gingivitis exacerbated by pregnancy in pregnant and nonpregnant women.

MATERIALS AND METHODS.

Study population

The study protocol was approved by the institutional Health Research Ethics Committee (Approval Number CEI-FE-014-019). Thus, the present work was conducted following the Helsinki Declaration of the World Medical Society and institutional standards. The study population consisted of females referred to the Periodontics Clinic, and Dentistry School, between March and August 2019. A total of 25 pregnant and 25 nonpregnant (control) women were enrolled in this study.

After each patient gave written informed consent, their medical and dental histories were recorded. The records included a questionnaire about practices related to gingival health, including frequency of tooth brushing, dichotomous questions (yes/no) about the use of dental flossing and mouth rinsing, and questions concerning dietary supplements that have either been self-prescribed or prescribed by a physician.

Inclusion criteria for the pregnant group were nonsmoking women of age >18 years, >15 weeks of pregnancy, mild gingivitis (Löe and Silness gingival index (GI) ranging from 0.1 to <1.1, probing pocket depth (PPD) ≤3 mm, and bleeding on probing (BOP) <20%),[7,12,16-18] and absence of alcohol abuse and dependence. Controls (non smoking females) were matched with the pregnant females for age and mild gingivitis. Also, the control women were free of pathological alcohol uses. Women with a history of preeclampsia, gestational diabetes, polycystic ovary syndrome, uterine malformation, heart murmur, valvular heart disease, renal disease, diabetes, hypertension, obesity, autoimmune disorders, antibiotic agents, or other use periodontal intervention within the previous three months were excluded. Finally, patients with a presence of oral lesions were also excluded.

Saliva collection

Whole saliva samples were obtained between 9 and 11 am. After abstaining from food, absence of oral hygiene, and lip cosmetic use for 12 hours alongside a fast from water for at least 2 hours, a saliva sample was allowed to accumulate in the floor of the mouth for 10 minutes. Subsequently, the patient spat it out into an ice-cold sterile container. Later, the samples were stored at –60°C until analysis.

Dental examination

After the saliva collection, the periodontal parameters were assessed in each patient. These parameters included GI, PPD, BOP, and plaque index (PI). A single calibrated examiner performed all these periodontal examinations. The GI, PPD, and BOP were evaluated using a manual periodontal probe (Hu Friedy, Chicago, IL, USA). Under the scoring criteria by Löe and Silness, each gingival area of the tooth was assigned with a score ranging from 0 to 3.[19] The PPD (in millimeters) was determined from the gingival margin to the bottom of the pocket. The BOP was expressed as the percentage of sites showing bleeding within 15 seconds after the probing.[12] The plaque disclosure was performed with a fuchsin tablet. Then, a score was assigned by the examiner (range from 0 to 5), according to the scoring criteria of the Turesky modification of the Quigley–Hein plaque index.[19,20] For the dental examination, each periodontal parameter was assessed at six sites of each tooth (mesiobuccal, mediobuccal, distobuccal, mesiolingual, mediolingual and distolingual). An average score of all teeth, except the third molars, was calculated for each patient.[16]

Biochemical analysis

The frozen saliva samples were thawed at room temperature (18°C ± 1°C). Subsequently, these samples were centrifuged at 1200 g for 10 minutes at 4°C. The supernatant was separated and immediately processed for the biochemical analyses.

Salivary antioxidant capacity (SAC)

The SAC was evaluated using the ferric reducing antioxidant power (FRAP) assay and 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radi-cal cation scavenging assay. The FRAP assay was performed as described previously.[21] In brief,

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one hundred microliters of saliva supernatant were added into a tube containing 250 µL of 300 mmol/L sodium acetate (pH 3.6), 25 µL of 10 mmol/L 2,4,6-tris(2-pyridyl)-s-triazine and 25 µL of 20 mmol/L iron (III) chloride hexahydrate. The mixture was incubated at 37°C for 4 minutes and then maintained at room temperature for 1 min. Subsequently, 200 µL of each mixture was placed in a well of a 96-well plate to read its absorbance at 596 nm. Meanwhile, the ABTS radical scavenging assay was performed as described by Gonzalez-Rivera *et al.*,[22]

Phosphotungstic acid reactive substances (PARS) in saliva

PARS technique was based on a previous methodology.[23] In brief, 100 µL of the saliva supernatant was added to 200 µL of phosphotungstic acid reagent. The mixture was incubated at 25°C for 15 minutes. After the incubation period, the mixture was centrifuged at 1200 g for 10 minutes at 4°C. Then, 200 µL of the supernatant was added to 100 µL of 140 g/L sodium carbonate solution. This mixture was incubated at 25°C for 15 minutes. After this second incubation period, 200 µL of each mixture were placed in a well of a 96-well plate to read its absorbance at 740 nm.

Salivary protein and polyphenol concentration

A Bradford microassay was performed as described previously to quantify proteins.[24] On the other hand, the measurement of salivary polyphenols was based on a previous technique.[25] In brief, 20 µL of the saliva supernatant was mixed with 280 µL of water and 50 µL of 50% Folin-Ciocalteu's reagent.

After incubation for 5 minutes at 25°C, 50 µL of 20% sodium carbonate solution was added to the mixture. Then, this mixture was incubated at 25°C for 20 minutes. After this second incubation period, 200 µL of each mixture were placed in a well of a 96-well plate to measure its absorbance at 760 nm.

Conversion from absorbance to concentration

The absorbance was monitored for all the biochemical assays using a Cytation™ 3 microplate reader and Gen5™ software (Biotek Instruments Inc., Vermont, USA). The salivary FRAP value, ABTS scavenging activity, PARS value, protein content, and polyphenol concentration were calculated using iron (II) sulfate, ascorbic acid, uric acid, albumin, and salicylic acid standard calibration curves, respectively. USA Food and Drug Administration guidelines, saliva-like buffers, and saliva samples were used to validate each assay.[26,27]

Statistical analysis

The antioxidant capacity values, PARS, proteins, polyphenols, age, and periodontal status were analyzed using the GraphPad Prism 5 software (San Diego, CA, USA). Also, this same software was used to perform the correlation analyses. Meanwhile, the data from the mineral intake, tooth brushing, dental flossing, and mouth rinsing were analyzed using the MedCalc software (MedCalc Software Ltd, Ostend, Belgium). The Shapiro-Wilk test evaluated the normal distribution of the data. Under a normal distribution, Pearson correlation analyses and Student's t-test were used; otherwise, Spearman correlation analyses and Mann-Whitney tests were applied. Chi-square tests were used for the differences between nominal variables. A p-value <0.05 was considered statistically significant.

RESULTS.

A total of 36 participants met the inclusion and exclusion criteria (17 pregnant and 19 nonpregnant women). Eight pregnant women were excluded because they recently received an antibiotic or dental intervention or presented a record of uterine malformation, pyogenic granuloma-like oral lesion, or GI score >1.1. Meanwhile, six nonpregnant women were excluded because they recently received an oral intervention or obtained a GI score <0.1.

The age of the included females was similar between the groups (29.4±6.1 and 28.5±5.3 years, p-value=0.6656), whereas the gestational age was 23.2±4.1 weeks in the pregnant group.

The percentage of subjects from the pregnant

Figure 1. Salivary antioxidant capacity (SAC) obtained from the pregnant and nonpregnant groups, using the ferric reducing antioxidant power (FRAP) and ascorbic acid equivalents (AAE) to inhibit the ABTS radical cation. ABTS, 2,2′-azino-bis 3-ethylbenzothiazoline -6-sulfonic acid.

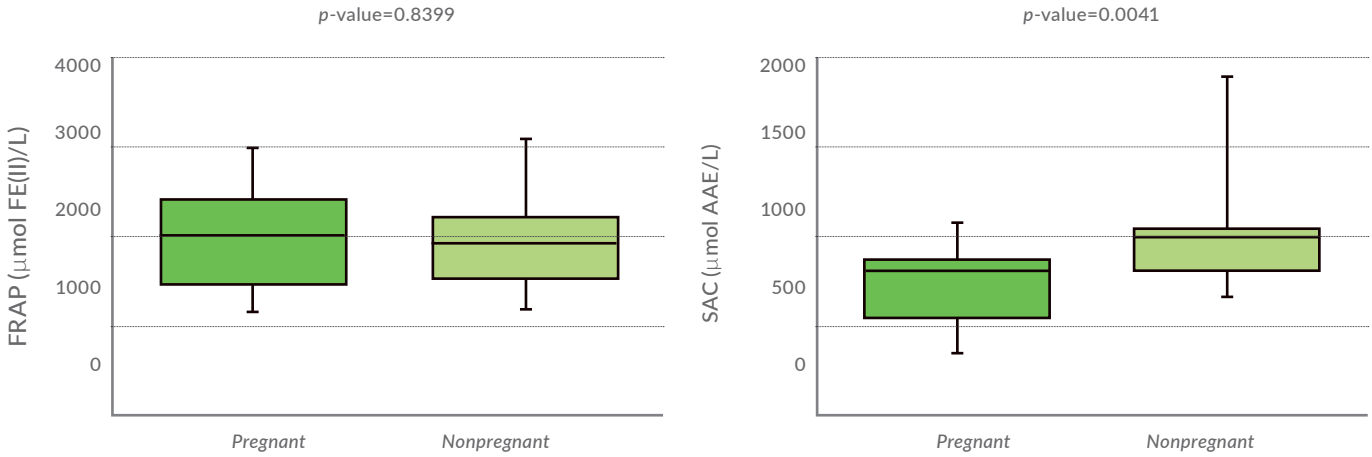

Figure 2. Concentration of phosphotungstic acid reactive substances (PARS), proteins, and polyphenols in saliva samples obtained from pregnant and nonpregnant females. SAE, salicylic acid equivalents.

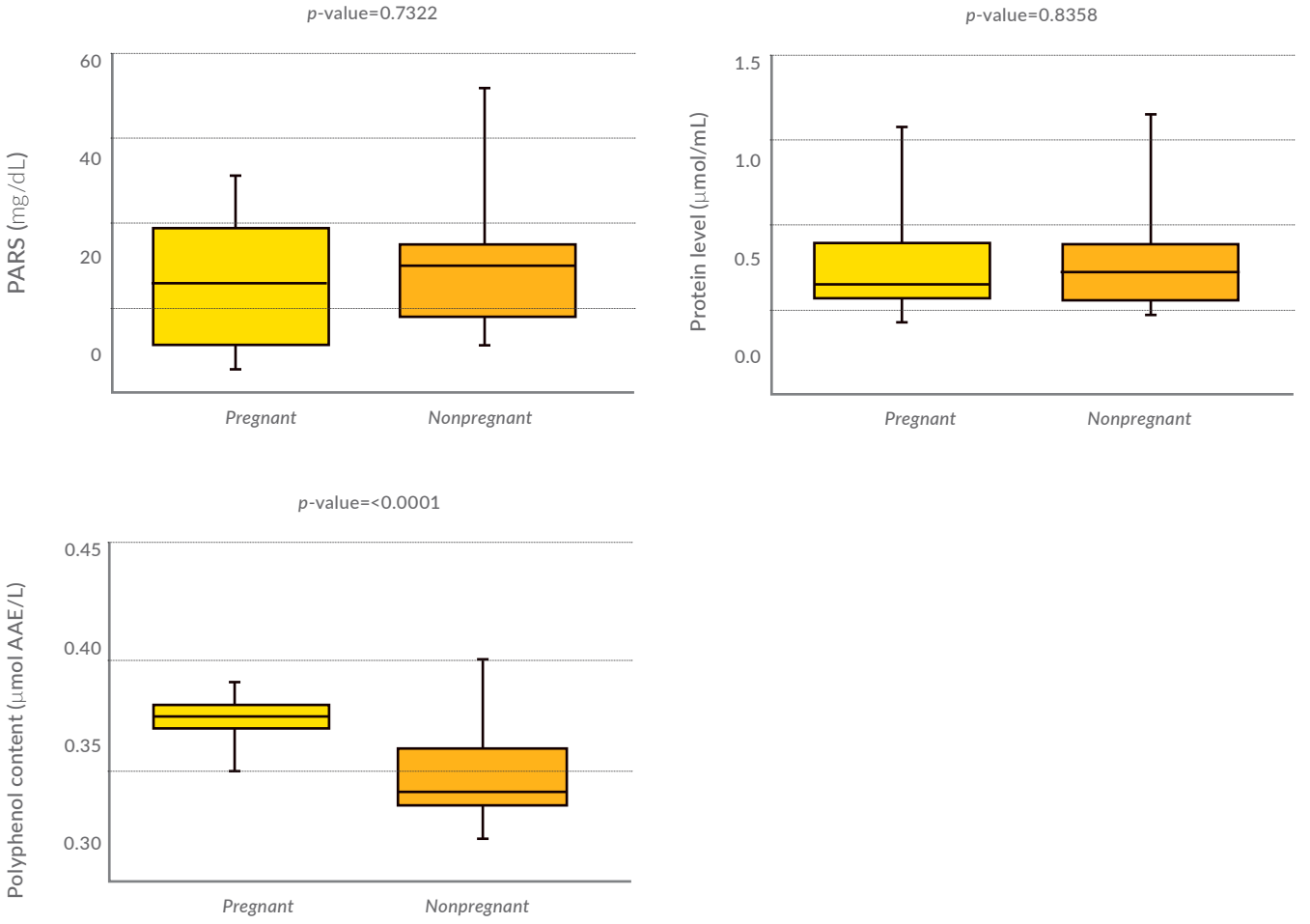

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Figure 3. Correlations between the gingival index (GI), bleeding on probing (BOP), salivary polyphenols (SP), and salivary antioxidant capacity (SAC) in females with mild gingivitis. AAE, ascorbic acid equivalents to inhibit the 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid radical cation; SAE, salicylic acid equivalents.

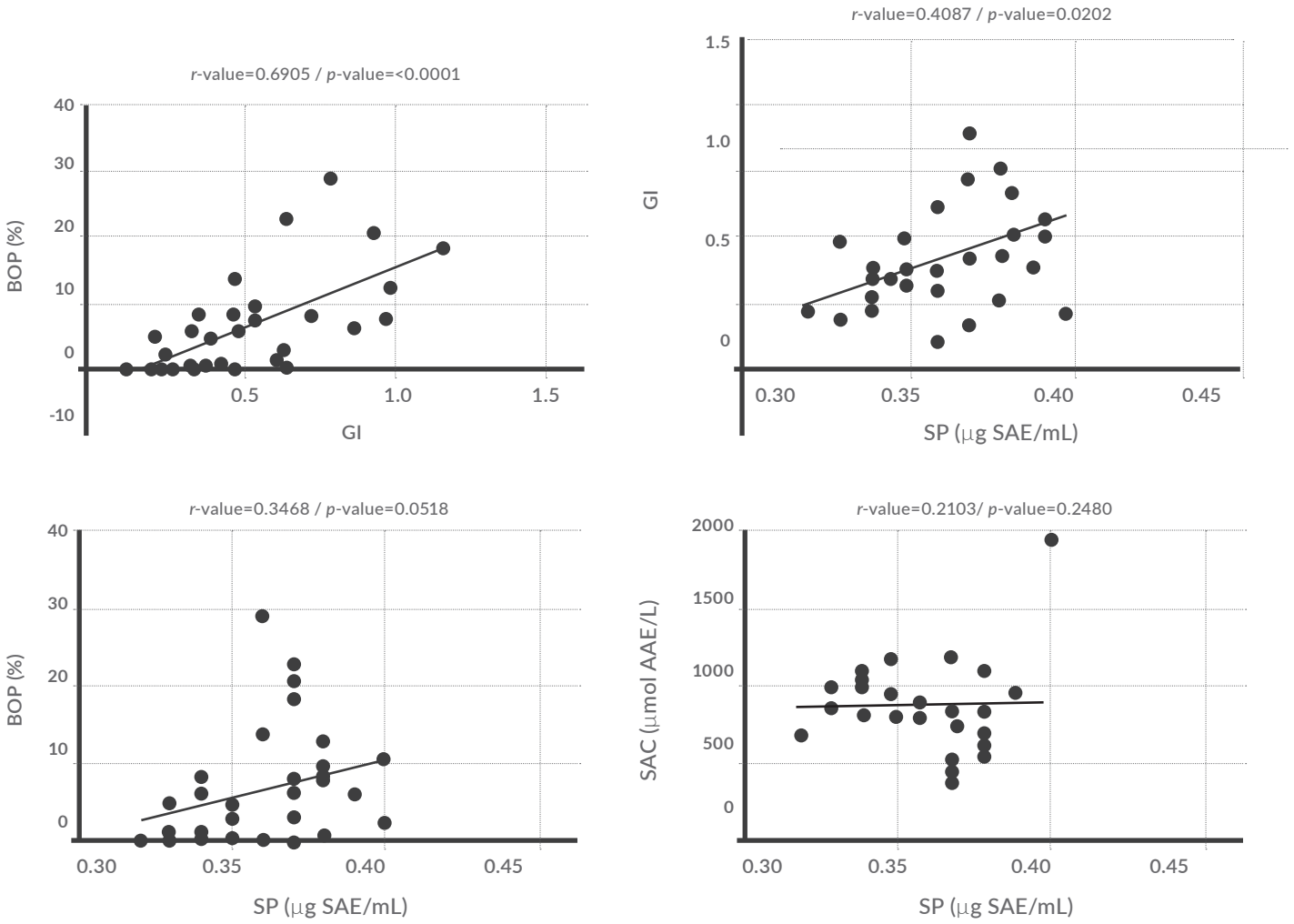

Table 1. Practices related to oral health and periodontal parameters of the included patients.

Parameter	Pregnant group (N=17)	Nonpregnant group (N=19)	*p*-value
Tooth Brushing (%)	88.2	100	0.1287
Dental flossing (%)	29.4	36.8	0.6428
Mouth rinsing (%)	23.5	10.5	0.3026
Mineral intake (%)	70.6	5.3	0.0001
Probing Pocket Depth (mm) (PPD)	1.86 ± 0.30	1.64 ± 0.37	0.0683
Plaque Index (PI)	1.96 ± 0.80	1.54 ± 0.54	0.0705
Gingival Index (GI)	0.65 ± 0.24	0.35 ± 0.16	0.0002
Bleeding on Probing(%) (BOP)	8.54 ± 8.15	4.35 ± 5.55	0.0466

Results are expressed as the mean and standard deviation.

group who brushed their teeth at least twice daily was similar to that of the control group (Table 1).

Also, the percentages of patients who use dental floss or mouth rinse in their oral hygiene routine were similar between the two groups (Table 1).

However, the pregnant group had a higher percentage of subjects who consumed mineral products than that the nonpregnant group (Table 1). The PI and PPD values were similar between the groups, but the GI and BOP values of the pregnant women were superior to those of the nonpregnant women (Table 1).

Our biochemical test results showed a lower SAC value in pregnant females than in nonpregnant females when the ABTS radical scavenging assay was used. Meanwhile, the antioxidant ability to reduce the ferric ion was similar for both groups (Figure 1). The salivary polyphenol concentration was higher in the pregnant group than the nonpregnant group, whereas the rest of the salivary parameters were similar between the two groups (Figure 2).

Our data analyses showed that the GI values correlated with the BOP and salivary polyphenol values, whereas the salivary polyphenol concentration was not associated with the SAC and BOP values (Figure 3).

DISCUSSION.

For the first time, our study shows that a reduced salivary antioxidant balance and increased gingival inflammation were presented in pregnant women with mild gingivitis, despite the salivary antioxidants were unchanged or raised, and independently of the dental plaque accumulation and oral hygiene habits, in comparison with the data from non-pregnant women with mild gingivitis.

Compared to controls, the pregnant women exhibited a lower SAC, reflected by the ABTS radical scavenging assay (ABTS-SAC). Meanwhile, the FRAP assay did not show a change in the salivary antioxidant capacity (Figure 1).

This situation was produced by the different sensitivity of the assays of the salivary antioxidant compounds. The salivary thiol antioxidants, like glutathione and albumin, reduce the ABTS radical cation efficiently, whereas the same antioxidants do not reduce the Fe^{3+}-TPTZ efficiently to Fe^{2+}-TPTZ.[6,22]

It is important to mention that one crucial advantage of a spectrophotometric measurement, like SAC, over the measure of an individual antioxidant compound using a liquid chromatographic or mass spectrometric technique is that it considers the additive, synergistic and antagonistic effects of antioxidant mixtures in actual samples.[22]

ABTS scavenging finding agreed with a previous report that shows a reduced antioxidant balance (low glutathione peroxidase activity) in pregnant women, compared with nonpregnant women.[7] The proteins and PARS were not shown to have a role in the reduced ABTS-SAC in pregnant women (Figure 1 and Figure 2).

The values of salivary protein in the pregnant females were inferior and the gingigival inflammation was higher to values reported in controls.[20] A decreased level of salivary proteins is due to a reduced inflammatory process in the gingiva of patients.[24]

The salivary PARS included uric acid, ascorbic acid, glucose, glutathione, and protocatechuic acid.[23] A previous study showed the salivary level of uric acid in normotensive pregnant women is equal to that found in healthy normotensive nonpregnant women.[28]

Despite uric acid being known as the main antioxidant in saliva, it has an oxidant-antioxidant paradox. In this way, uric acid can also act as a pro-oxidant agent since it can produce radicals by reacting with other biological fluid oxidants.[29] As observed in the PARS and ABTS-SAC results, the unchanged presence of the urate and other PARS does not preserve the salivary antioxidant ability during pregnancy (Figure 1 and Figure 2).

Besides, a high level of salivary polyphenols was found in pregnant women compared with the control group. The presence of these compounds was not positively associated with the ABTS-SAC

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value (Figure 1, Figure 2 and Figure 3).

A high concentration of several polyphenol compounds is available from fruits, vegetables, cocoa, tea, chocolate, coffee, herbal and fruit teas, and dietary supplements. All these foods can be part of a typical pregnant woman's diet.[30] Three hours after consuming a polyphenol-rich food, a transient increase in the plasma concentration of polyphenols is observed.[31]

However, salivary polyphenols are not derived from systemic absorption. The presence of polyphenols in the saliva is due to their release from oral surfaces. These compounds are binding to oral surfaces for long periods and under a constant salivary flow.[32]

The polyphenol compounds, recognized as antioxidants, can act as prooxidant agents under certain conditions.[13] A prooxidant environment can be induced by the reaction between the polyphenols and transition metal ions, such as copper and iron.[33]

The divalent copper and flavones can promote the formation of reactive oxygen species (ROS). The interaction between the iron and flavonoids produces a Fe (II)-flavonoid complex, which in turn can result in ROS formation.[13,33] Therefore, presence of high levels of polyphenols does not always produce a beneficial effect on the salivary antioxidant ability, especially under an iron and copper-rich environment, such as pregnancy.

During pregnancy, there is a critical mineral mobilization to generate fetal development. A prior study shows that a high salivary concentration of Fe ions is presented in the latest trimester of pregnancy, compared with postpartum data.[34] To date, there is no information about the salivary levels of copper in pregnancy. Still, serum copper in pregnant women is higher, compared with nonpregnant women.[35]

Moreover, products containing minerals were prescribed for the majority of pregnant women (Table 1), including Materna® (Societé des Produits Nestlé, Switzerland), Elevit® (Bayer HealthCare, Mexico), Previta Mom® (Laboratorios Liconsa SA, Spain) and Cyntelle O3® (Takeda Pharmaceuticals, Mexico). These products contain iron and copper, among many other macro and micronutrients. Nevertheless, the appropriate supplementation with iron and copper is essential to maintain a good pregnancy and fetal development.[35]

Under the inadequate salivary antioxidant protection in pregnant women, the overproduction of ROS can inhibit microbes involved in gingivitis and damage the periodontal tissues by various mechanisms, including lipid peroxidation, impairment of the gingival hyaluronic acid and proteoglycans, and stimulation of proinflammatory cytokine release.[14, 15]

This situation was reflected in the high GI and BOP values in pregnant women, under similar levels of plaque accumulation and oral hygiene habits, compared to those from the control group (Table 1). In addition, our study shows a positive correlation between the GI values and salivary polyphenol concentrations (Figure 3), where this situation can be explained by the proinflammatory actions of ROS derived from the prooxidant interaction between polyphenols and transition metal ions.

Under a low mineral content, the dietary polyphenols (*i.e.*, tannins, flavonoids, lignans, etc.) exhibit anti-inflammatory activity through different mechanisms, including the impairment of the nuclear factor-kB pathway, inhibition of C-reactive protein, and modulation of proinflammatory mediators.[36-37]

The beneficial or harmful effects of polyphenols on pregnancy are contradictory. In this regard, a recent review shows that a high concentration of polyphenols in diet may negatively affect the development of the fetus and the offspring's health.[30]

In contrast, another study shows that the prenatal intake of polyphenols may limit the placental oxidative injury, and consequently, it may confer protection to the fetus.[38] A randomized clinical trial shows that consumption of a high polyphenol diet does not alter predictors of cardiovascular

disease in healthy pregnant women, such as blood pressure.[31]

All those outcomes depended on the additive, synergistic or antagonistic interactions of the different types, abundance, and proportion of the polyphenols found in each tested intervention.[39] Dentists, physicians, and gynecologists should take precautions to avoid excessive harmful action on the gingiva of their pregnant patients by the prescription of diet supplements containing minerals.

CONCLUSION.

Salivary polyphenols correlate with plaque-induced gingivitis exacerbated by pregnancy, suggesting a deficiency of salivary antioxidant protection.

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Conflict of interests:

The authors declare that they have no conflict of interest.

Ethics approval:

Study was approved by the Health Research Ethics Committee of the Autonomous University of San Luis Potosi (approval number CEI-FE-014-019).

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Authors' contributions:

Aragon-Martineza O: Sample analysis, data analysis, drafting the work, revising of the manuscript, final approval of the version to be published.

Loredo-Escobar K: Sample collection, revising of the manuscript, final approval of the version to be published.

Cepeda-Bravo J: Revising of the manuscript, final approval of the version to be published.

Martinez-Morales F: Revising of the manuscript, final approval of the version to be published.

Gonzalez-Rivera M: revising of the manuscript, final approval of the version to be published.

Aranda-Romo S: Conception of the idea, design of the work, data analysis, drafting the work and revising it critically, final approval of the version to be published.

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