

Oral Health Status and Salivary Elemental Composition in Pregnancy: A Quantitative ICP-MS Study

Gebelikte Ağız Sağlığı Durumu ve Tükürük Element Kompozisyonu: Kantitatif Bir ICP-MS Çalışması

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ABSTRACT Objective: Pregnancy is associated with profound physiological and hormonal changes that significantly influence oral health, including both qualitative and quantitative alterations in the composition of oral fluids. The objective of this study is to investigate the relationship between salivary macro- and trace element levels and oral hygiene status during pregnancy. **Material and Methods:** A total of 100 participants were categorized into 4 groups: non-pregnant women with healthy periodontium, non-pregnant women with gingivitis, pregnant women with healthy periodontium, and pregnant women with gingivitis. Saliva samples were analyzed using inductively coupled plasma mass spectrometry to determine concentrations of Sodium (Na), potassium (K), magnesium (Mg), calcium, iron, zinc (Zn), copper, selenium (Se), cobalt, chromium, and manganese. Clinical periodontal parameters-plaque index (PI), gingival index (GI), bleeding on probing (BOP), clinical attachment level (CAL), probing depth (PD)-and the Decayed, Missed, Filled Teeth Index were also recorded. **Results:** PI, GI, BOP, CAL, and PD were significantly higher in pregnant women with gingivitis compared to other groups ($p<0.001$). Na, K, and Mg levels were significantly elevated in pregnant women with gingivitis, while Zn was lowest in non-pregnant women with gingivitis ($p<0.01$ or $p<0.05$). Se levels were significantly increased in the pregnant group ($p<0.01$). **Conclusion:** These findings suggest that pregnancy and periodontal health status are associated with alterations in salivary element composition, emphasizing the potential impact of gingival inflammation on oral biochemical profiles during pregnancy.

Keywords: Elements; gingivitis; oral health; pregnancy; saliva

ÖZET Amaç: Gebelik, ağız sağlığını önemli ölçüde etkileyen derin fizyolojik ve hormonal değişikliklerle ilişkilidir; bunlara ağız sıvılarının bileşimindeki nitel ve nicel değişiklikler de dahildir. Bu çalışmanın amacı, gebelik sırasında tükürük makro ve eser element seviyeleri ile ağız hijyeni durumu arasındaki ilişkiyi araştırmaktır. **Gereç ve Yöntemler:** Toplam 100 katılımcı 4 gruba ayrıldı: sağlıklı periodonsiyuma sahip gebe olmayan kadınlar, gingivitisli gebe olmayan kadınlar, sağlıklı periodonsiyuma sahip gebe kadınlar ve gingivitisli gebe kadınlar. Tükürük örnekleri, Sodyum (Na), potasyum (K), magnezyum (Mg), kalsiyum, demir, çinko (Zn), bakır, selenyum (Se), kobalt, krom ve manganez konsantrasyonlarını belirlemek için induktif olarak eşleşmiş plazma kütle spektrometrisi kullanılarak analiz edildi. Klinik periodontal parametreler-plak indeksi (PI), gingival indeks (GI), sondalamada kanama [bleeding on probing (BOP)], klinik ataşman seviyesi [clinical attachment level (CAL)], sondalama derinliği [probing depth (PD)]-ve Çürük, Kaçırılmış, Dolgulu Diş İndeksi de kaydedildi. **Bulgular:** PI, GI, BOP, CAL ve PD, gingivitisli hamile kadınlarda diğer gruplara kıyasla anlamlı derecede yüksekti ($p<0,001$). Na, K ve Mg düzeyleri gingivitisli hamile kadınlarda anlamlı derecede yükseldi, Zn düzeyleri gingivitisli hamile olmayan kadınlarda en düşüktü ($p<0,01$ veya $p<0,05$). Se düzeyleri hamile grupta anlamlı derecede artmıştı ($p<0,01$). **Sonuç:** Bu bulgular, hamilelik ve periodontal sağlık durumunun tükürük elementi bileşimindeki değişikliklerle ilişkili olduğunu düşündürmekte ve hamilelik sırasında gingival inflamasyonun oral biyokimyasal profiller üzerindeki potansiyel etkisini vurgulamaktadır.

Anahtar Kelimeler: Elementler; diş eti iltihabı; ağız sağlığı; gebelik; tükürük

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Pregnancy affects women's lives in almost every aspect, including oral health. During pregnancy, the pH, buffering capacity, and other chemical changes in the salivary structure increase the risk of caries and periodontal disease. The oral cavity harbours one of the most complex microbial communities in the human body, and the maintenance of this microbiome is paramount for a healthy pregnancy. An increase in total microbial counts has been observed in pregnant women, particularly during the first trimester, when compared to non-pregnant women.^{1,2} Hormonal changes during this period have been shown to increase susceptibility to various oral diseases, including gingivitis and periodontitis.^{1,3} Disturbances in the oral microbiome have been associated with adverse pregnancy outcomes, including preterm labour, pre-eclampsia and low birth weight.^{1,4} Therefore, vital to emphasize the significance of maintaining oral health during pregnancy for both the mother and foetus. The manifestation of oral health problems during pregnancy can present with symptoms such as increased tooth decay, bleeding gums and tooth mobility.⁵ Pregnancy has been clinically linked to an increase in the prevalence and/or severity of periodontitis, which is characterized by an increase in periodontal probing depth, clinical attachment, and bone loss, as well as an increase in the prevalence and/or severity of gingivitis, which is characterized by gingival bleeding and swelling.⁶

Dental caries, a condition characterized by the demineralization of tooth enamel due to the action of oral bacteria on dietary carbohydrates, affects approximately 25% of females of reproductive age. A multitude of factors have been posited as contributing to this phenomenon, including increased mouth acidity, a propensity for sweet foods, and diminished oral health awareness. A study by Silk et al. and Minozzi et al. revealed that pregnant women are more susceptible to developing tooth decay.^{7,8} Furthermore, calcium and phosphate concentrations in whole saliva appear to decrease during pregnancy. This decline in calcium and phosphate levels could be indicative of increased demineralization, which is a process that leads to reduced buffering capacity in saliva.

Hence, it is imperative to assess the composition of saliva with regard to remineralization or deminer-

alization. Pregnancy induced both qualitative and quantitative alterations in the composition of the oral fluid. Despite mounting evidence for the role of metal ions in periodontal health, our understanding of the changes in the ionic profile of saliva in periodontal disease remains limited.⁹ Saliva contains various macro and trace elements that play a role in periodontal hard and soft tissue homeostasis and regulate the concentration of inflammatory cytokines and oxidative stress markers responsible for periodontal tissue damage and decreased resistance to infection. In particular, it has been previously reported that trace elements such as zinc (Zn), copper (Cu), selenium (Se), cobalt (Co), chromium (Cr), and manganese (Mn) may be involved in immunological inflammatory pathways as components of antioxidant enzymes. Sodium (Na) and potassium (K) have been demonstrated to regulate the structural integrity of epithelial and connective tissue cells. Furthermore, magnesium (Mg), calcium (Ca), and iron (Fe) have been implicated in alveolar bone remodeling and periodontal ligament homeostasis. In view of these findings, it may be worthwhile to examine the ionic profile in periodontal disease in pregnancy. In addition to these, changes in dietary habits are thought to increase the incidence of caries by causing a decrease in the elements in the salivary structure. It has been found that salivary *Streptococcus mutans*, yeast, and *Lactobacilli* increase in the third trimester of pregnancy and during lactation, and the cariogenic flora changes during pregnancy. The present study aims to evaluate the relationship between ion levels in saliva and the occurrence of periodontal disease and caries in pregnant women, by comparing them to non-pregnant controls.

The objective of this study is to investigate the relationship between the levels of trace and macro elements in saliva during pregnancy and oral hygiene status.

MATERIAL AND METHODS

Following the approval of the Atatürk University Clinical Research Ethics Committee of the Faculty of Medicine (date: November 25, 2021; no: 00/551), the study population was established. Prior to participation, all subjects were thoroughly informed about the

study and provided written informed consent in accordance with the Declaration of Helsinki. The sample calculation conducted utilized the G*Power 3.1.9.7 (Franz Faul, Germany) programme, which determined that a total of 88 participants should be studied across 4 groups, with a minimum of 22 participants per group, an effect size of 0.4, 80% power and a 5% margin of error.¹⁰ The study included a total of 100 female participants, evenly distributed into 4 groups (n=25 each): Group 1 (G1)-non-pregnant women with healthy periodontium, Group 2 (G2)-non-pregnant women with gingivitis, Group 3 (G3)-pregnant women with healthy periodontium, and Group 4 (G4)-pregnant women with gingivitis. Non-pregnant participants were recruited from individuals who applied to the Periodontology Clinic at the Faculty of Dentistry, Atatürk University. Inclusion criteria for these groups were being between 20-30 years of age, systemically healthy, non-smokers, not using oral contraceptives, and having no history of pregnancy. Pregnant participants were selected from those attending routine prenatal follow-up at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Atatürk University. Eligibility criteria for these groups included being in the second trimester of pregnancy (20-30 weeks of gestation), aged between 20-30 years, systemically healthy, not taking any medications, and non-smokers. Oral examinations including clinical periodontal parameters [plaque index (PI), gingival index (GI), bleeding on probing (BOP), clinical attachment level (CAL), probing depth (PD)] and Decayed, Missed, Filled Teeth Index (DMFT) were performed on all participants. Unstimulated saliva samples were collected from all participants between 9:00-11:00 a.m., prior to clinical periodontal examination. Participants were instructed to abstain from eating, drinking, or performing any oral hygiene procedures for at least 2 hours before sample collection. For the collection procedure, each participant was asked to allow saliva to accumulate in the mouth and then passively drool into sterile collection tubes over a 5-minute period, without any external stimulation. A total of 3 ml of saliva sample was collected by transferring the collected saliva samples to the Eppendorf tube using an injector. Patients were asked not to eat, drink, or

brush their teeth for at least 1 hour before sampling. Saliva samples without stimulation were collected in 3 Eppendorf tubes for elemental analysis. The concentrations of the salivary elements Ca, Mg, Zn, Cu, Mn, Fe, Na, K, Cr, Co and Se were analysed by inductively coupled plasma mass spectrometry (ICP-MS) and stored at -80 °C until elemental analysis. Elemental analysis of collected saliva samples was stored at +4 °C before procedures. Sample weights were measured using a precision balance. The initial weight of the sample was recorded. Acid was added to each sample. All samples were allowed to react homogeneously with the acid for 10 minutes. The samples were allowed to dissolve in a microwave oven for 40 minutes at 1,600 watts, 100% power, 160 °C. After the samples were transferred to centrifuge tubes, ultrapure water was added and ICP-MS performed elemental analysis. The calibration curve was drawn from 8 different points of the standard solution containing the desired elements for calibration.

STATISTICALLY ANALYSES

The obtained results were analyzed using the SPSS 20.0 (IBM, New York, USA) program. The results were described as the mean±standard deviation. The normal distribution suitability of the parameters was determined using Kolmogorov-Smirnov tests. The clinical and laboratory data were analyzed by the one-way analysis of variance test. Principal Component Analysis (PCA) was conducted to reduce the dimensionality of the dataset and identify underlying patterns among the variables. Correlation coefficients were calculated to assess the relationships among the variables. The value of $p < 0.05$ was statistically significant.

RESULTS

Clinical parameters and mineral elements were compared between pregnant and control participants (Table 1, Table 2).

PI was statistically significantly higher in gingivitis than in healthy periodontium groups. A statistically significant difference was found in the pregnant healthy periodontium group than the non-pregnant healthy periodontium group. GI and BOP was statistically significantly the highest in the preg-

TABLE 1: The comparison of demographic and clinical data intra and inter-groups

| | G1 Nonpregnant-healthy periodontium | G2 Nonpregnant-gingivitis | G3 Pregnant-healthy periodontium | G4 Pregnant-gingivitis | p value |
|---------------------|--|------------------------------|-------------------------------------|---------------------------|---------|
| Age | 28.58±2.59 | 29.74±2.51 | 29.00±5.10 | 28.96±5.49 | 0.877 |
| Week | 0±0 ^b | 0±0 ^b | 24.06±4.76 ^a | 22.71±5.94 ^a | <0.001 |
| Number of pregnancy | 0±0 ^b | 0±0 ^b | 2.56±1.10 ^a | 3.04±1.62 ^a | <0.001 |
| PI | 0.87±0.59 ^c | 1.66±0.25 ^{ab} | 1.33±0.36 ^b | 1.92±0.76 ^a | <0.001* |
| GI | 1.01±0.26 ^c | 1.57±0.36 ^b | 1.13±0.76 ^c | 1.91±0.54 ^a | <0.001* |
| BOP | 6.35±2.98 ^c | 49.73±21.89 ^b | 7.12±3.51 ^c | 66.50±16.69 ^a | <0.001* |
| CAL | 0±0 ^b | 0.09±0.29 ^b | 0±0 ^b | 0.50±0.78 ^a | 0.001* |
| PD | 1.92±0.61 ^b | 1.74±0.49 ^b | 2.51±0.70 ^a | 2.88±0.49 ^a | <0.001 |
| DMFT | 0.05±0.04 | 0.07±0.04 | 0.08±0.66 | 0.07±0.68 | 0.235 |

*significantly different of intragroup comparison. PI: Plaque index; GI: Gingival index; BOP: Bleeding on probing; CAL: Clinical attachment level; PD: Probing depth; DMFT: Decayed, Missed, Filled Teeth Index

TABLE 2: Concentration of mineral elements in saliva of pregnant and non-pregnant gingivitis patients and healthy controls

| Elements | G1 Nonpregnant-healthy periodontium | G2 Nonpregnant-gingivitis | G3 Pregnant-healthy periodontium | G4 Pregnant-gingivitis | p value |
|----------|--|------------------------------|-------------------------------------|------------------------------|---------|
| Na | 140.39 ^b ±67.50 | 162.26 ^b ±114.36 | 191.87 ^b ±93.27 | 267.43 ^a ±107.92 | 0.00* |
| Mg | 8.81 ^{ab} ±5.51 | 6.37 ^b ±3.93 | 8.98 ^{ab} ±5.04 | 12.12 ^a ±9.41 | 0.04* |
| K | 682.65 ^b ±187.66 | 692.37 ^b ±197.26 | 793.36 ^b ±282.86 | 1042.21 ^a ±328.12 | 0.00* |
| Ca | 8.29±4.09 | 6.26±3.21 | 6.13±3.10 | 7.43±3.94 | 0.25 |
| Mn | 0.29±0.18 | 0.23±0.23 | 0.33±0.24 | 0.38±0.28 | 0.19 |
| Fe | 23.23±23.33 | 3.91±2.93 | 13.96±36.25 | 36.32±149.61 | 0.67 |
| Co | 0.60±1.37 | 0.28±0.84 | 0.59±1.48 | 0.15±0.55 | 0.41 |
| Cu | 236.22±81.85 | 262.42±65.18 | 263.21±68.46 | 242.03±74.23 | 0.57 |
| Zn | 0.30 ^{bc} ±0.10 | 0.25 ^c ±0.09 | 0.41 ^b ±0.13 | 0.73 ^a ±0.29 | 0.00* |
| Se | 0.33 ^b ±0.81 | 0.20 ^b ±0.66 | 2.50 ^a ±2.31 | 2.94 ^a ±4.23 | 0.00* |
| Cr | 0.04±0.07 | 0.04±0.09 | 0.01±0.03 | 0.02±0.03 | 0.34 |

Na: Sodium; Mg: Magnesium; K: Potassium; Ca: Calcium; Mn: Manganese; Fe: Iron; Co: Cobalt; Cu: Copper; Zn: Zinc; Se: Selenium; Cr: Chromium

nant gingivitis group. On the other hand, GI and BOP in the non-pregnant gingivitis group was observed statistically significant differences from healthy periodontium groups. CAL was significantly higher in the pregnant gingivitis than in non-pregnant gingivitis group and health periodontium groups. PD was significantly higher in pregnant groups compared to non-pregnant groups. No statistically significant difference was found between the groups in DMFT.

Salivary concentrations of Na, Mg, K, and Zn were significantly elevated in G4 compared to G3. No statistically significant differences were observed between G3 and G4 in terms of Ca, Mn, Fe, Co, Cu, and Cr concentrations. Se were lower non-pregnant groups than pregnant groups. Furthermore, G2 had notably lower salivary concentrations of Mg and Zn

compared to G1. No significant differences were found between G1 and G2 in the levels of Na, K, Ca, Mn, Fe, Co, Cu, Se, and Cr.

Table 3 illustrates the correlation coefficients indicating the relationships between the measured elemental concentrations. Correlation analysis revealed several statistically significant relationships among the measured elements. A strong positive correlation was observed between Mg and Ca, as well as between Na and K. Se also showed a strong positive correlation with Mg and a moderate correlation with Ca. A statistically significant negative correlation was found between Mn and Cu. In contrast, elements such as Zn and Cr exhibited generally weak or non-significant correlations with most of the other variables.

TABLE 3: Correlation coefficients among measured elemental concentrations

| | Na | Mg | K | Ca | Mn | Fe | Co | Cu | Zn | Se | Cr |
|----|---------------|---------------|---------------|---------------|--------|--------|--------|--------|--------|---------------|--------|
| Na | 1.000 | 0.531 | 0.746* | 0.505 | -0.196 | 0.035 | 0.092 | 0.191 | 0.100 | 0.397 | 0.033 |
| Mg | 0.531 | 1.000 | 0.433 | 0.896* | -0.241 | 0.283 | 0.052 | 0.002 | -0.045 | 0.804* | 0.155 |
| K | 0.746* | 0.433 | 1.000 | 0.518 | -0.263 | 0.341 | -0.097 | 0.304 | 0.159 | 0.212 | 0.232 |
| Ca | 0.505 | 0.896* | 0.518 | 1.000 | -0.189 | 0.440 | -0.046 | 0.271 | -0.015 | 0.642 | 0.287 |
| Mn | -0.196 | -0.241 | -0.263 | -0.189 | 1.000 | -0.119 | -0.133 | -0.375 | 0.038 | -0.068 | -0.077 |
| Fe | 0.035 | 0.283 | 0.341 | 0.440 | -0.119 | 1.000 | -0.020 | 0.327 | 0.007 | -0.153 | 0.043 |
| Co | 0.092 | 0.052 | -0.097 | -0.046 | -0.133 | -0.020 | 1.000 | -0.216 | -0.163 | 0.273 | -0.059 |
| Cu | 0.191 | 0.002 | 0.304 | 0.271 | -0.375 | 0.327 | -0.216 | 1.000 | 0.044 | -0.301 | 0.238 |
| Zn | 0.100 | -0.045 | 0.159 | -0.015 | 0.038 | 0.007 | -0.163 | 0.044 | 1.000 | -0.059 | 0.043 |
| Se | 0.397 | 0.804* | 0.212 | 0.642 | -0.068 | -0.153 | 0.273 | -0.301 | -0.059 | 1.000 | 0.129 |
| Cr | 0.033 | 0.155 | 0.232 | 0.287 | -0.077 | 0.043 | -0.059 | 0.238 | 0.043 | 0.129 | 1.000 |

Na: Sodium; Mg: Magnesium; K: Potassium; Ca: Calcium; Mn: Manganese; Fe: Iron; Co: Cobalt; Cu: Copper; Zn: Zinc; Se: Selenium; Cr: Chromium

DISCUSSION

This research aims to investigate the effects on oral health by examining the salivary ion profile in pregnant women. The findings of this study suggest that pregnancy is associated with elevated levels of Na, Mg, K, and Zn elements, particularly among individuals diagnosed with gingivitis. Notably, non-pregnant individuals with gingivitis exhibited lower concentrations of essential elements such as Mg and Zn. This observation may indicate a potential link between periodontal inflammation and deficiencies in key minerals. The significant differences in elemental concentrations observed between the study groups highlight the dual impact of both pregnancy and periodontal status on mineral homeostasis. These alterations in mineral balance may have important implications for both oral and systemic health, underscoring the need for further investigation into the complex interplay between pregnancy, oral health, and micronutrient status.

The measurement of ion levels in oral fluid is a significant diagnostic tool for the early identification of periodontal diseases. Levels of inorganic ions in saliva have been demonstrated to have a substantial role in the diagnosis and monitoring of periodontal diseases.¹¹ The salivary ion profile in pregnant women is of particular relevance to oral health. The oral cavity undergoes numerous transient alterations during pregnancy, a physiological state.³ The emergence of advanced sensors has facilitated direct ion

measurement, thereby enabling early diagnosis of periodontal diseases.¹² Furthermore, the correlation between ion concentrations in saliva and plaque fluid can influence the development of dental caries. These measurements can be utilized to ascertain the levels of these ions.¹²

The present study demonstrated that PI, GI, BOP, CAL, and PD were significantly elevated in pregnant individuals with gingivitis compared to other study groups, indicating a marked deterioration in periodontal health during pregnancy. These findings are consistent with previous research reporting increased GI and PD among pregnant women.¹³⁻¹⁵ However, discrepancies exist in the literature regarding clinical attachment levels, as some studies found no significant differences in CAL between pregnant and non-pregnant individuals.^{14,15} The observed increase in BOP, particularly during the second trimester, also aligns with prior findings, and these changes have been reported to reverse postpartum, suggesting a transient nature linked to pregnancy-related physiological changes.¹⁵

Periodontal diseases are characterized by inflammation of the tissues that support the teeth. This condition has been observed to result in significant alterations in the levels of inorganic ions present within the oral fluid. Increased levels of Ca, phosphate and bicarbonate have been documented in patients diagnosed with periodontitis. Furthermore, an increase in salivary flow rate has been reported in these patients.¹⁶ Concurrently, studies have shown

that individuals diagnosed with periodontitis exhibit higher levels of Na and K when compared to healthy individuals.¹⁷

The investigation of chronic periodontitis and associated ion levels, particularly inorganic ions such as Ca, Mg, K, and Na, has been a prominent focus in previous research. Aun reported increased levels of Ca, K, and Na, alongside decreased Mg levels, in individuals with periodontitis.¹⁸ Nevertheless, the findings of the study by Inonu et al. indicate an increase in the levels of Na, Mg, and K, which is consistent with the results obtained in the present study.¹¹ In contrast to our study, Inonu et al. also reported an increase in Ca levels with periodontal disease.¹¹ However, the levels of Ca in saliva were analysed in a population consisting of only female individuals with different levels of periodontal health in this study. No significant difference was found between the groups. One potential explanation for this discrepancy could be the exclusive focus on female subjects in the present study. In many studies, a mixed population of male and female subjects has been utilized. It is acknowledged that sex-related hormonal differences, particularly fluctuations in estrogen and progesterone levels, can influence both periodontal tissue response and saliva composition. Furthermore, the inclusion of pregnant individuals in the present study may have exerted direct or indirect influences on salivary Ca levels, attributable to the augmented Ca requirement during this physiological period, hormonal fluctuations, and variations in salivary gland function. This may have resulted in the observed relationship between periodontal status and salivary Ca levels deviating from the anticipated direction. Consequently, the observed discrepancy in findings when compared to existing literature may be attributable to the homogeneity of the study population with regard to gender and physiological status.

In a research by Inonu, it was reported that the Zn content in saliva was higher in patients with periodontal disease than in healthy patients.¹¹ Consistent with the study, in the present study a statistically significant increase was detected in the pregnant gingivitis group. However, a statistically significant decrease was observed in the non-pregnant gingivitis group. These deficiencies are particularly important

given the well-established roles of these elements in regulating immune function, inflammatory response, and maintaining periodontal tissue integrity.^{19,20} Zn contributes to epithelial barrier function and wound healing. Thus, lower levels of these elements may impair the host defense mechanisms, contributing to more severe periodontal inflammation in non-pregnant individuals.²¹ One possible reason for this discrepancy could be that the current study focused solely on female participants. Hormonal cycles in women, and especially fluctuations in oestrogen levels, may have a significant effect on microelement metabolism. Many studies that have been done with women and men may not show the full picture of how much gender affects immune and chemical reactions. Also, the extent of the gum disease, dietary habits of individuals, and other outside forces may have caused dissimilar results. The findings suggest that the connection of periodontal disease with Zn levels may be distinct in females, especially when the hormone level is high.

The recent study was reported that salivary Fe levels were similar in the gingivitis group to the healthy group.¹¹ The results of the present study were consistent with those of previous research regarding the Fe element level. Statistically significant differences were not identified between the gingivitis and healthy periodontium groups. Despite the administration of Fe supplements to the pregnant groups, no significant difference was found in the salivary levels. This phenomenon can be attributed to the fact that the maternal Fe level declines, necessitating the administration of supplements to address the augmented iron requirements of the foetus.²²

The study revealed no statistically significant differences in the element levels of Cr, Co, Mn and Cu between the groups. Despite the absence of research examining the saliva levels of these elements in pregnant women in the current literature, studies have been conducted on serum levels. Although Co, Cu and Mn levels tend to increase during pregnancy, a decrease was found in Cr levels.²³⁻²⁶ However, the results of these studies are not consistent with those of the present study. The underlying factors contributing to these variations may include the selection of different gestational weeks for analysis or variations in sample size.

Although previous studies have reported a decrease in salivary Ca levels during pregnancy, particularly in the 2nd and 3rd trimesters, attributed to increased fetal Ca demand, hormonal changes, and alterations in salivary gland function, present study findings demonstrated that there were no statistically significant disparities identified among the study groups with respect to calcium levels.^{27,28} This apparent contradiction may stem from several factors. Variations in analytical techniques, saliva collection protocols, or timing of sampling across studies may influence ion concentration measurements. Nutritional intake, or differences in overall maternal health may also account for interstudy variability.

Interestingly, the results did not show a statistically significant difference in DMFT scores between pregnant and non-pregnant individuals, which could be explained by the similarity in salivary Ca levels between the groups. Our findings were inconsistent with previous studies reporting an increase in DMFT scores during pregnancy.^{29,30} However, they align with the results of a study conducted by Yilmaz et al. which found no significant difference in DMFT indices between pregnant and non-pregnant women.³¹ These results suggest that, despite alterations in salivary mineral composition, clinical outcomes such as dental caries experience may not be directly or solely influenced by Ca concentration. Moreover, the inclusion of only female participants, specifically pregnant women at varying stages of gestation, introduces physiological complexities associated with hormonal fluctuations that differ substantially from the mixed-gender or non-pregnant populations examined in previous research. Collectively, these observations underscore the multifactorial nature of salivary biochemistry and oral health outcomes during pregnancy, highlighting the need for further investigation to elucidate the intricate interplay among hormonal status, salivary composition, and periodontal health. Conversely, the elevated trace element levels observed in pregnant individuals may not necessarily reflect improved systemic mineral status. Instead, these increases may result from enhanced metabolic demands associated with pregnancy, which could stimulate the mobilization of mineral stores from peripheral tissues into the bloodstream or exocrine flu-

ids such as saliva.^{32,33} This physiological adaptation may be driven by hormonal fluctuations, particularly elevated estrogen and progesterone levels, which are known to modulate mineral metabolism and distribution.³⁴

The findings of the present study indicated a statistically significant discrepancy between the pregnant and non-pregnant groups with respect to Se element levels. A marked increase in the presence of this element was detected in the saliva of pregnant subjects. Despite the existence of literature that suggests a decrease in salivary Se levels during pregnancy, this finding does not align with the results obtained in the present study.³⁵ The underlying factors contributing to these variations may include differences in gestational week or sample size. It has also been reported that pregnancy increases oxidative stress.³⁵ Given that Se is an antioxidant element, its increased presence in saliva may provide a plausible explanation for the observed differences.

Cumulatively, these results imply that both systemic changes with pregnancy and the status of periodontal health affect trace element equilibrium. Additionally, trace element availability changes, because of either deficiency or redistribution, could play an essential role in establishing the extent of periodontal inflammation.

This study has some limitations. Its cross-sectional design does not allow for causal conclusions. The sample was limited to a specific region, and dietary differences were not controlled, which may have affected salivary mineral levels. Hormonal levels were not measured. Including periodontitis patients in future studies could help compare different oral conditions more clearly. Also, selecting pregnant participants within a narrower gestational age range may reduce variability and improve data interpretation.

CONCLUSION

The altered composition of saliva during pregnancy could reflect and affect dental and periodontal health. In our study, pregnancy was linked with a high risk of pregnancy gingivitis, with elevated saliva Na, K, Mg, and Zn concentrations. In contrast, non-pregnant individuals with gingivitis exhibited lower concentra-

tions of essential elements such as Mg and Zn. These findings suggest that trace element alterations may play a role in the etiology of periodontal inflammation during different conditions of the body. More studies that follow over time and explain how things occur are needed to further delineate the effect of each element of saliva on dental and periodontal change during pregnancy and oral health.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

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REFERENCES

1. Saadaoui M, Singh P, Al Khodor S. Oral microbiome and pregnancy: a bidirectional relationship. *J Reprod Immunol.* 2021;145:103293. PMID: 33676065.
2. Fujiwara N, Tsuruda K, Iwamoto Y, Kato F, Odaki T, Yamane N, et al. Significant increase of oral bacteria in the early pregnancy period in Japanese women. *J Investig Clin Dent.* 2017;8(1). PMID: 26345599.
3. Silva de Araujo Figueiredo C, Gonçalves Carvalho Rosalem C, Costa Cantanhede AL, Abreu Fonseca Thomaz ÉB, Fontoura Nogueira da Cruz MC. Systemic alterations and their oral manifestations in pregnant women. *J Obstet Gynaecol Res.* 2017;43(1):16-22. PMID: 28074549.
4. AlHumaid GA, Alshehri T, Alwalmani RM, Alsubaie RM, Alshehri AD, Aljoghaiman E, et al. Assessment of oral health status and pregnancy outcomes among women in Saudi Arabia. *Patient Prefer Adherence.* 2024;18:1027-38. PMID: 38826504; PMCID: PMC11141704.
5. Pecci-Lloret MP, Linares-Pérez C, Pecci-Lloret MR, Rodríguez-Lozano FJ, Oñate-Sánchez RE. Oral manifestations in pregnant women: a systematic review. *J Clin Med.* 2024;13(3):707. PMID: 38337401; PMCID: PMC10856094.
6. Akcalı A, Akcalı Z, Batool F, Petit C, Huck O. Are sex steroid hormones influencing periodontal conditions? A systematic review. *Current Oral Health Reports.* 2018;5(10):1-6. doi:10.1007/s40496-018-0168-0
7. Silk H, Douglass AB, Douglass JM, Silk L. Oral Health During Pregnancy. *American Family Physician.* 2008;77(8):1139-44. <https://www.aafp.org/pubs/afp/issues/2008/0415/p1139.html>
8. Minozzi F, Chipaila N, Unfer V, Minozzi M. Odontostomatological approach to the pregnant patient. *Eur Rev Med Pharmacol Sci.* 2008;12(6):397-409. PMID: 19146202.
9. Goudouri OM, Kontonasaki E, Lohbauer U, Boccaccini AR. Antibacterial properties of metal and metalloid ions in chronic periodontitis and peri-implantitis therapy. *Acta Biomater.* 2014;10(8):3795-810. PMID: 24704700.
10. Serdar CC, Cihan M, Yücel D, Serdar MA. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem Med (Zagreb).* 2021;31(1):010502. PMID: 33380887; PMCID: PMC7745163.
11. Inonu E, Hakkı SS, Kayis SA, Nielsen FH. The association between some macro and trace elements in saliva and periodontal status. *Biol Trace Elem Res.* 2020;197(1):35-42. PMID: 31848920.
12. Totu EE, Isildak I, Nechifor AC, Cristache CM, Enachescu M. New sensor based on membranes with magnetic nano-inclusions for early diagnosis in periodontal disease. *Biosens Bioelectron.* 2018;102:336-344. PMID: 29172141.
13. Taani DQ, Habashneh R, Hammad MM, Batieha A. The periodontal status of pregnant women and its relationship with socio-demographic and clinical variables. *J Oral Rehabil.* 2003;30(4):440-5. PMID: 12631171.
14. Ho CC, Chou MY. Periodontal status in Taiwanese pregnant women. *J Dent Sci.* 2016;11(2):146-51. PMID: 30894963; PMCID: PMC6395188.
15. Gürsoy M, Pajukanta R, Sorsa T, Könönen E. Clinical changes in periodontium during pregnancy and post-partum. *J Clin Periodontol.* 2008;35(7):576-83. PMID: 18430046.
16. Amalina R, Mahdalena M, Aditya G. Differences in the salivary inorganic ions levels and salivary flow rate of the periodontitis and non-periodontitis patients. *Padjadjaran Journal of Dentistry.* 2020;32(2):160-5. doi:10.24198/pjd.vol30no3.21201
17. Baima G, Iaderosa G, Corana M, Romano F, Citterio F, Giacomino A, et al. Macro and trace elements signature of periodontitis in saliva: a systematic review with quality assessment of ionomics studies. *J Periodontal Res.* 2022;57(1):30-40. PMID: 34837226; PMCID: PMC9298699.
18. Aun WAA. Inorganic ions level in saliva of patients with chronic periodontitis and healthy subjects. *First National Conference For Iraqi Dental Colleges;* 2012; Volume: 24 (Sp. Issue 2).

19. Fekete M, Lehoczki A, Csipó T, Fazekas-Pongor V, Szappanos Á, Major D, Mózes N, Dósa N, Varga JT. The role of trace elements in COPD: pathogenetic mechanisms and therapeutic potential of zinc, iron, magnesium, selenium, manganese, copper, and calcium. *Nutrients*. 2024;16(23):4118. PMID: 39683514; PMCID: PMC11644833.
20. Romano F, Castiblanco A, Spadotto F, Di Scipio F, Malandrino M, Berta GN, et al. ICP-mass-spectrometry ionic profile of whole saliva in patients with untreated and treated periodontitis. *Biomedicines*. 2020;8(9):354. PMID: 32942752; PMCID: PMC7555328.
21. Berg Y, Gabay E, Božić D, Shibli JA, Ginesin O, Asbi T, et al. The impact of nutritional components on periodontal health: a literature review. *Nutrients*. 2024;16(22):3901. PMID: 39599688; PMCID: PMC11597335.
22. Sungkar A. The role of iron adequacy for maternal and fetal health. *World Nutrition Journal*. 2021;5(S1):10-5. <https://worldnutrijournal.org/OJS/index.php/WNJ/article/view/260>
23. Fort M, Grimalt JO, Casas M, Sunyer J. Interdependence between urinary cobalt concentrations and hemoglobin levels in pregnant women. *Environ Res*. 2015;136:148-54. PMID: 25460631.
24. Dokumov SI. Serum copper and pregnancy. *Am J Obstet Gynecol*. 1968;101(2):217-22. PMID: 5652955.
25. Spencer A. Whole blood manganese levels in pregnancy and the neonate. *Nutrition*. 1999;15(10):731-4. PMID: 10501283.
26. Saner G. Urinary chromium excretion during pregnancy and its relationship with intravenous glucose loading. *Am J Clin Nutr*. 1981;34(9):1676-9. PMID: 7282592.
27. Yousefi M, Parvaie P, Riahi SM. Salivary factors related to caries in pregnancy: a systematic review and meta-analysis. *J Am Dent Assoc*. 2020;151(8):576-88.e4. PMID: 32718487.
28. Guidozi F, MacLennan M, Graham KM, Jooste CP. Salivary calcium, magnesium, phosphate, chloride, sodium and potassium in pregnancy and labour. *S Afr Med J*. 1992;81(3):152-4. PMID: 1734555.
29. Liang L, Aris IM. Association between age at first birth and long-term dental caries experience among women in the United States. *J Womens Health (Larchmt)*. 2024;33(10):1409-16. PMID: 38853662.
30. Geevarghese A, Baskaradoss JK, Sarma PS. Oral health-related quality of life and periodontal status of pregnant women. *Matern Child Health J*. 2017;21(8):1634-42. PMID: 28155025.
31. Yilmaz F, Carti Dorterler O, Eren Halici S, Kasap B, Demirbas A. The effects of pregnancy on oral health, salivary pH and flow rate. *BMC Oral Health*. 2024;24(1):1286. PMID: 39455976; PMCID: PMC11515415.
32. King JC. Physiology of pregnancy and nutrient metabolism. *Am J Clin Nutr*. 2000;71(5 Suppl):1218S-25S. PMID: 10799394.
33. Black RE. Micronutrients in pregnancy. *Br J Nutr*. 2001;85 Suppl 2:S193-7. PMID: 11509110.
34. Prentice A. Calcium in pregnancy and lactation. *Annu Rev Nutr*. 2000;20:249-72. PMID: 10940334.
35. Awn BH. Salivary protein carbonyl and selected antioxidants in relation to dental caries among pregnant women. *Journal of Baghdad College of Dentistry*. 2023;35(1):27-35. <https://doi.org/10.26477/jbcd.v35i1.3312>