

# Gingival Crevicular Fluid and Placental Tissue Levels of Interleukin-17 as a Possible Marker for Preterm Labor in Patients with Chronic Periodontitis



Ahmed Y. Gamal,<sup>a</sup> Mohamed Agoor,<sup>a</sup> Olfat G. Shaker,<sup>b</sup> Fatma H. El-Demerdash,<sup>a</sup> and Mohamed Samir Sweed<sup>c</sup>

<sup>a</sup>Department of Periodontology, Faculty of Dental Medicine, Ain Shams University, Cairo, Egypt.

<sup>b</sup>Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt.

<sup>c</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

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## Abstract

**Background:** The present study was conducted to evaluate the levels of interleukin (IL) – 17 in gingival crevicular fluid (GCF) and placental tissue samples of pregnant females as a possible marker in determining whether or not an association exists between chronic periodontitis and preterm labor.

**Methods:** This case-control study included a random sample of 40 female patients, aged 18 to 35 years, who were assigned to one of the following four groups (10 subjects each): **group 1** included patients who underwent spontaneous preterm birth (PB) and were diagnosed with chronic periodontitis upon clinical examination (preterm/periodontitis); **group 2** included patients who underwent spontaneous PB and who had a healthy periodontium upon clinical examination (preterm/healthy periodontium); **group 3** included patients who underwent spontaneous normal term birth and were diagnosed with chronic periodontitis upon clinical examination (term/periodontitis); and **group 4** included patients who underwent spontaneous normal term birth and who had a healthy periodontium upon clinical examination (term/healthy periodontium). GCF and placental tissue samples were obtained from each patient and IL-17 levels were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** GCF levels of IL-17 were significantly higher ( $P=0.010$ ) in patients with chronic periodontitis compared to those with a healthy periodontium. No significant differences were observed in IL-17 levels in placental tissue samples of all study groups. **Conclusion:** An association between chronic periodontitis and preterm labor could not be established based on IL-17 levels measured in the present study.

**Key Words:** Preterm labor; periodontal medicine; periodontitis; interleukin-17; gingival crevicular fluid.

## Introduction

Pregnancy is normally a healthy physiological process that sometimes has adverse outcomes including low birth weight (LBW) (<2500 grams) and preterm birth (PB) (<37 weeks). PB is now the second most common cause of death worldwide in children younger than five years after pneumonia.<sup>1</sup> As normal pregnancy progresses, amniotic fluid levels of prostaglandin E2 (PGE2) and inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) rise

steadily until a critical threshold is reached to induce rupture of the amniotic sac membranes, uterine contraction, cervical dilation, and finally delivery.<sup>2</sup> Thus, normal parturition is controlled by inflammatory signaling and this process represents a triggering mechanism that can be modified by external stimuli including infection and inflammatory stressors.<sup>3</sup>

Two major pathways have been proposed to trigger an inflammatory/immune response and/or suppression of local growth factors in the fetal-placental unit. The direct pathway suggests that oral microorganisms

and/or their components reach the fetal-placental unit via hematogenous dissemination from the oral cavity, or via the genitourinary tract. The indirect pathway suggests that inflammatory mediators locally produced in periodontal tissues, for example PGE2 and TNF- $\alpha$ , circulate and impact the fetal-placental unit, or inflammatory mediators and/or microbial components circulate to the liver, enhancing cytokine production (e.g. IL-6) and acute phase response (e.g. C-reactive protein), which then impact the fetal-placental unit.<sup>1,3</sup> Numerous studies have associated an increase in the levels of local and systemic markers of inflammation with adverse pregnancy outcomes (APOs). Elevated levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , PGE2, fibronectin, and  $\alpha$ -fetoprotein in the amniotic fluid have been associated with PB.<sup>4</sup> Increased maternal serum levels of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF- $\alpha$ , have also been reported to be associated with prematurity or LBW.<sup>5</sup> Moreover, C-reactive protein (CRP), which is an acute phase reactant synthesized by the liver in response to pro-inflammatory cytokines, and hence a marker of systemic inflammation, was also reported to be associated with PB.<sup>6</sup>

Periodontal diseases are a group of infectious/inflammatory diseases involving Gram negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area and cause local and systemic elevations of pro-inflammatory prostaglandins and cytokines, resulting in tissue destruction.<sup>3,7</sup> During pregnancy, due to physiological hormonal changes, there is a systematic inclination to periodontal disease. There is a global rise in anaerobic Gram-negative agents in particular, such as *Fusobacterium nucleatum*, *Treponema denticola*, *Tannerella forsythia*, and *Campylobacter rectus*.<sup>8</sup> Moreover, especially during pregnancy, the hormonal changes due to elevated levels of estrogens and progesterone increase vascular permeability in the gingival tissues and, as a consequence, bacteria and/or their products can more readily diffuse through the tissues. Evidence indicates that periodontal pathogens/byproducts may reach the fetal-placental unit.<sup>3</sup> In this context, Madianos et al. found that maternal serum immunoglobulin G (IgG) specific to oral organisms was associated with decreased PB and increased birth weight. In the presence of maternal oral organisms, the lack of a protective maternal IgG response could increase fetal exposure which may in turn contribute to a fetal immune response that could lead to PB.<sup>9</sup> *Porphyromonas gingivalis* and

*Aggregatibacter actinomycetemcomitans*, both established periodontal pathogens, have also been found in the placenta of women with PB or pre-eclampsia.<sup>10</sup>

In periodontally affected patients, there is an increase in the production of pro-inflammatory cytokines and mediators from periodontal tissues. Once released, they may diffuse in the gingival crevicular fluid (GCF) or enter the blood circulation and reach the placenta-fetus interface. The production of prostaglandins could be stimulated by IL-1, IL-6, and TNF- $\alpha$  in the chorion, and in conjunction with the maternally derived PGE2 generated at the gingival level and released in the circulation, may exacerbate cervical ripening and uterine contraction leading to an increased risk for PB.<sup>3</sup>

Interleukin (IL) – 17 is an important pro-inflammatory cytokine which induces the expression of many mediators of inflammation and is critical to host defense. It has been reported that amniotic IL-17 levels were significantly higher in cases of preterm delivery compared to term delivery. In humans, IL-17 was identified first in CD4<sup>+</sup>T cells followed by CD8<sup>+</sup>T cells,  $\gamma\delta$  T cells, and monocytes. This cytokine has been linked to various chronic inflammatory conditions including: periodontitis, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and cancer. IL-17 binds to its receptor, IL-17 receptor A (IL-17 RA), and signals are transmitted through nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and the mitogen-activated protein kinase (MAPK).<sup>11</sup> IL-17 has been reported to up-regulate IL-1 $\beta$ , TNF- $\alpha$ , and the tissue destructive matrix metalloproteinases (MMP) – 1 and 3 in periodontal disease. It also induces the production IL-6 and IL-8 from gingival fibroblasts.<sup>12</sup> An increase has been reported in the amount of IL-17 in GCF samples and in the culture supernatants of gingival cells in periodontal disease.<sup>13</sup>

Although a large number of epidemiological and intervention studies demonstrate a positive association between periodontal disease and APOs, the results are not always consistent.<sup>14</sup> Therefore, the present study will be conducted to investigate the correlation between periodontal disease and PB by measuring the levels of IL-17, a highly sensitive novel inflammatory marker, in both GCF and placental tissues samples.

## **Subjects and Methods**

## I - Patient Selection

This case-control study was conducted at the Maternal-Fetal Medical Clinic at the Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Forty eligible female patients aged 18 to 35 years (mean: 25.95 years) were consecutively recruited to this study from December 2014 – March 2015. Inclusion criteria included: 1) patients free of any systemic diseases as referenced from Burket's Oral Medicine health history questionnaire;<sup>15</sup> 2) spontaneous normal term birth at  $\geq 37$  weeks of gestation without any obstetrical or medical complications; 3) spontaneous PB at  $< 37$  weeks of gestation after uterine contraction or rupture of membranes.<sup>16</sup> Exclusion criteria included: 1) history of medications that may affect the study outcome, such as: current use of systemic

corticosteroids or antibiotics for at least one month; 2) genital and urinary tract infections; 3) existing hypertension and diabetes mellitus before pregnancy, autoimmune disease, asthma, and chronic renal disease; 4) multiple pregnancies, cervical cerclage, abnormal placentation, past history of preterm delivery, or antepartum hemorrhage; and 5) low or high maternal body mass index. All procedures performed in the present study were in accordance with the protocol approved by the Ain Shams University Research Ethics Committee. The nature and aim of the study was explained to each individual patient, and both verbal and written consents were obtained from all of the participants. All of the obtained information was treated with confidentiality. The study protocol was registered and identified as NCT03171675 at the U.S. National Institutes of Health Clinical Trials Registry.

**Table 1.** Descriptive statistics and test of significance for age, gestation duration, and parity of all groups

Parameter	Group	Mean	SD	Min.	Max.	dt
Age (Years)	1	28.40	4.648	21	33	a
	2	24.50	4.503	18	35	a
	3	26.40	4.115	21	31	a
	4	24.50	4.905	18	32	a
Gestation Duration (Weeks)	1	34.20	2.845	28.714	36.857	b
	2	33.20	4.188	23.857	36.714	b
	3	39.314	1.449	37.714	41.286	a
	4	38.457	0.796	37.429	39.857	a
Parity	1	1.60	1.174	0	3	a
	2	1.00	0.943	0	3	a
	3	1.70	1.252	1	5	a
	4	1.30	0.823	0	3	a

**SD** = Standard deviation; **Min.** = Minimum value; **Max.** = Maximum value; **dt** = Duncan's Multiple Range Test for the effect of group; Means with the same letter within each parameter are not significantly different at  $p=0.05$ .

## II - Clinical Evaluation and Patient Grouping

Patients who met the eligibility criteria received full-mouth periodontal examinations at the maternity ward within 24 hours postpartum. Full mouth baseline periodontal parameters were recorded for each patient, which included: **plaque index (PI)**,<sup>17</sup> **gingival index (GI)**,<sup>18</sup> **probing depth (PD)**,<sup>19</sup> and **clinical attachment**

**level (CAL)**.<sup>19</sup> All parameters were examined using a University of Michigan 'O' Probe with William's markings. Patients with  $\geq 30\%$  full arch probing depth of  $> 3$  mm, who demonstrated periodontal clinical attachment loss, and who had a gingival index of 1-3, were diagnosed with chronic periodontitis.<sup>20</sup> Subjects with a probing depth of  $\leq 3$  mm, who lacked periodontal clinical attachment loss, and who

had a gingival index of 0-0.5, were deemed to have a healthy periodontium.

All participants were assigned to one of the following four groups (10 patients each): **group 1** included patients who underwent spontaneous PB and were diagnosed with chronic periodontitis upon clinical examination (preterm/periodontitis); **group 2** included patients who underwent spontaneous PB and

who had a healthy periodontium upon clinical examination (preterm/healthy periodontium); **group 3** included patients who underwent spontaneous normal term birth and were diagnosed with chronic periodontitis upon clinical examination (term/periodontitis); and **group 4** included patients who underwent spontaneous normal term birth and who had a healthy periodontium upon clinical examination (term/healthy periodontium).

**Table 2.** Descriptive statistics and test of significance for the effect of group on different clinical periodontal parameters

Clinical Periodontal Parameters	Group	Mean	SD	Min.	Max.	dt
Plaque Index (PI)	1	2.6	0.516	2	3	a
	2	0.2	0.422	0	1	c
	3	1.9	0.316	1	2	b
	4	0.4	0.516	0	1	c
Gingival Index (GI)	1	2.0	0.471	1	3	a
	2	0.0	0.000	0	0	b
	3	2.0	0.000	2	2	a
	4	0.0	0.000	0	0	b
Probing Depth (PD)	1	4.1	0.316	4	5	a
	2	2.0	0.667	1	3	b
	3	4.2	0.422	4	5	a
	4	2.2	0.632	1	3	b
Clinical Attachment Level (CAL)	1	1.2	0.422	1	2	a
	2	0.0	0.000	0	0	b
	3	1.3	0.483	1	2	a
	4	0.0	0.000	0	0	b

**SD** = Standard deviation; **Min.** = Minimum value; **Max.** = Maximum value; **dt** = Duncan's Multiple Range Test for the effect of group; Means with the same letter within each measurement are not significantly different at  $p=0.05$ .

### III - Sample Collection

GCF and placental tissue samples were obtained from each subject. Sterile absorbing paper strips<sup>d</sup> were used to obtain GCF samples. For patients diagnosed with chronic periodontitis, a single sample was taken from the site possessing the highest PD along with the most clinical attachment loss; while for patients exhibiting a healthy periodontium, a random site was used to obtain the sample. Cotton gauze sponges were used to isolate the teeth being sampled and the absorbing paper strips were

gently placed into the gingival sulcus of the collection site until mild resistance was encountered, and were left in place for approximately 15 seconds.<sup>21</sup> Each sample was placed into a cooled Eppendorf tube and stored at  $-80^{\circ}\text{C}$  for future use. Using a scalpel, a small piece of villous placental tissue (approximately  $1\text{ cm}^3$ ) was dissected free of the maternal decidua from the placental bed after delivery. Each sample was placed into a cooled Eppendorf tube and stored at  $-80^{\circ}\text{C}$  for future use.

<sup>d</sup> Periopaper™, Oraflow, Smithtown, NY, USA 11787

Enzyme-linked immunosorbent assay (ELISA)<sup>e</sup> was used to assess the level of IL-17 in each sample. The unopened kit was stored at approximately 5 °C. This immunoassay is a 4.5 hour solid phase ELISA built to measure human IL-17 in cell culture supernates, serum, and plasma. It is comprised of *E. coli*-expressed human IL-17 and antibodies raised against the recombinant factor.

The duplicate readings for each standard, control, and sample were averaged, and the average zero standard optical density (O.D.) was subtracted. A standard curve was created by reducing the data using computer software capable of producing log/log curve-fit.

#### IV - Statistical Analysis

The primary objective of the present study was to evaluate the levels of IL-17 in GCF and placental tissue samples of both healthy and periodontally affected females who underwent PB. The secondary objective was to correlate clinical parameters with IL-17 levels in GCF and placental tissue samples. A power analysis was designed to have adequate power to apply a 2-sided statistical test of the research hypothesis (Null hypothesis) that there was no difference between the two groups (chronic periodontitis and healthy periodontium). According to the results of Vernal et al. (2005) and Beklen et al. (2007), and using a significance level ( $\alpha$ ) of 0.05 and a power level ( $\beta$ ) of 0.80, the predicted minimum sample size ( $n$ ) was a total of 4 cases i.e. 2 cases in each group (chronic periodontitis and healthy periodontium). Oversampling was done to overcome any suspected samples insufficiency. Sample size calculation was performed using the G\*Power software (Version 3.1.9.2).

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) program (Release 17.0.0). Descriptive statistics was calculated using Descriptive Statistics of SPSS (SPSS, analyze, Descriptive Statistics). Kolmogorov-Smirnov test of normality (SPSS, analyze, descriptive statistics, explore, plots, normality plots with tests) was run to test the normality of IL-17 in different groups. One way analysis of variance (SPSS, analyze, compare means, one way ANOVA) was used to test the effect of group on different measurements. Duncan Post-Hoc Multiple Comparisons (Post-Hoc) at  $p \leq 0.05$  was used for comparison of means. Student t test (SPSS,

analyze, compare means, independent-samples t test) was used to test the effect of periodontal status and gestation duration on mean IL-17 levels in GCF and placental tissue samples. Spearman correlation (SPSS, analyze, correlate, bivariate) was used to test the correlation between mean IL-17 levels in GCF and placental tissue samples, gestation duration, and clinical periodontal parameters of all groups.

#### Results

No statistically significant differences were recorded in the periodontal parameters of the preterm and term/healthy periodontium groups. Similarly, the preterm/periodontitis group demonstrated insignificant differences compared to the term/periodontitis group, at  $p \leq 0.05$ , regarding the mean GI, PD, and CAL. The preterm/periodontitis group had a significantly higher mean PI compared to term/periodontitis study group (Table 2).

Regarding the mean IL-17 levels in the GCF samples, a statistically significant difference was found between the term/periodontitis and term/healthy periodontium groups, with values at  $55.92 \pm 15.54$  pg/ml and  $42.94 \pm 8.25$  pg/ml respectively ( $p < 0.05$ ). A statistically significant difference between the preterm/healthy periodontium and the term/periodontitis groups ( $p < 0.05$ ) was also recorded, while the preterm/periodontitis group demonstrated an insignificant difference when compared to the rest of the study groups at  $p = 0.05$  (Table 3). The chronic periodontitis and the healthy periodontium study groups demonstrated a statistically significant difference regarding the mean IL-17 levels in the GCF samples ( $p = 0.01$ ) (Table 4). However, no statistically significant difference was recorded for the preterm delivery and the term delivery groups ( $p = 0.123$ ) (Table 5).

Regarding the mean IL-17 levels in the placental tissue samples, no statistically significant differences were demonstrated between all study groups at  $p = 0.05$ . The highest level was found in the preterm/periodontitis study group, followed by the preterm/healthy periodontium study group, while the term/periodontitis study group demonstrated the lowest level (Table 3). No statistically significant difference was demonstrated between the chronic periodontitis and the healthy periodontium study groups regarding the mean

<sup>e</sup> Quantikine® ELISA, R&D Systems, Human IL-17

IL-17 levels in the placental tissue samples ( $p=0.550$ ) (Table 4). Comparing the preterm delivery with the term delivery groups, no statistically significant difference was recorded ( $p=0.234$ ) (Table 5).

The term/periodontitis group demonstrated a positive, statistically significant correlation between the mean IL-17 level in

placental tissue samples and the mean CAL at the 0.01 level. No other statistically significant correlations were recorded for IL-17 levels in GCF and placental tissue samples, gestation duration, and clinical periodontal parameters of all groups. No statistically significant correlations were observed between IL-17 levels in GCF samples and placental tissue samples of all study groups (Table 6).

**Table 3.** Descriptive statistics and test of significance for the effect of group on mean IL-17 levels in GCF and placental tissue samples

Measurement	Group	Mean	SD	Min.	Max.	dt
IL-17 in GCF (pg/ml)	1	47.092	15.969	23.86	66.14	ab
	2	38.264	8.415	25.42	51.88	b
	3	55.920	15.537	20.78	75.78	a
	4	42.938	8.250	28.50	55.04	b
IL-17 in Placental Tissue (pg/g)	1	136.520	30.824	98.30	197.50	a
	2	124.985	16.266	94.40	145.50	a
	3	120.035	33.877	59.65	169.35	a
	4	121.470	21.116	90.55	165.35	a

**SD** = Standard deviation; **Min.** = Minimum value; **Max.** = Maximum value; **dt** = Duncan's Multiple Range Test for the effect of group; Means with the same letter within each measurement are not significantly different at  $p=0.05$ .

**Table 4.** Effect of periodontal status on mean IL-17 levels in GCF and placental tissue samples

Measurement	Chronic Periodontitis Groups (Groups 1 & 3)		Healthy Periodontium Groups (Groups 2 & 4)		P Value
	Mean	SD	Mean	SD	
IL-17 in GCF (pg/ml)	51.506	15.989	40.601	8.457	0.010*
IL-17 in Placental Tissue (pg/g)	128.278	32.637	123.228	18.433	0.550 NS

**SD** = Standard deviation; **P** = Probability level for the effect of periodontal status (Student t test); **NS** = Insignificant ( $p>0.05$ ); \* = Significant at  $p\leq0.01$ .

**Table 5.** Effect of gestation duration on mean IL-17 levels in GCF and placental tissue samples

Measurement	Preterm Delivery Groups (Groups 1 & 2)		Term Delivery Groups (Groups 3 & 4)		P Value
	Mean	SD	Mean	SD	
IL-17 in GCF (pg/ml)	42.678	13.223	49.429	13.818	0.123 NS
IL-17 in Placental Tissue (pg/g)	130.753	24.706	120.753	27.484	0.234 NS

**SD** = Standard deviation; **P** = Probability level for the effect of gestation duration (Student t test); **NS** = Insignificant ( $p>0.05$ ).

**Table 6.** Spearman ranked correlation coefficients between mean IL-17 levels in GCF and placental tissue samples, gestation duration, and clinical periodontal parameters of all groups

Correlations		Group 1	Group 2	Group 3	Group 4
GCF IL-17 & GD	r	.267	-.259	-.488	-.110
	p	.455	.470	.153	.763
GCF IL-17 & PI	r	.178	.576	-.175	.036
	p	.622	.087	.629	.922
GCF IL-17 & GI	r	-.273	.	.	.
	p	.445	.	.	.
GCF IL-17 & PD	r	.058	-.276	.000	-.296
	p	.873	.440	1.000	.407
GCF IL-17 & CAL	r	-.175	.	.229	.
	p	.629	.	.525	.
GCF IL-17 & PT IL-17	r	.373	-.277	.229	.488
	p	.288	.438	.525	.153
PT IL-17 & GD	r	-.104	.195	.268	-.537
	p	.776	.589	.453	.110
PT IL-17 & PI	r	-.107	-.175	.218	-.428
	p	.768	.629	.545	.218
PT IL-17 & GI	r	.196	.	.	.
	p	.588	.	.	.
PT IL-17 & PD	r	.350	.248	.218	-.452
	p	.321	.489	.545	.190
PT IL-17 & CAL	r	.525	.	1.000*	.
	p	.119	.	.	.
GD & PI	r	.569	.218	.468	.606
	p	.086	.545	.172	.063
GD & GI	r	.156	.	.	.
	p	.668	.	.	.
GD & PD	r	.406	.469	.571	.379
	p	.244	.171	.085	.280
GD & CAL	r	.261	.	.268	.
	p	.466	.	.453	.

r = Correlation coefficient; p = Probability level; GD = Gestation duration; PT = Placental tissue; \* Correlation is significant at the 0.01 level.

## Discussion

Serious health problems are associated with preterm low birth weight (PLBW) infants including, neurodevelopment disturbances, ear infections, respiratory infections, asthma, and death. Premature birth causes 10% of neonatal mortality worldwide.<sup>22</sup> Despite immense medical advances, the rate of PB has not declined in the United States over the past several decades. The rate actually rose to more than 12% of all births in the year 2003. Thus, identifying risk factors for PB, especially those that can be controlled or treated, would certainly have far-reaching and long-lasting effects.<sup>23</sup>

Although it has been asserted that periodontopathogens from oral infection can disseminate to distant body sites and thus result

in the delivery of PLBW infants, results have been inconclusive.<sup>24, 25, 26, 27</sup> Some studies have demonstrated positive correlations, while others have not.<sup>28, 29, 30, 31, 32, 33, 34, 35</sup> Although these results seem contradictory, factors such as race, geographical location, and socioeconomic standards have not been adequately adjusted for. Consequently, studies conducted in the United States and Europe differ from those conducted in Latin America or Africa, mainly due to a lack of adjustment for socio-economic factors, such as accessibility to adequate and affordable health care.<sup>23</sup>

In the present study, IL-17 was chosen as a possible marker for preterm labor in patients with chronic periodontitis for several reasons. Since IL-17 has been found to up-regulate IL-1 $\beta$

and TNF- $\alpha$ , it was proposed that its level increases in periodontitis, leading to an up-regulation of these cytokines and tissue destructive matrix metalloproteinases (MMP) in local migrant and resident cells. Elevated levels of IL-1 $\beta$ , TNF- $\alpha$ , and IL-17 have been found in periodontitis via immunocytochemistry. These cytokines induced proMMP-1, and particularly MMP-3 in gingival fibroblasts. Although IL-17 was less potent as a direct MMP-inducer than IL-1 $\beta$  and TNF- $\alpha$ , it induced the production of IL-1 $\beta$  and TNF- $\alpha$  from macrophages, and IL-6 and IL-8 from gingival fibroblasts. It was reported that gingival fibroblasts may play a significant role in periodontal tissue destruction through cytokine-inducible MMP-1 and MMP-3 production, in which IL-17 acts as an important regulatory cytokine.<sup>12</sup>

Cellular and humoral responses in both healthy individuals and those with periodontal disease have been detected through GCF analysis.<sup>21</sup> In the present study, GCF was used to measure IL-17 levels associated with periodontitis since it is considered an excellent undispersed media for evaluating the released IL-17. Brill and Krasse inserted filter paper into the gingival sulci of dogs that had previously been given intramuscular fluorescein injections. The fluorescent material was absorbed on the paper strips within 3 minutes. This demonstrated that fluid passed from the bloodstream through the tissues, and subsequently into the gingival sulcus. In later studies, Brill verified the presence of GCF in humans and considered it a "transudate."<sup>36, 37, 38, 39</sup> Sterile gingival fluid collection absorbing paper strips were used in the present study to obtain GCF samples due to their simplicity, convenience, and ease of use. The Brill technique, which involves inserting the absorbing paper strip into the sulcus until resistance is encountered, was used to obtain the GCF samples. This technique was chosen because it irritates the sulcular epithelium to some degree, subsequently leading to the flow of fluid.<sup>21</sup>

In the present study, placental tissue samples were obtained from the subjects rather than amniotic fluid samples, due to the invasiveness of amniocentesis. Gram-negative periodontal bacteria and/or their virulence factors (e.g. endotoxins) have been shown to reach the systemic circulation, producing a low-grade bacteremia. This is especially true during pregnancy, during which elevated levels of estrogen and progesterone increase vascular permeability in the gingival tissues, and

consequently, diffusion of bacteria and/or their products occurs more readily. Immunological data has indicated that periodontal pathogens/byproducts may reach the fetal-placental unit. Upon exposure of the mother to bacterial pathogens, an attempt will be made by the host's immune response to contain and resolve the infection. If this is unsuccessful, then bacterial-specific antibodies will be produced by the more efficient adaptive immune response.<sup>3</sup>

Although Keelan et al. reported increased cytokine levels in the gestational membranes of both term and preterm labor patients, signifying an inflammatory process, this process was not evident in the villous placenta. Thus, cytokine concentrations within placental tissues were not affected by labor.<sup>16</sup> His study however lacked an exposure factor, as is the case in the present study, where two of the study groups were diagnosed with chronic periodontitis, a disease which has been associated with increased IL-17 levels.<sup>12, 13</sup>

It has been hypothesized that PB may be precipitated by cytokines produced by the infected periodontium, which may reach the systemic circulation and subsequently target the placenta and fetus.<sup>40</sup> Our results however, suggest that this is not the case given that the IL-17 levels in GCF fluid did in fact increase in chronic periodontitis compared to our healthy periodontium groups, whereas this increase was not demonstrated in the IL-17 levels in placental tissue samples.

The present study demonstrated a statistically significant difference between the term/periodontitis and the term/healthy periodontium groups regarding the mean IL-17 levels in the GCF samples, with higher levels having been detected in the term/periodontitis study group. A statistically significant difference was also found ( $p=0.010$ ) between mean IL-17 levels in the GCF samples of the chronic periodontitis and healthy periodontium study groups, with levels being considerably higher in the chronic periodontitis study groups. These findings conform to those of Beklen et al. and Vernal et al., both of whom reported elevated IL-17 levels in periodontitis.<sup>12, 13</sup> However, no statistically significant differences were found ( $p=0.123$ ) regarding the mean IL-17 levels in the GCF samples of both preterm and term delivery groups. Furthermore, the results demonstrated no statistically significant differences, at  $p=0.05$ , between all study



groups regarding the mean level of IL-17 in placental tissue samples. These findings are consistent with those of Wood et al., Bassani et al., Abati et al., Souccar et al., and other authors who found no association between periodontitis and preterm labor.<sup>32, 33, 34, 35</sup>

Conclusively, we could not establish an association between chronic periodontitis and preterm labor based on the measured IL-17 levels in GCF and placental tissue samples in the present study. Despite limitations, including a small sample size and lack of intervention, this is a pioneer case-control study that can be added to the database of evidence-based research in this particular field of periodontology.

### References

1. Sanz M, Kornman K, and on behalf of working group 3 of the joint EFP/AAP workshop. Periodontitis and adverse pregnancy outcomes: consensus report of the joint EFP/AAP workshop on periodontitis and systemic diseases. *J Periodontol.* 2013;84(4 Suppl.):S164-S169.
2. Haram K, Mortensen JH, Wollen AL. Preterm delivery: an overview. *Acta Obstetrica et Gynecologica Scandinavica.* 2003;82, 687–704.
3. Madianos PN, Bobetsis YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. *J Clin Periodontol.* 2013;40(SUPPL. 14):170-180. doi:10.1111/jcpe.12082.
4. Gürsoy M, Könönen E, Gürsoy UK, Tervahartiala T, Pajukanta R, Sorsa T. Periodontal status and neutrophilic enzyme levels in gingival crevicular fluid during pregnancy and postpartum. *J Periodontol* 2010;81:1790–1796.
5. Hitti J, Tarczy-Hornoch P, Murphy J, Hillier SL, Aura J, Eschenbach D. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. *Obstetrics and Gynecology.* 2001;98:1080–1088.
6. Pitiphat W, Gillman MW, Joshipura KJ, Williams PL, Douglass CW, Rich- Edwards JW. Plasma C-reactive protein in early pregnancy and preterm delivery. *American Journal of Epidemiology.* 2005;162:1108–1113.
7. Smalley JW. Pathogenic mechanisms in periodontal disease. *Adv Dent Res.* 1994;2:320-328.
8. Carta G, Persia G, Falciglia K, Iovenitti P. Periodontal disease and poor obstetrical outcome. *Clinical and Experimental Obstetrics and Gynecology.* 2004;31:47–49.
9. Madianos PN, Lieff S, Murtha AP, et al. Maternal periodontitis and prematurity. Part II: maternal infection and fetal exposure. *Annals of Periodontology.* 2001;6: 175–182.
10. Swati P, Thomas B, Vahab SA, Kapaettu S, Kushtagi P. Simultaneous detection of periodontal pathogens in subgingival plaque and placenta of women with hypertension in pregnancy. *Archives of Gynecology and Obstetrics.* 2012;285:613–619.
11. Ito M, Nakashima A, Hidaka T, et al. A role for IL-17 in induction of an inflammation at the fetomaternal interface in preterm labour. *J Reprod Immunol.* 2010;84(1):75-85. doi:10.1016/j.jri.2009.09.005.
12. Beklen A, Ainola M, Hukkanen M, Gürkan C, Sorsa T, Kontinen YT. MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. *J Dent Res.* 2007;86(4):347-351. doi:10.1177/154405910708600409.
13. Vernal R, Dutzan N, Chaparro A, Puente J, Valenzuela MA, Gamonal J. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. *J Clin Periodontol.* 2005; 32: 383–389. doi: 10.1111/j.1600–051X.2005.00684.x.
14. Polyzos NP, Polyzos IP, Zavos A, et al. Obstetric outcomes after treatment of periodontal disease during pregnancy: systematic review and metaanalysis. *British Medical Journal.* 2010;341, c7017.
15. Glick M, Greenberg MS, Ship JA. Introduction to oral medicine and oral diagnosis: evaluation of the dental patient. In: Greenberg MS, Glick M, Ship JA. *Burket's Oral Medicine.* 11th Edition. Hamilton, Ontario: BC Decker; 2008:3.
16. Keelan JA, Marvin KW, Sato TA, Coleman M, McGowan L, Mitchell DM. Cytokine abundance in placental tissues: evidence of inflammatory activation in gestational

- membranes with term and preterm parturition. *American Journal of Obstetrics and Gynecology*. 1999; 181:1530-1536.
17. Silness J, Løe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica*. 1964;22:121-35.
  18. Løe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica*. 1963;21:533-51.
  19. Glavind L, Løe H. Errors in the clinical assessment of periodontal destruction. *J Pedodont Res*. 1967;2:180-184.
  20. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4:1-6.
  21. Bulkacz J, Carranza FA. Defense mechanisms of the gingiva. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA. *Carranza's Clinical Periodontology*. Twelfth Ed. St. Louis, Missouri : Elsevier; 2015:214-218.
  22. Africa CWJ, Kayitenkore J, Bayingana C. Examination of maternal gingival crevicular fluid for the presence of selected periodontopathogens implicated in the pre-term delivery of low birthweight infants. *Virulence*. 2010;1(4):254-259. doi:10.4161/viru.1.4.12004.
  23. Babalola DA, Omole F. Periodontal disease and pregnancy outcomes. *J Pregnancy*. 2010;2010:293439. doi:10.1155/2010/293439.
  24. Scannapieco FA, Bush RB, Paju S. Periodontal disease as a risk factor for adverse pregnancy outcomes. A systematic review. *Ann Periodontol*. 2003;8(1):70-78. doi:10.1902/annals.2003.8.1.70.
  25. Àgueda A, Echeverría A, Manau C. Association between periodontitis in pregnancy and preterm or low birth weight: review of the literature. *Med Oral Patol Oral Cir Bucal*. 2008;13(9):609-615.
  26. Chambrone L, Guglielmetti MR, Pannuti CM, Chambrone LA. Evidence grade associating periodontitis to preterm birth and/or low birth weight: I. A systematic review of prospective cohort studies. *J Clin Periodontol*. 2011;38(9):795-808. doi:10.1111/j.1600-051X.2011.01755.x.
  27. Stadelmann P, Alessandri R, Eick S, Salvi GE, Surbek D, Sculean A. The potential association between gingival crevicular fluid inflammatory mediators and adverse pregnancy outcomes : a systematic review. *Clin Oral Invest*. 2013;1453-1463. doi:10.1007/s00784-013-0952-0.
  28. Offenbacher S, Boggess KA, Murtha AP, et al. Progressive periodontal disease and risk of very preterm delivery. *Obstet Gynecol*. 2006;107(1):29-36. doi:10.1097/01.AOG.0000190212.87012.96.
  29. Marakoglu I, Gursoy UK, Marakoglu K, Cakmak H, Ataoglu T. Periodontitis as a risk factor for preterm low birth weight. *Yonsei Med J*. 2008;49(2):200-203. doi:10.3349/ymj.2008.49.2.200.
  30. Alves RT, Ribeiro RA. Relationship between maternal periodontal disease and birth of preterm low weight babies. *Braz Oral Res*. 2006;20(4):318-323.
  31. Agueda A, Ramón JM, Manau C, Guerrero A, Echeverría JJ. Periodontal disease as a risk factor for adverse pregnancy outcomes: a prospective cohort study. *J Clin Periodontol*. 2008;35(1):16-22. doi:10.1111/j.1600-051X.2007.01166.x.
  32. Wood S, Frydman A, Cox S, et al. Periodontal disease and spontaneous preterm birth: a case control study. *BMC Pregnancy and Childbirth*. 2006;6:24. doi:10.1186/1471-2393-6-24.
  33. Bassani DG, Olinto MTA, Kreiger N. Periodontal disease and perinatal outcomes: a case-control study. *J Clin Periodontol*. 2007;34(1):31-39. doi:10.1111/j.1600-051X.2006.01012.x.
  34. Abati S, Villa A, Cetin I, et al. Lack of association between maternal periodontal status and adverse pregnancy outcomes: a multicentric epidemiologic study. *J Matern Neonatal Med*. 2013;26(4):1-4. doi:10.3109/14767058.2012.733776.
  35. Souccar NM, Chakhtoura M, Ghafari JG, Abdelnoor AM. Porphyromonas gingivalis in dental plaque and serum C-reactive protein levels in pregnancy. *J Infect Dev Ctries*. 2010;4(6):362-366.
  36. Brill N, Krasse B. The passage of tissue fluid into the clinically healthy gingival pocket. *Acta Odontol Scand*. 1958;16:223.
  37. Brill N. Effect of chewing on flow of tissue fluid into gingival pockets. *Acta Odontol Scand*. 1959;17:277.
  38. Brill N, Bronnestam R. Immunoelectrophoretic study of tissue fluid from gingival pockets. *Acta Odontol Scand*. 1960;18:95.

39. Brill N. The gingival pocket fluid: studies of its occurrence, composition and effect. *Acta Odontol Scand.* 1969;20 (Suppl 32):159.
40. Goepfert AR, Jeffcoat MK, Andrews WW, et al. Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. *Obstet Gynecol.* 2004;104(4):777-783.  
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**Corresponding author:**

Ahmed Y. Gamal  
Professor of Periodontology  
Department of Periodontology  
Faculty of Dental Medicine, Ain Shams University  
Cairo, Egypt  
[hgamal1@hotmail.com](mailto:hgamal1@hotmail.com)

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