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Research Article

# Changes in Levels of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) in the Serum of Preterm Delivery Pregnant Women Affected by Gingivitis

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**Abstract**

**Background:** Periodontal disease increases local and systemic inflammatory responses in pregnant women, which may lead to premature delivery.

**Objectives**: To detect maternal serum levels of proinflammatory cytokine (Interleukine-6, Interleukin-8) with preterm delivery in pregnant women suffering from gingivitis.

**Methods:** In this case-control study, a total of 85 pregnant women, were included in the study, of whom 55 had gingivitis: 25 preterm deliveries and 30 full-term deliveries. Thirty healthy pregnant women with healthy gingiva were in the control group. They were selected from Al-Ramadi Teaching Hospital for Gynecology and Pediatrics, from November 2022 to May 2023. Blood samples were collected from all participants and the biomarkers cytokines Interleukine-6 and Interleukin-8 were detected by enzyme-linked immunosorbent assay (ELISA) kits.

**Results:** The current study showed an increased level of interleukin-6 and interleukin-8 in the pre-term delivery gingivitis group followed by full-term-delivery pregnant groups having gingivitis compared with the control group, with statistically highly significant differences.

**Conclusion:** Pregnant women with gingivitis with the highest levels of Interlukine-6 and Interlukine-8 were more prone to have premature delivery than those with lower levels of Interleukins and those without gingivitis.

**Keywords:** Full-term Delivery; Gingivitis; Interleukine-6; Interleukin-8; Periodontal disease; Preterm delivery.

## Introduction:

Periodontal diseases are bacterial infections of tooth-supporting tissues that inflame and destroy the periodontium. The complex bacterial structure of a protective and adhesive matrix is what maintains this bacterial infection affixed to the periodontal tissue (1). Bacterial infection severely degrades the periodontium and creates a continual and systemic assault with bacterial compounds, and host-derived inflammatory mediators may start and perpetuate systemic diseases (2). Because periodontal disease affects 90% of the population (either gingivitis or periodontitis), dental health and its relationship to systemic health are global health problems (3). New studies links periodontal disease to heart disease, diabetes, respiratory infections, and pregnancy complications.

Pregnancy can also negatively impact gingival health, as variations in hormone levels during pregnancy can induce gingivitis. Pregnant women may also have a higher risk of developing new periodontal diseases, as preexisting periodontal diseases become active during pregnancy (4,5).

Hill suggested that early-stage microorganisms' alterations of sub-gingival biofilm during pregnancy linked to gingivitis and periodontitis may trigger a placental response, leading to premature delivery. Hormonal alterations are well-established to cause a host response to infection, but the growth and are poorly understood (6). Forty percent of pregnant women have periodontitis (7,8), and their babies are seven times more likely to be premature or underweight. Pregnancy hormones cause inflammation, which promotes periodontal disease. Gingivitis affects 50-70% of pregnant women due to hormonal changes. Pregnancy-induced plasma progesterone and estrogen levels may alter subgingival bacteria, the maternal immune system, and pro-inflammatory mediator production (9,10). Cytokines are well-known mediators that govern local, systemic, and inflammatory responses and play a role in numerous biological processes, such as inflammation, by regulating the length and intensity of the inflammatory response (11). Cytokines control homeostasis and inflammation. Female sex hormones' ability to enhance cytokines production by human gingival fibroblasts at high concentrations comparable to pregnant women's plasma suggested that they may contribute to periodontal disease progression during pregnancy (12,13). Interlukin-8 is a cytokine generated in response to inflammation by several immunological inflammatory cells. Interlukin-8 functions begin with neutrophil

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activation and play a role in polymorphonuclear neutrophils (PMNs) migration to the inflammatory region (14). Chronic and excessive IL-8-mediated chemotactic and triggering impact on neutrophils in the inflammatory gingiva may generate local periodontal tissue injury (15). According to Shahshahan's research, maternal blood interleukin-6 and interleukin-8 concentrations may be employed as acceptable indicators for predicting preterm labor (16). In industrialized and developing nations, preterm birth (PTB) is a major public health issue and the most important biological factor of neonatal survival. They affect families and the healthcare system. Preventable risk factors for preterm birth must be continuously sought (17). Preterm birth is important because it predicts increased mortality and morbidity in infants born with this condition and reflects the mother's exposure to other risk factors like socioeconomic conditions, malnutrition, and diseases (18). In 1996, Offenbacher et al. found that periodontal disease is a risk factor for preterm low birth weight (9). Hill, also observed that periodontal bacteria may cause upper genital tract infections in pregnant women, resulting in premature delivery (6). Thus, this study aims to detect maternal serum levels of proinflammatory cytokine (Interleukine-6, Interleukin-8) with preterm delivery in pregnant women suffering from gingivitis.

**Subjects, Materials, and Methods:**

The current study was performed on 85 pregnant women 55 of whom had gingivitis and was divided into two groups: 25 preterm deliveries and 30 full-term deliveries, in addition to 30 healthy pregnant women with healthy gingiva as a control group.

The cases were taken from Al-Ramadi Teaching Hospital for Gynecology and Pediatrics, Al-Ramadi, Iraq, during the period November 2022 to May 2023.

**Inclusion Criteria for the gingivitis groups**:

1. Pregnant women in their third trimester months (term pregnancies were those who delivered at $\geq$ 37 weeks of gestation, preterm pregnancies were those who delivered at < 37 weeks of gestation) (17).
2. Good general health.
3. 18 – 42 years old.
4. Iraqi nationality.
5. Has at least 20 teeth in the oral cavity
6. Having gingivitis with Pocket depth $\leq$ 3 mm

**Exclusion Criteria:**

1. Periodontal therapy for the past 3 months.
2. History of smoking or alcohol drinking.
3. Use of antibiotics during the past three months.
4. Wearing orthodontic appliances or prosthodontics
5. Twin pregnancy.
6. Genital tract infection.
7. Urinary tract infection.
8. No systemic or autoimmune disease.
9. Not diabetic, no history of allergy or rhinosinusitis.

**Oral Examination:** A clinical examination was performed by a dentist for every subject under the study.

The periodontal status of all teeth was assessed using a periodontal probe, the periodontal parameters include (8,9): Gingival index (GI), bleeding on probing (BOP), and probing pocket depth (PPD).

**1. Gingival Index:** The occurrence of gingival inflammation at 4-surfaces of each tooth was assessed using the criteria of the gingival index system (9).

Score 0: normal gingiva

Score 1: mild inflammation, slight change in color, slight edema, no bleeding on probing.

Score 2: moderate inflammation, redness, edema, and glazing, bleeding on probing.

Score 3: severe inflammation, marked redness and edema, ulceration, tendency to spontaneous bleeding.

**2. Bleeding on Probing:** A periodontal probe is inserted into the base of the periodontal pocket/sulcus for six surfaces of each tooth (8,9) (mesiofacial, facial, distofacial, mesiolingual, lingual, and distolingual) and is moved lightly along the tooth (root) surface. After probing, the site was given a score:

a. If bleeding occurred within 30 seconds (score 1)
b. For the non-bleeding site (Score 0)

**3. Probing Pocket Depth:** Refers to the distance from the gingival margin to the most apical penetration of the periodontal probe. The sites of measurements were Mesiofacial, facial, distofacial, mesiolingual, lingual, and distolingual (8,9). No pressure was used to insert the periodontal probe.

**Blood Sample Collection:** Five milliliters of venous blood were collected from each subject under aseptic conditions before delivery, moved to a sterile gel tube, and serum was separated by centrifugation at 3000rpm, divided into small aliquots, and stored until analysis (18).

**Enzyme-Linked Immunosorbent Assay (ELISA):** Detection of pro-inflammatory biomarkers by using Human Interleukine-6 and Interleukin-8 enzyme immunoassay kit (ELK Biotechnology, company) in serum samples.

**Principle of the Procedure:** This kit uses sandwich enzyme immunoassay. This package includes a microtiter plate pre-coated with either IL-6 or IL-8 antibody. Microtiter plate wells containing standards or samples get a biotin-conjugated IL-6 or IL-8 antibody. Incubate microplate wells with avidin-HRP. TMB substrate solution only colours wells containing IL-6, IL-8, biotin-conjugated antibodies, and enzyme-conjugated avidin. Sulfuric acid blocks the enzyme-substrate reaction, and the color change is measured at 450 nm±10 nm. The concentration of biomarkers in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

**Statistical analysis**

The Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Excel 2010 were employed for data processing. Differences between group means were studied. Fisher's exact test, ANOVA, and Pearson correlation were also used.

**Results**

Table 1 compares the three study groups in terms of their age, bleeding on probing, Interlukine-6, and Interlukine-8. The differences between the mean ages of the three study groups were not statistically significant ($p>0.05$). The level of BOP % in preterm-delivery pregnant groups having gingivitis was higher than in the full-term delivery group having gingivitis and the healthy pregnant control group, with significant differences ($p<0.05$). The mean±SE was (0.19±0.019), (0.17±0.016), and (0.09±0.028), respectively. The mean±SE value of IL-6 level in pre-term delivery was higher than in full-term-delivery pregnant groups having gingivitis compared with healthy pregnant control group, with significant differences ($p<0.05$). The mean±SE was (45.2±7.90), (22.2±9.64), and (7.7±0.75), respectively. Likewise, the mean±SE IL-8 levels in the pre-term delivery group were higher than the full-term-delivery groups with gingivitis and the control group, (128.5±11.72), (115.1±0.93) and (13.9±1.44) respectively, with significant differences ($p<0.05$).

**Table (1): The mean value of age, BOP%, and the biomarkers in the three groups**

| Variable | Group | | | |
|---|---|---|---|---|
| | Full term Mean±SE | Preterm Mean±SE | Controls Mean±SE | ANOVA test (*p. value*) |
| Age (year) | 25.07±1.097 | 26.12±1.400 | 27.73±1.250 | F =0.553 0.296 |
| Bleeding on probing (BOP)% | 0.17±0.016 | 0.19±0.019 | 0.09±0.028 | F =5.751 0.005 |
| Interlukine-6 (ng/ml) | 22.2±9.64 | 45.2±7.90 | 7.7±0.75 | F =7.950 0.001 |
| Interlukine-8 (ng/ml) | 115.1±0.93 | 128.5±11.72 | 13.9±1.44 | F =77.750 0.0001 |

Table 2 reveals the correlation between IL-6 and IL-8 in Full-term and Preterm delivery having gingivitis groups. There was a negative non-significant correlation between IL-6 and IL-8 in both groups ($r=-0.015$), ($r= -0.072$), ($p>0.05$).

**Table (2): Correlation between IL-6 and IL-8 in the gingivitis study groups**

| Biomarkers | Full term | | Pre term | |
|---|---|---|---|---|
| | R | p | r | p |
| IL-6 and IL-8 | -0.015 Ns | 0.943 | -0.072 Ns | 0.706 |

r= Pearson correlation, Ns= non-significant ($p>0.05$)

**Discussion**

The present study showed the percentage of BOP % in preterm-delivery pregnant groups having gingivitis was significantly higher than in the full-term delivery group having gingivitis compared with the healthy pregnant group. This indicates that the pregnant women in this study had suffered the bad outcomes of gingivitis.

The significantly higher level of interleukin-6 in preterm-delivery than full-term delivery pregnant groups having gingivitis compared with the healthy pregnant woman group suggests that the local generation of these cytokines in the periodontal pocket due to periodontal disease may raise blood concentration and amniotic fluid concentration (19,20). Several prior studies back up these findings, showing that any infection that causes chorioamnionitis and leads to higher cytokine levels in amnionic fluid, particularly high levels of IL-6, may cause preterm rupture of membranes and delivery (20,21). The results are consistent with those of Latorre Uriza et al., who found that cytokines (interleukin-2, IL-6, IL-10, and TNF-α) increased as the periodontal disease progressed from healthy through gingivitis to chronic periodontitis, with the highest levels in pregnant patients (22). Greig et al. and Von Minckwitz *et al*. revealed that periodontal disease patients release proinflammatory cytokines and mediators into the gingival crevicular fluid (GCF) or the bloodstream, reaching the placenta-fetus interface (23). IL-1, IL-6, and TNF-α stimulate chorion prostaglandin synthesis, cervical ripening, and uterine contraction, increasing preterm labor risk (24,25). An earlier study by Van Dyke et al. agrees with the present investigation, showing that periodontal infection increases systemic inflammatory markers IL-3, IL-4, IL-5, IL-6, IL-10, and TNF-α (26). Even though elevated levels of these mediators in the serum, amniotic fluid, and GCF have been linked to a variety of adverse pregnancy outcomes. Deortbudak *et al*. and Madianos *et al*. found no evidence that periodontitis patients with elevated mediators in GCF, serum, or amniotic fluid had pregnancy difficulties (27,28). Our findings showed a noticeable increase in the IL-8 levels in pre-term delivery compared with full-term-delivery pregnant groups having gingivitis and the control group. This is a line with a previous study by Hebisch *et al*. who demonstrated that periodontal infections linked with preterm delivery during cervical dilatation increased maternal serum IL-8 and IL-6 levels in serum, amniotic fluid, maternal, and retroplacental blood (29). Although the increased percentage of periodontopathic bacteria in subgingival plaque might have produced the elevation of such cytokines, the causes for the rise were not obvious and should be investigated further (29). Another study, found these cytokines IL-6, IL-8, and TNF-α have a rise during periodontal disease and also chorioamnionitis (30). Furthermore, Early signals to enhance the production of pro-inflammatory molecules such as IL-6 and IL-8, according to Hasegawa *et al*., lead to uterine contraction and cervical ripening, which leads to parturition (21). Monocytes, fibroblasts,

lymphocytes, and endothelial cells release interleukin-8, which draws neutrophils to the infection site. Its pro-inflammatory and neutrophil chemotactic characteristics may cause periodontitis (31,32). Lagdive *et al*. discovered higher IL-8 levels in gingival tissue, which has been linked to progressive periodontitis (15). Actually, pregnancy increases gingival edoema and bleeding. Pregnancy-induced high oestrogen levels increase gingival inflammation and gram-negative anaerobe growth., particularly *Prevotella nigrescens* (33,34). Nevertheless, the oestrogen hormone inhibits neutrophilic chemotaxis as well as enzyme activity in periodontal tissues (33, 35).

Periodontal tissues upregulate endothelium adhesion molecules, enhance chemotactic agent secretion, and exacerbate leukocyte chemotaxis in response to bacterial infection, secreting IL-8 and macrophage chemoattractant peptide-1 to reduce estrogen concentrations (36-39).

**Conclusion**

The findings of this study suggest a potential association between maternal oral health (gingivitis) and adverse pregnancy outcomes (premature delivery) mediated by elevated levels of proinflammatory cytokines (Interleukin-6 and Interleukin-8).

**Authors' declaration:**

Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (the college's in-house ethics committee of Dentistry, University of Baghdad (Project No.).) according to the code number (713822).

**Author contributions:**

Study conception & design: (Ghada I.Taha). Literature search: (Shaden H. Maddah). Data acquisition: (Shaden H. Maddah). Data analysis & interpretation: (Shaden H. Maddah & Ghada I.Taha). Manuscript preparation: (Shaden H. Maddah & Ghada I.Taha). Manuscript editing & review: (Ghada I.Taha).

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# التغييرات في مستويات IL-6 و IL-8 في مصل النساء الحوامل اللواتي ولدن قبل الأوان المصابات بالتهاب اللثة

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**الخلاصة**

**الخلفية** : أمراض اللثة هي عدوى مهمة عند النساء الحوامل، قد تعمل كعامل خطر للولادة المبكرة عن طريق زيادة الاستجابات الالتهابية الموضعية والجهازية.

**المواد والطرق**: تم تقسيم إجمالي 85 امرأة حامل إلى ثلاث مجموعات. 25 من الولادات قبل الأوان و 30 من الولادات الكاملة ومصابات بالتهاب اللثة، بالإضافة إلى 30 من النساء الحوامل اللواتي يتمتعن بصحة جيدة مع اللثة السليمة كمجموعة ضابطة. كان متوسط عمر المرضى (25.07 ± 1.097)، (26.12 ± 1.400) سنة، و (27.73 ± 1.250) سنة للمجموعة الضابطة. تم جمع عينة الدم من جميع المشاركين وتم استكشاف المؤشرات الحيوية السيتوكينات Interleukine-6 و Interleukin-8 بواسطة مجموعات مقايسة الممتز المناعي المرتبط بالإنزيم.(ELISA)

**النتائج:** أظهرت الدراسة الحالية زيادة أعلى في مستويات IL-6 و IL-8 في الولادة المبكرة المصابة بمجموعة التهاب اللثة تليها مجموعة الولادات الكاملة والتي تعاني من التهاب اللثة مقارنة بمجموعة التحكم، مع وجود فروق ذات دلالة إحصائية عالية.($p < 0.01$)

**الخاتمة**: كانت النساء الحوامل المصابات بالتهاب اللثة مع أعلى مستويات إنترلوكين -6 وإنترلوكين -8 أكثر عرضة للولادة المبكرة مقارنة بالنساء اللائي لديهن مستويات منخفضة من الإنترلوكين وأولئك اللواتي لا يعانين من التهاب اللثة.

**الكلمات المفتاحية**: التهاب اللثة، أمراض اللثة، الولادة المبكرة، الولادة الكاملة، إنترلوكين 6، إنترلوكين 8