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Original Article

The Influence of the Menstrual Cycle on Inflammatory Markers: the Cytokines Il-1β, IL-6, and TNF-α in the Gingival Crevicular Fluid

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Abstract

Hormonal fluctuations, primarily in progesterone and estrogen during the menstrual cycle, may influence periodontal tissues, with inflammatory cytokines playing a crucial role. Therefore, our primary objective was to assess clinical periodontal parameters and measure levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in gingival crevicular fluid (GCF) throughout the menstrual cycle. This longitudinal prospective study was conducted from February to April 2022 and included 50 participants. We assessed clinical periodontal parameters—plaque index (PI), gingival index (GI), pocket depth (PD), clinical attachment loss (CAL), and tooth mobility—at three stages of the menstrual cycle: menstruation day, ovulation day, and premenstrual day. Additionally, GCF samples were collected using paper points. These samples were then stored and analyzed for levels of IL-1β, IL-6, and TNF-α using enzyme-linked immunosorbent assays. There were 25 participants in our study. The GI, PD, and CAL increased significantly during the menstrual cycle and were significantly higher during the premenstrual phase than in the ovulation phase (P<0.05). The levels of GCF IL-1β (P=0.012), IL-6 (P=0.002), and TNF-α (P=0.015) showed statistically significant throughout the menstrual cycle compared to baseline which was the menstrual (follicular) phase. Furthermore, the GCF levels of IL-1β and IL-6 reached their peak during the luteal or premenstrual phase, whereas TNF-α peaked during the ovulation phase. The increase in biological markers was more pronounced between the menstruation phases than the clinical periodontal markers. All clinical periodontal parameters, except for the PI, showed a slight increase from the follicular phase to the luteal phase, with significant differences observed between each phase. The levels of GCF IL-1β (P=0.012), IL-6 (P=0.002), and TNF-α (P=0.015) were statistically significant, with increases in IL-1β and IL-6 throughout the menstrual cycle, peaking in the luteal phase. This demonstrates the influence of the menstrual cycle on clinical periodontal and GCF inflammatory markers.

Key words: Periodontal disease, Menstrual cycle, Interleukin-1 beta, Interleukin-6, Tumor necrosis factor-alpha, Gingival crevicular fluid

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Introduction

Gingivitis and periodontitis are 2 forms of periodontal disease, a chronic oral infection that affects the tissues supporting and surrounding the teeth. If left untreated, gingivitis—an inflammation of the gingival tissue—can progress to periodontitis,

which destroys the structures that support the teeth, leading to tooth loosening and eventual loss [1, 2]. The primary cause of this disease is the buildup of plaque, which consists of anaerobic gram-negative bacteria [3]. Periodontal disease is a leading cause of tooth loss, significantly affecting quality of life, mastication, appearance, and self-confidence.

The global burden of periodontal disease increased by 57.3% between 1990 and 2010. Additionally, periodontitis affects 23% of women aged 23 to 54. Factors such as smoking, poor hygiene, diabetes, medications, age, and other risk factors contribute to the prevalence, progression, and severity of periodontal disease. Conversely, sex hormones have been identified as significant modifiable factors that may influence the pathogenesis of periodontal disease. These hormones exert substantial biological effects that can impact various organ systems, including the oral cavity [2]. A woman's reproductive system undergoes regular cyclic changes, which are considered periodic preparations for pregnancy and fertilization. During the menstrual cycle, fluctuations in hormones, specifically estrogen and progesterone, occur, with the most significant impact on the periodontium observed during the second phase.

Much of the research on hormone behavior in the periodontium has focused on the effects of androgens, estrogens, and progestins on the gingiva [1]. Consequently, our understanding of the specific actions of sex steroid hormones in the periodontal ligament, cementum, and alveolar bone remains limited. From the mid- to late-twentieth century, there was a surge in clinical research exploring the etiology of sex steroid hormone-induced inflammatory changes in the periodontium [1]. Studies have also documented the effects of estrogen and progesterone on periodontal tissues, saliva, oral mucosa, wound healing, periodontal disease progression, and bone health [4]. Additionally, fluctuations in estrogen and progesterone levels during the menstrual cycle have been shown to influence gingival inflammation and clinical changes during pregnancy [5]. However, to fully understand the impact of hormones on the periodontium, it is crucial to first elucidate the actions and interactions of various hormones with immune cells and the resident cell populations within specific tissues [6]. The mechanisms by which androgens, estrogens, and progesterone interact with immune cells and periodontal resident cells are still largely unknown.

Several studies have assessed periodontal clinical parameters and cytokine levels in gingival crevicular fluid (GCF) throughout the menstrual cycle [4, 7-9].

Thus, the goal of our study was to describe the effects of the menstrual cycle on the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in the periodontium of young women.

Materials and Methods

We conducted a longitudinal prospective study from February 2022 to April 2022. Our study was conducted in the Implantology Laboratory at the Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. Here, participants were received, consulted, and samples were collected. The samples were then stored and analyzed at the Biochemistry Laboratory of the Yaoundé University Teaching Hospital.

The study population consisted of young women aged 18 to 35 years who had regular menstrual cycles of 28±3 days, were available for clinical examinations, did not smoke, had no systemic diseases, and possessed a healthy periodontium. We carried out consecutive and convenience sampling. The use of oral contraceptives, participant withdrawal, and recent antibiotic therapy or non-steroidal anti-inflammatory drug use within the past 6 months were considered exclusion criteria.

Participants underwent full mouth scaling and received oral hygiene instructions prior to the study, with their plaque index (PI) scores recorded over one month. Additionally, the duration and regularity of their menstrual cycles were monitored during the same period. GCF sample collection was conducted by the principal investigator in the morning. After the participants rinsed their mouths, the sample collection area was isolated using cotton rolls and dried with a gentle stream of air. Paper points were then placed in the gingival sulcus of the teeth for 60 seconds. GCF samples were collected during three phases of the menstrual cycle: the follicular phase (FP) on days 2–7, ovulation phase (OP) on days 13–15 (confirmed using an ovulation test), and the luteal phase (LP) on days 22–24. These samples were then analyzed using the sandwich enzyme-linked immunosorbent assay (ELISA) technique.

Clinical periodontal parameters, including the PI, gingival index (GI), pocket depth (PD), clinical attachment loss (CAL), and tooth mobility, were evaluated at 3 points in the menstrual cycle.

Data collection and analysis

Data collection was conducted using predesigned questionnaires, the responses to which were entered into a Microsoft Excel 2016 spreadsheet along with the laboratory analyses. The analysis was performed using IBM-SPSS software, version 26 (IBM Corp., Armonk, NY, USA). Variables were compared using Kruskal-Wallis tests. *P* values less than 0.05 were considered to indicate statistical significance.

Ethical approval of the study and informed consent

Ethical authorizations for this study were obtained from the Ethical Committee of the Faculty of Medicine and Biomedical Sciences at the University of Yaoundé I (N°194/UY1/FMSB/VDRC/DAASR/CSD).

Results and Discussion

We initially included 50 participants in the study; however, 25 were excluded, leaving a final cohort of 25 individuals.

Socio-demographic characteristics

The participants' ages ranged from 18 to 26 years, with an average age of 22±2.5 years. Among the 25 participants included in the study, 22 (88%) were single, and 3 (12%) were married. All participants resided in Yaoundé, as indicated in **Table 1**.

Table 1. Socio-demographic characteristics

Variable	Frequency	Percentage
Marital status		
Single	22	88.0
Married	3	12.0
Widow	0	0.0
Residence		
Yaoundé - urban	25	100.0
Yaoundé - rural	0	0.0

Dental history

Of the 25 participants included in the studies, 13 (52.0%) had never visited a dentist. Among the 12 (48.0%) who had visited a dentist, 6 (24.0%) had gingivitis, 5 (20.0%) had dental caries, and 1 (4.0%) had periodontitis. Among all participants, 13 (52.0%) brushed their teeth once per day. Additionally, 12 (48.0%) used a soft toothbrush, while 13 (52.0%) used a medium toothbrush. Twelve participants (48.0%) reported oral discomfort during the menstrual cycle, with the highest percentage occurring during premenstrual day (PmD) (n=8, 32%), followed by ovulation day (OD) (n=3, 12.0%), and the lowest during menstruation day (MD) (n=1, 4.0%). The most common complaint was bleeding during brushing, as reported by 11 participants (44.0%), as shown in **Table 2**.

Table 2. Dental history

Variable	Category	Frequency	Percentage
Dental consultation	Yes	12	48.0
	No	13	52.0
Diagnosis	Dental caries	5	20.0
	Gingivitis	6	24.0
	Periodontitis	1	4.0

	Others	0	0.0
Treatment plan	Full mouth scaling	8	32.0
	Extraction	4	16.0
	Obturation	0	0.0
Frequency of brushing	Once per day	12	48.0
	Twice per day	12	48.0
	Three times per day	0	0.0
Type of toothbrush	Soft	12	48.0
	Medium	13	52.0
	Hard	0	0.0
Oral discomfort during menstruation	Yes	12	48.0
	No	13	52.0
If yes, what phase?	During menstruation	1	4.0
	During ovulation	3	12.0
	Premenstrual phase	8	32.0
If yes, what symptoms?	Bleeding during brushing	11	44.0
	Tooth mobility	0	0.0
	Painful gum	1	4.0
	Other	0	0.0
Therapy used	Dental consultation	0	0.0
	Traditional methods	0	0.0
	Nothing	12	48.0
Duration of menstruation	3 days	12	48.0
	4 days	13	52.0

Gynecological history

In our study population, 12 participants (48.0%) reported a menstruation duration of 3 days, while 13 participants (52.0%) experienced menstruation for 4 days. We observed menstrual cycle durations ranging from 25 to 31 days.

Social habits

Most participants, 19 (76.0%), reported not consuming alcohol, while 6 (24.0%) did consume alcohol. Of those who drank, 5 (20.0%) consumed 1 drink per week, and 1 (4.0%) consumed more than 1 drink per week, as presented in **Table 3**.

Table 3. Social habits

Variable	Possibilities	Number	Percentage
Alcohol consumption	Yes	6	24.0
	No	19	76.0
Quantity	A glass	5	20.0
	More	1	4.0
Tobacco use	Yes	0	0.0
	No	25	100.0

Periodontal evaluation

Both clinical periodontal parameters and GCF inflammatory markers demonstrated a significant association with the phases of menstruation. The GI, PD, and CAL significantly increased during the menstrual cycle. These parameters were notably higher during the OP compared to the menstrual (follicular) phase, and from the premenstrual (luteal) phase compared to the OP **(Table 4)**, indicating a progressive increase throughout the menstrual cycle.

Table 4. Clinical periodontal parameter levels during the menstrual cycle

Variable	MD	OD	PmD	*P* value			
				MD, OD, PmD	PmD-MD	PmD-OD	MD-OD
Plaque index	0±0	0.3±0.34	0.28±0.46	0.002	0.012	0.012	0.001
Gingival index	0±0	1±0.83	1.40±0.58	<0.001	0.034	0.022	0.001
Pocket depth	0±0	0.42±0.58	0.8±0.71	<0.001	0.011	0.049	0.017
Clinical attachment loss	0±0	0.46±0.59	0.8±0.71	<0.001	0.003	0.023	0.026

MD: menstruation day, OD: ovulation day, PmD: premenstrual day.

Frequency of clinical periodontal indices in the different phases

For our study, we considered several periodontal indices as parameters of periodontal disease during the different phases of the menstrual cycle, as presented below and illustrated in **Figure 1**.

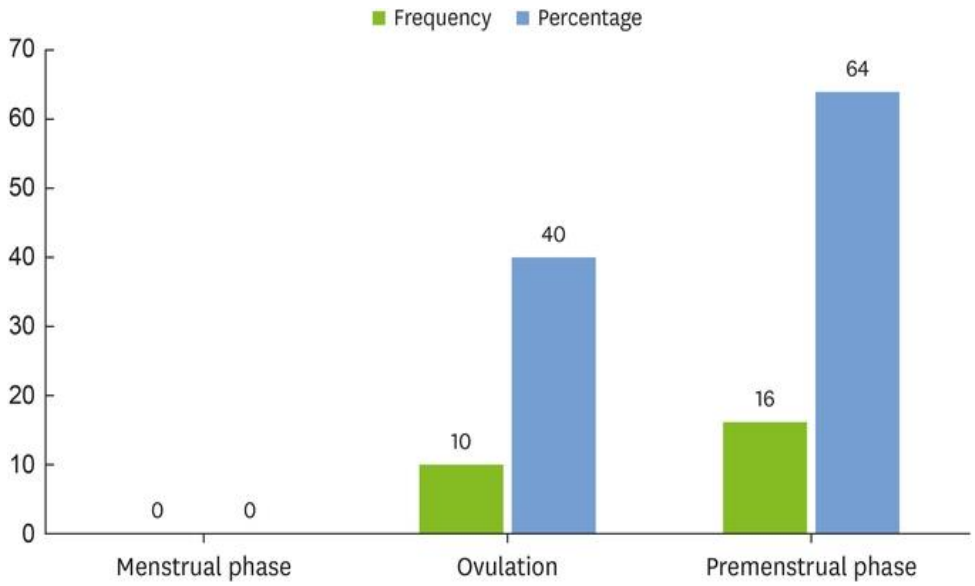

Figure 1. Freqency of periodontal diseases in the different phases of the menstrual cycle.

Symptoms in the different phases

FP: There were no significant changes in any periodontal parameters, which could be attributed to the fact that all participants underwent a full mouth scaling prior to the study.

OP: The PI remained relatively unchanged, with a thin film of plaque observed on the surface of some teeth. Approximately 40% of the participants exhibited slight inflammation of the gingival mucosa, and 20% reported painful gums and bleeding. Additionally, there was an increase in PD and CAL compared to the FP.

In the LP, there was no significant plaque accumulation; however, we observed an increase in both PD and CAL. Additionally, the GI rose from the FP to the LP **(Table 4)**, characterized by mild to moderate inflammation and bleeding, which complicated the collection of some samples.

In our study, 40% of participants experienced oral changes during the OP, and 64% reported similar changes during the LP.

Il-1β variation during the different phases of the menstrual cycle

There was a considerable increase in IL-1β levels throughout the 3 phases of the menstrual cycle (FP: 120.53 pg/mL, OP: 187.10 pg/mL, and LP or premenstrual phase: 243.38 pg/mL) **(Table 5, Figure 2)**.

Table 5. Levels of biological markers in gingival crevicular fluid during the menstrual cycle

Entities	FP		OP		LP		*P* value
	Median	IQR	Median	IQR	Median	IQR	
IL-1β (pg/mL)	120.53	105.93–165.59	187.10	134.07–256.01	243.38	123.39–491.99	**0.012** [a)]
IL-6 (pg/mL)	1.81	1.28–4.74	3.30	2.70–4.92	3.62	3.05–4.63	**0.002** [a)]
TNF-α (pg/mL)	9.2	6.1–11.2	12.00	9.37–14.28	10.20	8.12–12.24	**0.015** [a)]

Bold-faced $P<0.05$ denotes statistical significance.

FP: follicular phase, OP: ovulation phase, LP: luteal phase, IQR: interquartile range, IL: interleukin, TNF: tumor necrosis factor.

[a)] Statistically significant difference compared to the baseline.

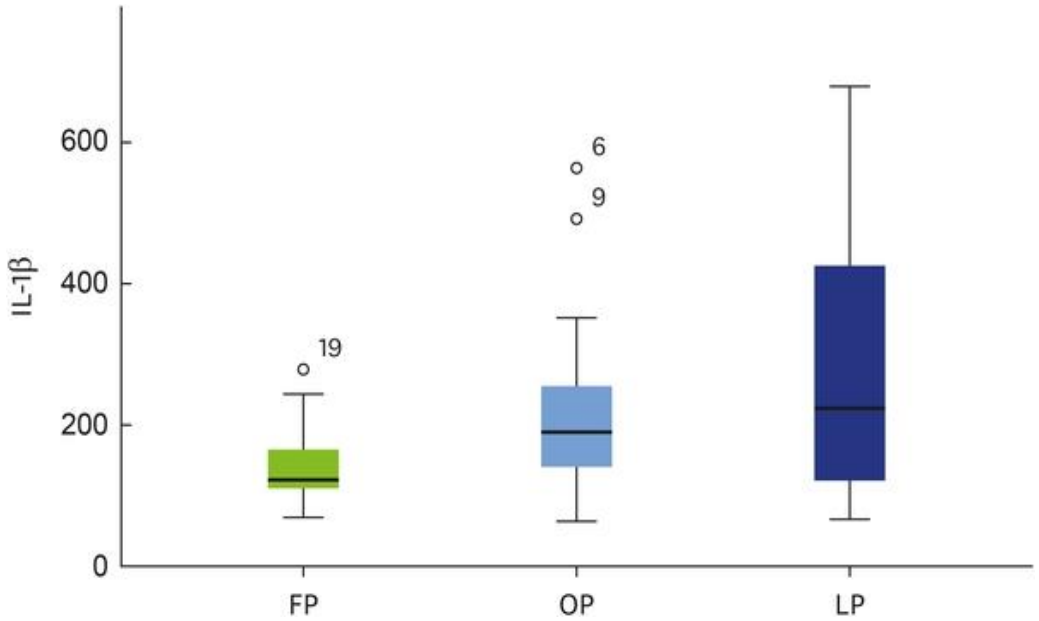

Figure 2. IL-1β variations during the different phases of the menstrual cycle. IL: interleukin, FP: follicular phase, OP: ovulation phase, LP: luteal phase.

Il-6 variation during the different phases of the menstrual cycle

IL-6 levels increased throughout the cycle, with a peak observed during the LP (FP: 1.81 pg/mL, OP: 3.30 pg/mL, and LP: 3.62 pg/mL) **(Table 5, Figure 3)**.

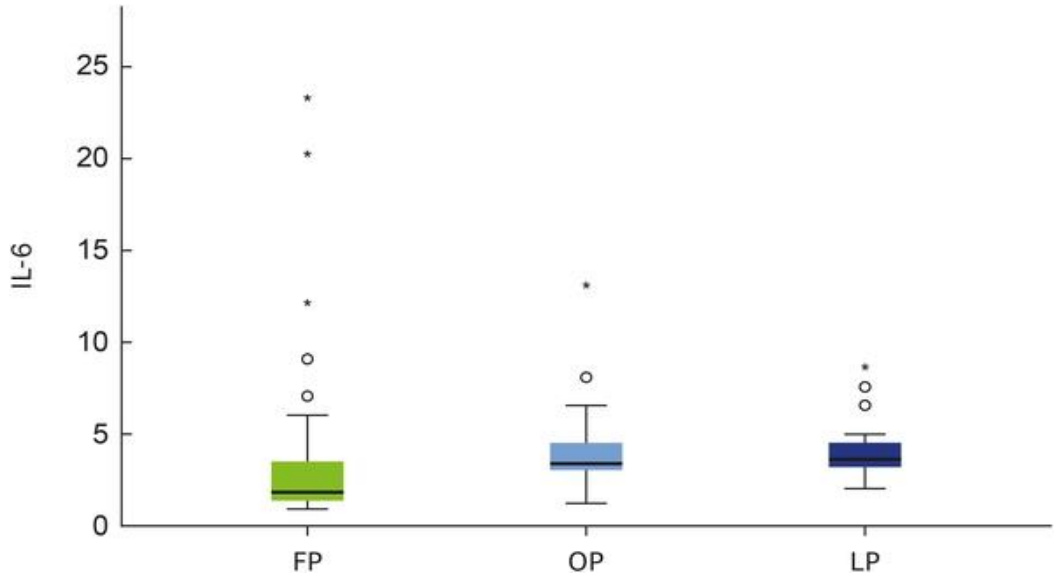

Figure 3. IL-6 variations during the different phases of the menstrual cycle. IL: interleukin, FP: follicular phase, OP: ovulation phase, LP: luteal phase.
*P<0.05.

TNF-α variation during the different phases of the menstrual cycle
TNF-α levels in the GCF were the highest during the OP (12.0 pg/mL), followed by the LP (10.2 pg/mL), and FP (9.2 pg/mL) **(Table 5, Figure 4)**.

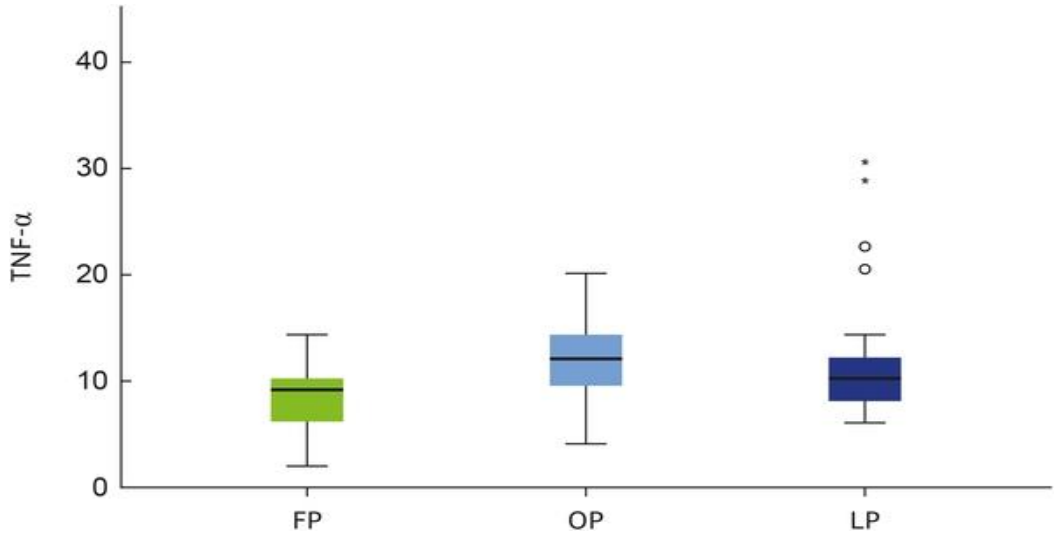

Figure 4. TNF-α variations during the different phases of the menstrual cycle. TNF: tumor necrosis factor, FP: follicular phase, OP: ovulation phase, LP: luteal phase.
*P<0.05.

It has been demonstrated that there are fluctuations in the production and secretion of estrogen and progesterone during the menstrual cycle; however, few studies have examined how these alterations affect gingival inflammation. Therefore, the purpose of this longitudinal prospective study was to evaluate the effects of the menstrual cycle on GCF levels of IL-1β, IL-6, and TNF-α, as well as clinical periodontal parameters in periodontally healthy women. A total of 25 participants were

recruited from the University of Yaoundé 1. The mean age of the participants was 22±2.5 years, with 88% being married and all residing in Yaoundé.

Biomarkers found in the GCF can serve as reliable tools for distinguishing between periodontal health and disease status [10]. Paper points have been identified as an efficient way to sample oral fluid biomarkers [11, 12]. Antibody-based ELISA assays have been shown to be the gold standard for detecting oral inflammatory markers [13, 14].

GCF is derived from serum, and studies have shown that the production of inflammatory GCF cytokines increases in the presence of smoking and stress [15], as well as diabetes [16]. Pertaining to stress, cortisol—a hormone associated with stress—can compromise immune function and elevate blood glucose levels, thereby increasing susceptibility to periodontal disease. Furthermore, psychological stress can impact periodontal health through behavioral changes, such as neglecting dental hygiene, increasing smoking and alcohol consumption, and making poor nutritional choices [17]. Glycemic levels and periodontitis are closely interconnected. Diabetes can adversely affect immune function and inflammatory pathways, which in turn can deteriorate periodontal health. Conversely, periodontitis can negatively influence glycemic control in diabetic patients [18].

In this study, the phases of the menstrual cycle were considered when evaluating periodontal indicators such as PI, GI, periodontal PD, and CAL. The PI increased from the menstrual day to the OD and then slightly decreased from the OD to the PmD. This decrease may be attributed to the fact that all participants underwent full mouth scaling at the beginning of the study. Additionally, a minor change in the GI was observed from the menstruation period to the OP and the premenstrual phase. However, there were significant changes in the CAL and periodontal PD across these three periods.

These results are consistent with those reported by Sahin Aydınyurt *et al*. [4] from Turkey, who observed modest variations in the PI and GI across the three menstrual phases. However, these findings contradict those of Khosravisamani *et al*. [19], who noted a significant increase in the GI on the day of ovulation in their study.

The minor increase in specific periodontal markers may be attributed to the rise in estrogen during the OP and the subsequent increase in both progesterone and estrogen during the premenstrual phase.

Periodontitis is an opportunistic inflammatory disease that has been shown to be associated with IL-6 levels [20].

The relationship between IL-6 polymorphism and periodontitis varies, with different alleles exerting distinct impacts [20]. Additionally, IL-1β single nucleotide polymorphisms may lead to varied immune responses [21], indicating that further research is necessary to enhance our understanding of how IL polymorphisms influence periodontitis.

In this study, we focused on 3 inflammatory markers due to their role in the inflammatory response of the periodontium. Among these markers, TNF-α exhibited a slight decrease from the OP to the premenstrual phase. Conversely, the levels of the cytokines IL-1β and IL-6 increased significantly from the menstrual phase through to the ovulation and premenstrual phases. These results are consistent with our clinical observations, which indicated that oral discomfort during the menstrual cycle was most severe during the premenstrual phase (n=8, 32.0%), followed by the OP (n=3, 12.0%), and was least during menstruation (n=1, 4.0%) **(Table 2)**. The changes in inflammatory markers observed in this study align with the findings of Shourie *et al*. [9], who reported an increase in IL-1β, and Markou *et al*. [22], who noted an increase in IL-6 from the menstrual phase to the premenstrual phase.

Our findings do not support those of Khosravisamani *et al*. [19], who reported an increase in TNF-α from the menstruation phase to the premenstrual phase.

Changes in TNF-α and IL-1β levels may be attributed to variations in progesterone and estrogen production and secretion observed throughout the menstrual cycle [23].

Progesterone affects polymorphonuclear leukocytic activity and elevates inflammatory cytokine levels by enlarging small blood capillaries and increasing vascular permeability. Estrogen stimulates polymorphonuclear cell phagocytosis [24].

The PI, GI, PD, and CAL all exhibited statistically significant across the 3 menstrual phases in our study **(Table 4)**. Additionally, GCF levels of all 3 inflammatory markers (IL-1β, IL-6, and TNF-α) showed statistically significant variations corresponding to the menstruation phase, OP, and premenstrual phase. These results align with those reported by Khosravisamani *et al*. [19], who found significant differences in GI values between the premenstrual and menstrual phases. Moreover, Baser *et al*. [7] observed an increase in IL-1β levels during the PmD phase, noting that levels were significantly higher in the premenstrual phase compared to the OD and MD phases, which corroborates our findings. Similarly, our results

are consistent with those of Sahin Aydınyurt *et al*. [4], who identified statistically significant variations in TNF-α levels across all 3 phases of the menstrual cycle.

Hormonal fluctuations during the menstrual cycle may exacerbate gingival inflammation [4]. However, the changes in the GI reported during the menstrual cycle have been a subject of debate in the literature. In the current study, the observed increases in GI, PD, and CAL across the 3 phases of the menstrual cycle are believed to be linked to rises in estrogen and progesterone levels. Becerik *et al*. [8] noted a gradual increase in serum estradiol levels during menstruation, peaking during ovulation, followed by a sharp decline. A second peak in estradiol levels occurred on days 22–24 [8].

A study by Becerik *et al*. [8] found that progesterone, which reaches its highest levels during the premenstrual phase, increases blood vessel permeability. Therefore, the elevated levels of the GI, PD, CAL, IL-1β, IL-6, and TNF-α observed during this phase may be associated with increased progesterone. Changes in progesterone levels could account for the observed increases in IL-1β and TNF-α production. Progesterone specifically regulates the stimulation, production, and roles of TNF-α and IL-1β in the inflammation of periodontal tissues. Additionally, fluctuations in hormone levels may impact clinical parameters, as evidenced by the increase in gingival inflammation during the menstrual cycle [25].

The variability in results across different studies in various populations may be attributed to genetic diversity, environmental factors, and lifestyle influences on sex steroid hormone fluctuations during the menstrual cycle, periodontal health, and the rate of inflammatory cytokine secretion in GCF.

It is crucial to understand how patients respond to surgical and non-surgical treatments at different stages of the menstrual cycle. For example, Eshghpour *et al*. [26] noted that the menstrual cycle might influence the incidence of alveolar osteitis after third molar surgery. Consequently, the menstrual cycle could elevate the risk of developing alveolar osteitis and periodontal disease.

Because the gums are highly protective, it is crucial to exercise caution when using implants and to specifically assess the periodontal status, as indicated by the increased periodontal friability observed during menstruation.

The health of the gums is crucial for the longevity of an implant. If conditions such as mucositis and peri-implantitis are present, they may compromise the long-term success of the patient's rehabilitation.

Although these scenarios do not always occur, this information can be applied in a preventative manner.

Our study had several limitations: First, the small sample size may affect the generalizability of the results. A larger sample would enhance the statistical power and provide more robust data for analysis. Additionally, the selection of participants might not accurately represent the broader population. The specific demographic characteristics of our sample could skew the results and limit their applicability to other groups. We did not account for all potential confounding variables, such as participants' overall health, lifestyle factors, or genetic predispositions, which could influence the outcomes. The methods used to measure certain variables may also have limitations in accuracy or sensitivity, potentially leading to inaccuracies in the data collected. Furthermore, the high costs prevented the inclusion of estrogen and progesterone analyses. The geographic scope of our study was limited, which might lead to regional variations that do not reflect broader contexts. Additionally, our study may not adequately represent the diversity of different ethnic or cultural groups, potentially affecting the presentation and progression of periodontal diseases. Finally, the absence of long-term follow-up complicates the ability to assess the enduring impact of the observed factors on periodontal health. Conducting an analysis of the proteins in the same patients across various menstrual cycles would have provided a more reliable statistical evaluation.

Conclusion

In conclusion, at the conclusion of this study, which aimed to describe the effects of the menstrual cycle on inflammatory cytokines—IL-1β, IL-6, and TNF—in the GCF, our findings support the initial hypothesis regarding hormonal fluctuations. We can therefore conclude that:

- There was an increase in clinical periodontal parameters, indicating the presence of periodontal disease.
- There were increases in pro-inflammatory cytokine levels throughout the menstrual cycle.
- Hormonal fluctuations during the menstrual cycle might have a temporary effect on gingival inflammation and pro-inflammatory cytokines, which could be of clinical importance for therapeutics involving the gums, such as dental implants.

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