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ORIGINAL RESEARCH

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Gingival crevicular fluid cytokines as non-invasive biomarkers for the identification of gingivitis in pediatric patients: a cross-sectional clinical study

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Abstract

Background: Gingivitis is the most common periodontal disease in children and adolescents, characterized by reversible inflammation caused by plaque accumulation. Because traditional clinical indices, such as gingival and plaque scores, detect inflammation only after visible tissue changes occur, identifying early biomarkers of subclinical inflammation is essential. Gingival crevicular fluid (GCF), a serum-derived exudate enriched with host inflammatory mediators, has emerged as a valuable non-invasive diagnostic medium. This study aimed to compare GCF concentrations of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) between children with clinically healthy gingiva and those with plaque-induced gingivitis, and to evaluate correlations between cytokine levels and clinical periodontal parameters. **Methods**: A cross-sectional study was conducted in 58 children aged 10–13 years, classified as healthy (n = 30) or with gingivitis (n = 28). Clinical parameters—including plaque index, gingival index, probing depth, bleeding on probing, and GCF volume were recorded. GCF samples were collected from the mesiobuccal sites of the upper incisors and analyzed using enzyme-linked immunosorbent assay (ELISA). Statistical tests included Mann-Whitney U, Spearman correlation, and receiver operating characteristic (ROC) curve analysis ($p < 0.05$). **Results**: All clinical parameters were significantly higher in the gingivitis group ($p < 0.001$). IL-1β and IL-8 levels were significantly elevated in gingivitis ($p = 0.001$ and $p = 0.035$, respectively), whereas IL-6 and TNF-α showed no significant differences. IL-1β correlated with bleeding on probing ($r = 0.47$) and pocket depth ($r = 0.34$). IL-1β demonstrated the highest diagnostic accuracy, with an area under the curve (AUC) = 0.76. **Conclusions**: IL-1β appears to be a sensitive and reliable biomarker of gingival inflammation in children, reflecting local immune activation before irreversible tissue damage.

Keywords

Gingivitis; Children; Gingival crevicular fluid; Cytokines; Interleukin-1β; Biomarkers; Pediatric dentistry

1. Introduction

Gingivitis is the most common periodontal disease in children and adolescents and is characterized by reversible inflammation of gingival tissues primarily driven by dental plaque accumulation. Although it is often mild and may remain unnoticed, persistent plaque-induced gingival inflammation may contribute to periodontal tissue breakdown later in life [1]. Reported prevalence rates vary widely (approximately 20% to >90%), depending on diagnostic criteria, age group, oral hygiene habits, and socioeconomic factors [1–3]. Studies from different regions indicate that inadequate oral hygiene, limited parental awareness, and restricted access to preventive care are among the main contributors to pediatric gingival inflammation [2, 4].

Although gingivitis is reversible with adequate plaque control, its often subtle course in childhood underscores the importance of timely diagnosis and preventive care. Routine dental visits and oral health programs can help detect early clinical signs (*e.g.*, redness, edema, and bleeding) before attachment loss develops [5, 6]. Plaque accumulation and gingival inflammation are influenced by behavioral and structural factors, including irregular toothbrushing, high sugar intake, and orthodontic appliances, as well as socioeconomic conditions affecting oral health literacy and access to care [6–8]. Accordingly, preventive strategies emphasizing education and family-based oral hygiene interventions remain essential for maintaining gingival health and supporting long-term periodontal

stability [6].

Traditional assessment of gingivitis relies on clinical indices such as the gingival index, plaque index, and bleeding on probing. However, these indices largely reflect inflammation after observable tissue changes occur, which may limit their ability to capture subtle inflammatory activity across individuals [9]. This limitation has increased interest in biologically based approaches that can provide objective information on local inflammatory status. Gingival crevicular fluid (GCF), a non-invasive and child-friendly sampling medium, reflects the local immune and inflammatory environment of the gingival tissues and has therefore emerged as a promising biomarker source [10].

GCF is a serum-derived exudate or ultrafiltrate that seeps through the gingival sulcus and contains host- and pathogen-derived molecules, including cytokines, enzymes, and antibodies [10, 11]. Its composition changes with inflammatory activity, and molecular alterations may occur before overt clinical findings become pronounced [12]. Among GCF constituents, cytokines play central roles in host responses to bacterial biofilms. IL-1β and TNF-α are key pro-inflammatory mediators that promote vascular changes, leukocyte recruitment, and connective tissue breakdown [13]. Elevated IL-1β has been associated with increased bleeding on probing and pocket depth even without clinical attachment loss, suggesting that it may reflect active gingival inflammation [14]. TNF-α contributes to macrophage activation and cytokine amplification [11], whereas IL-6 has context-dependent pro- and anti-inflammatory functions related to acute-phase responses and tissue remodeling [15]. IL-8 functions as a major chemoattractant for neutrophils and supports the initial immune response to bacterial challenge [16]. Experimental gingivitis models have shown that several cytokines rise during plaque accumulation and decrease after oral hygiene re-establishment [15], and IL-1β and IL-8 may respond relatively rapidly and correlate with clinical signs such as redness and bleeding [17]. Importantly, cytokine responses in children may differ from adults because of immune developmental characteristics and the relative immaturity of periodontal tissues [6]. Beyond periodontal applications, GCF biomarker approaches have also been explored in systemic inflammatory conditions, reinforcing interest in GCF as a non-invasive diagnostic source [17–20].

Despite growing interest, pediatric studies assessing GCF cytokines remain limited compared with adult research. Prior findings suggest that IL-1β and IL-8 may be more closely associated with gingival inflammation than IL-6 or TNF-α, yet these associations have not been consistently validated in children [11]. Clarifying which cytokines most closely reflect gingival inflammatory status—and how well they discriminate between gingivitis and periodontal health—may help reduce subjectivity in clinical assessment and inform future biomarker-focused research in pediatric dentistry.

Therefore, the present study aimed to evaluate and compare the concentrations of IL-1β, TNF-α, IL-6, and IL-8 in the GCF of children with clinically healthy gingiva and those with plaque-induced gingivitis, and to examine their associations with clinical periodontal parameters (gingival index, plaque index, and bleeding on probing). We hypothesized that IL-1β and IL-8 would show higher levels and stronger associations with clinical inflammatory indices than IL-6 and TNF-α, and that these markers would demonstrate comparatively better discriminatory performance between gingivitis and periodontal health.

2. Materials and methods

2.1 Study design and ethical approval

This cross-sectional clinical study was conducted jointly by the Department of Periodontology at Kahramanmaraş Sütçü İmam University Faculty of Dentistry and the Department of Pediatric Dentistry at Gaziantep University Faculty of Dentistry. Participants were recruited and examined in the outpatient clinical settings of the participating departments between July and October 2025. Eligible participants were enrolled using a non-consecutive (clinic-based) sampling according to the predefined inclusion and exclusion criteria. All clinical periodontal recordings and gingival crevicular fluid sampling were performed using a standardized protocol by calibrated examiners to ensure consistency across centers. The study protocol (No. 2025/119) was approved by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University. The study was conducted in full accordance with the ethical standards outlined in the Declaration of Helsinki (2013 revision) and the institutional requirements of both participating universities. Written informed consent was obtained from the parents or legal guardians of all participants prior to inclusion. The study is reported in accordance with the Strengthening the Reporting of Observational Epidemiology (STROBE) guidelines for cross-sectional studies.

2.2 Inclusion and exclusion criteria

Children who met the study requirements were considered eligible for inclusion. Periodontal status was defined based on the percentage of bleeding on probing (BOP). Participants were classified as having no gingivitis or healthy gingiva when BOP% was $<$10%, localized gingivitis when BOP% was $\geq$10% and $\leq$30%, and generalized gingivitis when BOP% was $>$30%. Only children diagnosed with generalized plaque-induced gingivitis (BOP% $>$30%) were included in the study; children with localized gingivitis (BOP% $\geq$10% and $\leq$30%) were excluded.

Inclusion criteria further comprised the absence of clinical attachment loss and the ability to cooperate with both clinical examination and gingival crevicular fluid sampling procedures. Children presenting with any degree of clinical attachment loss were not included in the study.

Participants were excluded if they had any systemic disease or condition known to influence periodontal status or immune response, or if they had used antibiotics, anti-inflammatories, or immunosuppressive medications within the previous six months. In addition, children who had received any dental treatment within the last 6 months were excluded. Patients presenting with cervical (root) caries, deep carious lesions requiring endodontic treatment, or an existing indication for root canal therapy were also excluded to avoid potential confounding effects on inflammatory and biochemical parameters.

2.3 Sample size calculation

The sample size was calculated using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, NRW, Germany). The calculation was based on differences in gingival crevicular fluid (GCF) interleukin-1β (IL-1β) levels between periodontally healthy and gingivitis groups reported by Gündoğar *et al.* [21]. In that study, mean $\pm$ standard deviation (SD) IL-1β values were 21.73 $\pm$ 17.53 for healthy subjects and 44.63 $\pm$ 40.63 for gingivitis. A standardized effect size (Cohen's *d*; dimensionless) was derived using the mean difference divided by the pooled standard deviation (SD_pooled = $\sqrt{[(SD_1{}^2 + SD_2{}^2)/2]}$), yielding an estimated *d* of approximately 0.73. To avoid overestimating the expected effect due to the relatively high variability in the reference data, a more conservative standardized effect size of $d = 0.60$ was used for the a priori power analysis. With $\alpha = 0.05$ and power = 80% ($1 - \beta = 0.80$), the required sample size was estimated as at least 25 participants per group (total n = 50). Because this study involved GCF collection and cytokine measurements, insufficient sample volume or sample loss may occur due to technical reasons during sampling/processing. Therefore, slightly higher number of participants were enrolled than the minimum required sample size to ensure an adequate number of valid GCF samples for biochemical analysis.

2.4 Clinical examination

All clinical measurements were performed by two calibrated examiners (MU and ZUG). Prior to the study, calibration was conducted on 10 patients who were not included in the main sample. Intra-examiner reliability was excellent (intraclass correlation coefficient (ICC) = 0.92 for MU and ICC = 0.91 for ZUG), and inter-examiner agreement was also high (ICC = 0.89). Clinical parameters included probing pocket depth (PPD), plaque index (PI) [22], gingival index (GI) [23], and bleeding on probing (BOP) [24]. Clinical measurements were recorded only for fully erupted teeth. Teeth undergoing eruption were not assessed for any clinical indices (PPD, BOP, GI, or PI) and were excluded from BOP% calculations to avoid potential measurement bias. Measurements were obtained at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) using a Williams-type periodontal probe (PW and PW7, Hu-Friedy, Chicago, IL, USA) with a 0.5 mm tip diameter. All data were recorded digitally using a customized periodontal charting form.

2.5 Gingival crevicular fluid (GCF) collection and sample analysis

Gingival crevicular fluid (GCF) samples were collected from all participants prior to clinical measurements to prevent contamination from probing. Sampling was performed from the mesiobuccal sites of the upper first incisors, which were free of caries or restorations.

Each sampling site was carefully isolated with cotton rolls to avoid salivary contamination, and supragingival plaque was gently removed. The area was then air-dried, and sterile prefabricated paper strips (Periopaper; GCF Collection Strips, Oraflow Inc., Plainview, NY, USA) were gently inserted into the gingival sulcus until mild resistance was felt and left in place for 30 seconds. Mechanical irritation was avoided throughout the procedure. Strips contaminated with blood or saliva were discarded.

The GCF volume was measured immediately after collection using a calibrated Periotron 8000 (Oraflow Inc., Plainview, NY, USA). The paper strips were then placed in sterile Eppendorf tubes and stored at −80 °C until biochemical analysis.

Biochemical analyses were performed at the Department of Biochemistry, Faculty of Medicine, Gaziantep University. The concentrations of IL-1β, TNF-α, IL-6, and IL-8 in the GCF samples were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Each sample was thawed once and centrifuged at 3000 rpm for 10 minutes at 4 °C prior to analysis. Absorbance was measured at 450 nm using a microplate spectrophotometer (Varioskan ALF Multimode Microplate Reader, Thermo Fisher Scientific, Waltham, MA, USA). Cytokine levels were calculated from standard curves and expressed as pg/30 s of GCF.

2.6 Statistical analysis

All statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). The normality of data distribution for clinical and biochemical variables was assessed using the Shapiro-Wilk test. Demographic variables (age and gender) were compared between groups using the chi-square test for categorical variables and the Mann-Whitney U test for continuous variables. Intergroup comparisons of clinical and biochemical parameters were conducted using the Mann-Whitney U test, as most variables did not follow a normal distribution. Correlations between clinical and biochemical parameters were evaluated using Spearman's rank correlation coefficient, and results were visualized with a heatmap. Receiver operating characteristic (ROC) curve analyses were performed for each biochemical marker to assess their diagnostic performance in distinguishing gingivitis from healthy status; the area under the curve (AUC) was calculated for each marker. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Demographic characteristics

A total of fifty-eight systemically healthy children were included in this cross-sectional clinical study, which was jointly conducted by the Department of Periodontology, Kahramanmaraş Sütçü İmam University Faculty of Dentistry, and the Department of Pedodontics, Gaziantep University Faculty of Dentistry. The participants had a mean age of 11.5 $\pm$ 1.1 years (range: 10–13 years) and were categorized into two groups based on gingival health status: the healthy group (n = 30) and the gingivitis group (n = 28). There were no statistically significant differences in age or gender distribution between the groups ($p > 0.05$ for both). These comparable demographic characteristics support the validity of subsequent comparisons of clinical and biochemical parameters between groups.

Gender distribution is shown in Table 1. Among the healthy group, 8 participants (26.7%) were female, and 22 (73.3%) were male. Within the gingivitis group, females accounted for 11 (39.3%) and males for 17 (60.7%). There was no statistically significant difference in gender distribution between the two groups (Chi-square test, $p = 0.457$).

Age data are presented in Table 2. The mean age was 11.3 ± 1.0 years in the healthy group and 11.7 ± 1.2 years in the gingivitis group. The median age (minimum–maximum) was 12 (10–13) years for both groups. The difference in age distribution between the groups was not statistically significant (Mann-Whitney U test, $p = 0.197$).

3.2 Comparison of clinical parameters between groups

The comparison of clinical parameters between the healthy and the gingivitis groups is summarized in Table 3. All clinical parameters, including GCF volume, pocket depth (both tooth and whole-mouth), gingival index, plaque index, and bleeding on probing, were significantly higher in the gingivitis group compared to the healthy group ($p < 0.001$). Bleeding on probing showed the most pronounced difference between the groups.

3.3 Comparison of biochemical parameters between groups

The comparison of biochemical parameters between the healthy and the gingivitis groups is presented in Table 4. IL-1β and IL-8 levels were significantly higher in the gingivitis group compared to the healthy group ($p = 0.001$ and $p = 0.035$, respectively). No significant differences in IL-6 or TNF-α concentrations were observed between the groups ($p > 0.05$).

3.4 Correlation between clinical and biochemical parameters

Spearman correlation analysis demonstrated that IL-1β levels showed moderate and statistically significant positive correlations with multiple clinical periodontal parameters, including GCF volume measured by Periotron ($r = 0.53$, $p < 0.05$), pocket depth measured at the tooth level ($r = 0.34$, $p < 0.05$), pocket depth at the mouth level ($r = 0.47$, $p < 0.05$), gingival index ($r = 0.45$, $p < 0.05$), plaque index ($r = 0.38$, $p < 0.05$), and bleeding on probing ($r = 0.47$, $p < 0.05$) (Fig. 1).

In contrast, IL-6 did not demonstrate significant correlations with most clinical parameters. IL-8 exhibited weak but statistically significant correlations with GCF volume ($r = 0.27$, $p < 0.05$) and pocket depth at the mouth level ($r = 0.26$, $p < 0.05$), while TNF-α showed weak correlations that did not reach statistical significance. Overall, IL-1β emerged as the biomarker most consistently associated with clinical indicators of gingival inflammation (Fig. 1).

TABLE 1. Distribution of gender by periodontal status.

Gender	Healthy (n = 30)	Gingivitis (n = 28)	*p*-value*
Female	8 (26.7%)	11 (39.3%)	0.457
Male	22 (73.3%)	17 (60.7%)	

**Chi-square test.*

TABLE 2. Distribution of age by periodontal status.

Age (yr)	Healthy (n = 30)	Gingivitis (n = 28)	*p*-value*
Mean ± SD	11.3 ± 1.0	11.7 ± 1.2	0.197
Median (Minimum–Maximum)	12 (10–13)	12 (10–13)	

**Mann-Whitney U test. SD: Standard Deviation.*

TABLE 3. Comparison of clinical parameters between the healthy and the gingivitis groups.

Clinical Parameter	Healthy (n = 30)	Gingivitis (n = 28)	*p*-value
GCF volume (μL)	0.08 ± 0.07	0.23 ± 0.09*	<0.001
Pocket Depth (Teeth, mm)	1.07 ± 0.25	1.89 ± 0.57*	<0.001
Pocket Depth (Mouth, mm)	1.84 ± 0.13	2.33 ± 0.20*	<0.001
Gingival Index	0.93 ± 0.28	1.53 ± 0.23*	<0.001
Plaque Index	1.08 ± 0.33	2.05 ± 0.30*	<0.001
Bleeding on Probing (%)	3.85 ± 2.45	56.44 ± 11.13*	<0.001

**Statistically significant difference between groups ($p < 0.05$, Mann-Whitney U test). Data are presented as mean ± standard deviation (SD). GCF: gingival crevicular fluid.*

TABLE 4. Comparison of biochemical parameters between the healthy and the gingivitis groups.

Biochemical Parameter	Healthy (n = 30)	Gingivitis (n = 28)	*p*-value
IL-1β (pg/30 s)	17.33 ± 21.07	43.67 ± 40.25*	0.001
IL-6 (pg/30 s)	4.86 ± 3.34	8.52 ± 13.70	0.507
IL-8 (pg/30 s)	115.38 ± 166.44	167.19 ± 155.24*	0.035
TNF-α (pg/30 s)	1.15 ± 0.86	1.28 ± 0.88	0.421

**Statistically significant difference between groups ($p < 0.05$, Mann-Whitney U test). Data are presented as mean ± standard deviation (SD). IL: interleukin; TNF-α: tumor necrosis factor-α.*

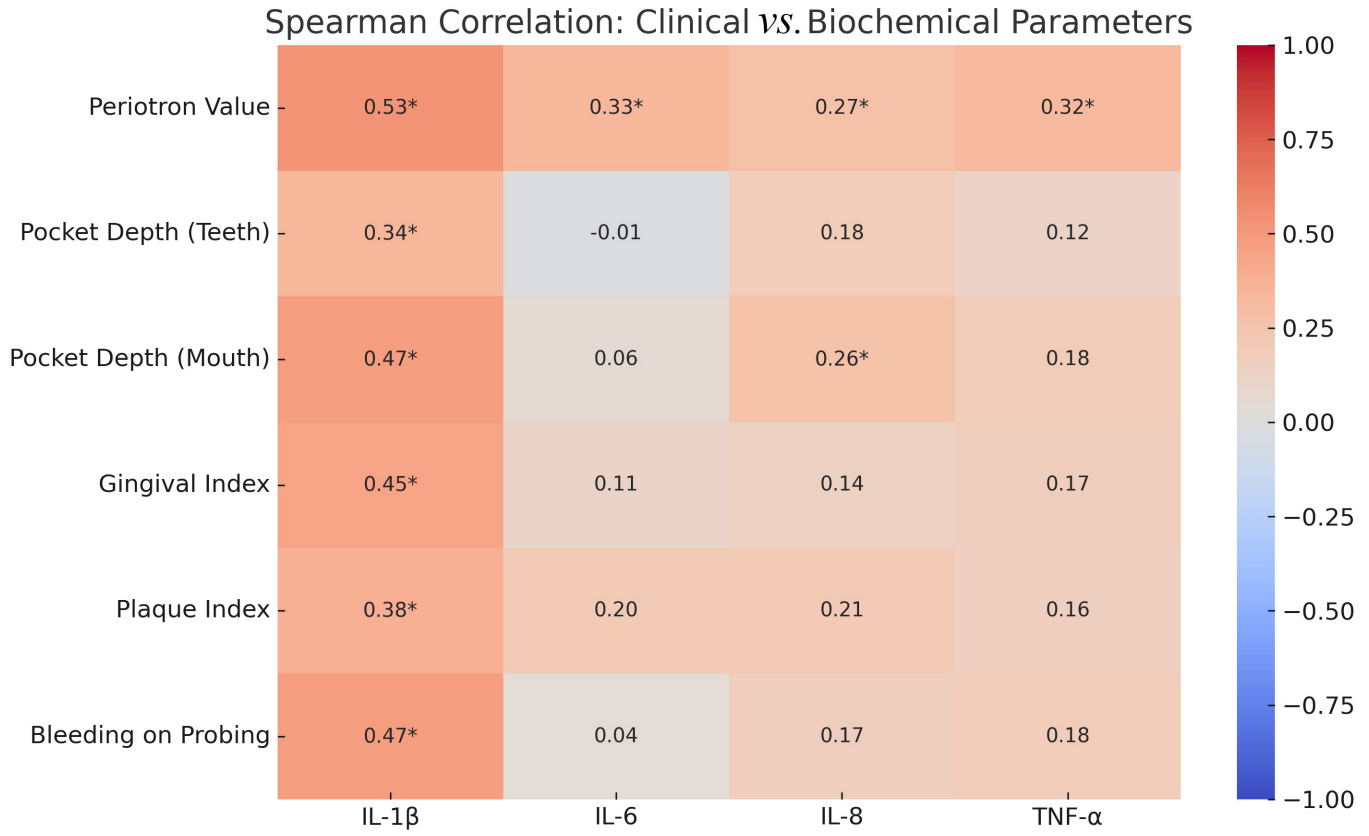

FIGURE 1. Spearman correlation heatmap between clinical periodontal parameters and GCF cytokine levels. Color intensity indicates the strength and direction of the correlation, with warmer colors representing positive correlations and cooler colors representing weaker or negative correlations. Correlation coefficients (*r* values) are displayed within each cell. An asterisk (*) denotes statistically significant correlations ($p < 0.05$). IL: interleukin; TNF-α: tumor necrosis factor-α.

3.5 Diagnostic performance of biochemical markers

Receiver operating characteristic (ROC) analysis, together with cut-off-based diagnostic indices, was performed to assess the ability of GCF cytokines to distinguish gingivitis from periodontal health. IL-1β demonstrated the strongest diagnostic validity, with an optimal cut-off value of 27.87 pg/30 s, yielding an AUC of 0.76, sensitivity of 64%, and specificity of 83%, along with acceptable positive predictive value (PPV) (78%) and negative predictive value (NPV) (71%). These values indicate that IL-1β possesses the most balanced and clinically meaningful discriminatory profile among the evaluated biomarkers. IL-8 showed moderate diagnostic capability, at its optimal cut-off (65.85 pg/30 s), IL-8 achieved an AUC of 0.64, with sensitivity of 68% and specificity of 60%. In contrast, IL-6 (cut-off: 7.61 pg/30 s, AUC 0.55) and TNF-α (cut-off: 1.06 pg/30 s, AUC 0.56) demonstrated only marginal discriminatory ability (Table 5 and Fig. 2).

4. Discussion

This study evaluated the clinical and biochemical profiles of children with gingivitis and compared them with those of periodontally healthy peers to explore the diagnostic potential of gingival crevicular fluid (GCF) cytokines in the detection of inflammation. The findings revealed that all clinical parameters, including GCF volume, pocket depth, gingival index, plaque index, and bleeding on probing, were significantly higher in the gingivitis group, confirming the clinical manifestation of active inflammation. Among the

TABLE 5. Diagnostic performance of GCF cytokines for distinguishing gingivitis from periodontal health.

Marker	Cut-off (pg/30 s)	AUC	95% CI	Sensitivity	Specificity	PPV	NPV
IL-1β	27.87	0.76	0.63–0.88	0.64	0.83	0.78	0.71
IL-6	7.61	0.55	0.38–0.71	0.46	0.87	0.76	0.63
IL-8	65.85	0.64	0.50–0.78	0.68	0.60	0.61	0.67
TNF-α	1.06	0.56	0.42–0.71	0.68	0.53	0.58	0.64

AUC: Area under the curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; IL: interleukin; TNF-α: tumor necrosis factor-α.

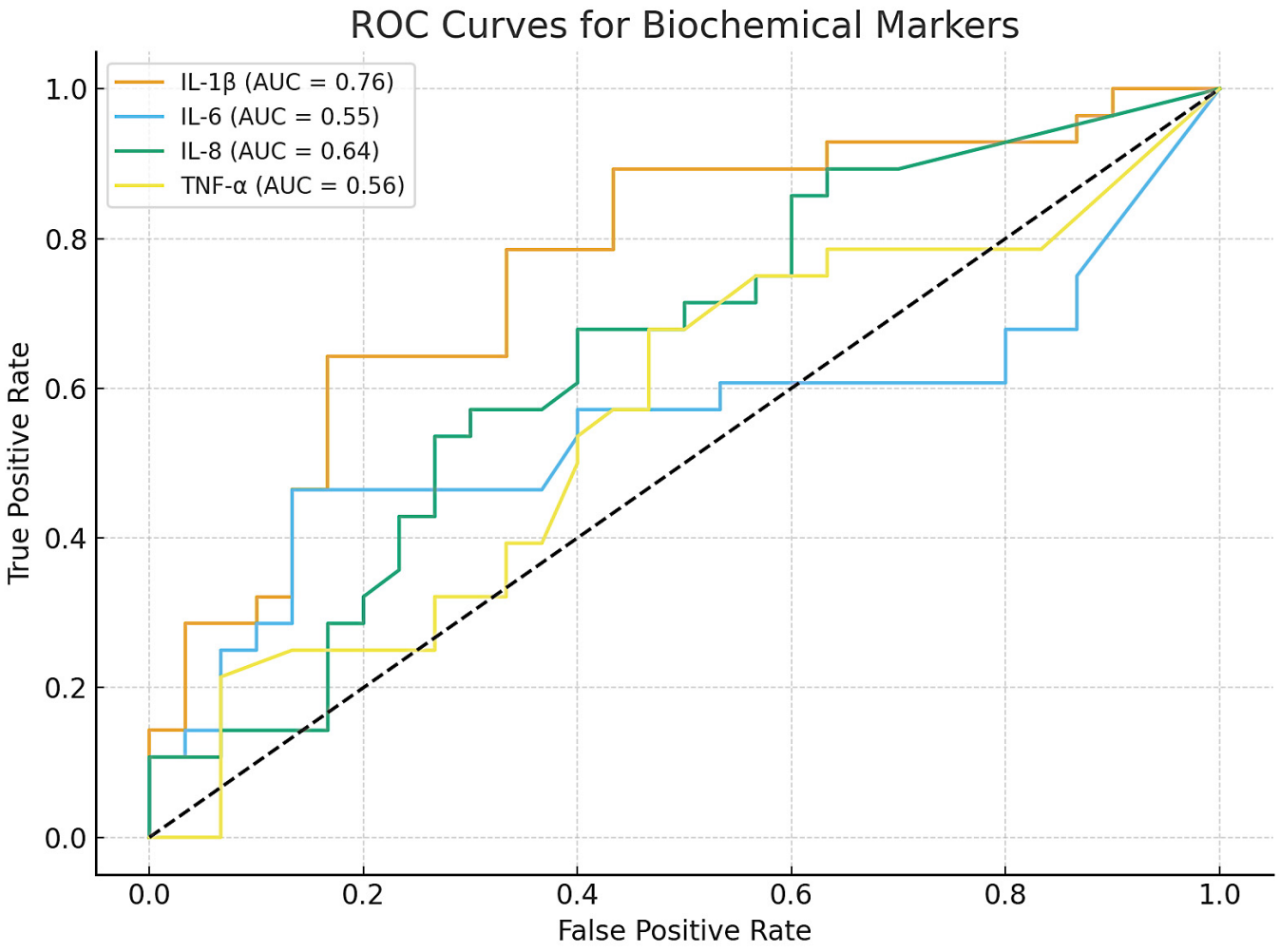

FIGURE 2. Receiver operating characteristic (ROC) curves for GCF cytokine biomarkers. IL: interleukin; AUC: Area under the curve; TNF-α: tumor necrosis factor-α.

cytokines analyzed, interleukin-1β (IL-1β) and interleukin-8 (IL-8) levels were significantly elevated in the gingivitis group, while interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) showed no significant intergroup differences. Moreover, IL-1β levels demonstrated strong positive correlations with multiple clinical indicators—especially bleeding on probing and pocket depth—highlighting its potential as a sensitive biomarker of gingival inflammation. Receiver operating characteristic (ROC) analysis further supported this, as IL-1β showed the highest diagnostic accuracy (AUC = 0.76), outperforming other cytokines, which exhibited limited or poor diagnostic discriminatory capacity.

These findings are consistent with previous studies that have identified IL-1β as a central mediator in the pathogenesis of plaque-induced gingival inflammation. Hsu *et al.* [11] demonstrated that IL-1β concentrations in GCF rise proportionally with plaque accumulation and clinical severity, returning to baseline after plaque removal. Similarly, Gamonal *et al.* [14] and Kowalczyk *et al.* [25] reported that elevated IL-1β levels correlate strongly with bleeding on probing and pocket depth, even in the absence of attachment loss, supporting its role as a marker of subclinical gingival inflammation. In the present study, the positive association between IL-1β and multiple clinical parameters reinforces the concept that this cytokine

reflects local vascular and immune activity before irreversible tissue changes occur. Therefore, IL-1β quantification in GCF may represent a clinically feasible and biologically meaningful method for the identification of gingivitis in the pediatric population.

In contrast, IL-6 and TNF-α did not show significant differences between groups, whereas IL-8 displayed a moderate increase in the gingivitis group, reflecting a more biologically plausible pattern than initially observed. These outcomes differ from some findings in the adult population, where IL-6 and TNF-α have been consistently implicated in both acute and chronic periodontal inflammation [12, 13]. Several biological and methodological explanations may account for these discrepancies. First, cytokine kinetics differ across the periodontal disease continuum; IL-6 and TNF-α tend to rise more prominently during advanced or chronic stages, rather than in early gingivitis. Second, pediatric immune profiles differ from adults, as children exhibit distinctive gingival tissue composition, vascularity, and immune responsiveness, potentially leading to lower or more variable cytokine output [6]. Third, the standardized 30-second GCF collection period and sampling from the mesiobuccal sites of upper incisors may have captured early-phase cytokine fluctuations or produced diluted signals for short-lived molecules.

The behavior of IL-8 is consistent with its mechanistic role as a potent neutrophil chemoattractant during the early inflammatory response. Experimental gingivitis models have demonstrated that IL-8 rises rapidly following plaque accumulation and can fluctuate dynamically within short time intervals [11]. Thus, the timing of sampling relative to inflammation onset may still influence IL-8 concentrations, and some variability is expected. Additionally, increased GCF volume in inflamed sites may alter absolute cytokine concentrations, although in the present dataset, IL-8 remained elevated despite potential dilution effects. Taken together, these findings support that IL-8 contributes meaningfully to the early inflammatory milieu in pediatric gingivitis; however, its diagnostic performance remains moderate, suggesting that IL-8 may be most informative when interpreted alongside other cytokines within a multiplex biomarker framework.

Although gingivitis in children is often considered a chronic condition, the inflammatory burden is typically mild to moderate and largely confined to superficial gingival tissues. Cytokines such as IL-6 and TNF-α are more closely associated with higher levels of inflammation, tissue destruction, or advanced periodontal pathology, which may explain the absence of significant group differences observed in the present cohort. The absence of significant differences for TNF-α and IL-6 also reflects their context-dependent expression. TNF-α primarily contributes to macrophage activation, endothelial adhesion, and amplification of inflammatory signaling in chronic inflammatory environments [13]. In contrast, IL-6 exhibits both pro- and anti-inflammatory properties, playing roles in acute-phase responses as well as tissue repair. Given the generally mild and reversible nature of plaque-induced gingivitis in children, it is plausible that the expression levels of these cytokines remain below diagnostic thresholds during early or low-grade inflammatory states. These findings are consistent with previous reports indicating that IL-1β functions as a more immediate and sensitive marker of gingival inflammation, whereas IL-6 and TNF-α tend to reflect later or more systemic phases of immune activation [15, 26].

Previous experimental gingivitis (EG) models have consistently reported that IL-1β increases rapidly and markedly in the gingival crevicular fluid (GCF), whereas IL-6, IL-8, and TNF-α display heterogeneous responses across studies. Such inconsistencies are likely influenced by contextual and biological factors, including age, stress, metabolic status, microbial composition, and differences in sampling or assay methodology [11]. Leite *et al*. [27] further proposed that the variation in cytokine behavior may reflect distinct host-response phenotypes. Specifically, “fast” EG responders tend to demonstrate a disorganized cytokine profile characterized by elevated IL-6 and IL-8 levels, while “slow” responders exhibit a more regulated and organized network dominated by TNF-α and IL-10. Considering this framework, the attenuated IL-8 response observed in our pediatric cohort may represent a predominance of “slow” or low-reactivity gingival phenotypes, which could partially explain the absence of severe tissue breakdown despite clinical inflammation.

The combined clinical and biochemical findings of this study provide important insights into the identification and characterization of plaque-induced gingival inflammation in pediatric patients. The most pronounced clinical differences between groups were observed in the gingival index, plaque index, and bleeding on probing, which showed consistent separation and remained the most practical tools for routine clinical identification of gingivitis in pediatric settings [5]. Among the biochemical markers, IL-1β demonstrated the most robust performance, showing significant group differences and the highest discriminatory accuracy (AUC = 0.76), supporting its role as a key mediator of periodontal inflammation and its potential utility as an adjunctive biomarker in research and future diagnostic frameworks. Additionally, IL-8 levels were moderately higher in the gingivitis group and showed moderate discrimination (AUC = 0.64), consistent with its role in neutrophil recruitment during inflammatory responses [27]. While these results do not support IL-8 as a standalone clinical marker, it may provide complementary value when included in a multiplex biomarker panel. In contrast, IL-6 and TNF-α showed minimal discriminatory performance, suggesting context-dependent expression that may be less informative in mild plaque-induced gingivitis and potentially more evident under conditions of higher inflammatory burden or more extensive periodontal involvement. Overall, cytokine profiling may enrich the biological understanding of pediatric gingival inflammation; however, under the current study conditions, IL-1β appears to be the most promising single-marker candidate, whereas other cytokines—particularly IL-8—may be more useful as supportive components within broader biomarker-based models. Importantly, traditional clinical indices remain the foundation for gingivitis diagnosis in daily pediatric dental practice, with biochemical markers serving as adjuncts rather than replacements.

This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design does not allow causal inference or assessment of within-subject cytokine changes over time. Second, although the study was

conducted jointly in two university outpatient clinical settings, the clinic-based recruitment and non-consecutive sampling may limit generalizability to broader community pediatric populations. Third, periodontal status was defined using BOP% thresholds, and the gingivitis group was restricted to generalized plaque-induced gingivitis (BOP% $>$30%), while localized gingivitis cases were not included; therefore, the results may not be directly extrapolated to milder forms or the full clinical spectrum of gingival inflammation. In addition, clinical indices were recorded only for fully erupted teeth, and teeth under eruption were excluded from all periodontal measurements and BOP% calculations to avoid measurement bias; however, this approach may have led to an underestimation of gingival inflammation in children with a higher proportion of erupting teeth. Moreover, GCF sampling was standardized to a single site per participant, which improves methodological consistency but may not fully capture intra-oral (site-to-site) variability in gingival inflammation. Future studies incorporating multi-site and/or repeated sampling designs may better characterize cytokine dynamics and improve external validity. Finally, cytokine quantification was performed using laboratory-based ELISA, and the biomarker panel was limited to four analytes, which may constrain immediate clinical feasibility and the breadth of host-response characterization [21].

Future research should address these limitations by adopting longitudinal and multicenter designs with larger and more diverse pediatric populations. Repeated sampling during both plaque accumulation and resolution phases would help clarify cytokine dynamics and their predictive value for disease progression. Expanding biomarker panels and applying advanced analytical approaches—such as proteomics or machine learning-based classification—may further enhance diagnostic accuracy. Developing standardized pediatric cut-off values for IL-1β and other relevant markers will be critical for successful clinical implementation and integration into chairside diagnostic tools. In this regard, the present study aimed to provide preliminary evidence supporting the potential future use of chairside or rapid diagnostic approaches for early detection of gingival inflammation in children, and we believe that this perspective represents an important initial step in this area of research. Early identification of gingival inflammation prior to the appearance of overt clinical signs may contribute to improved preventive strategies and reduce the risk of progression to periodontitis.

5. Conclusions

Identification of plaque-induced gingival inflammation in pediatric patients is considered important for informing preventive strategies and supporting positive oral health behaviors. The present study suggests that combining conventional clinical periodontal indices with gingival crevicular fluid-based cytokine profiling, particularly IL-1β assessment, may provide a sensitive, non-invasive, and child-friendly approach for characterizing inflammatory activity. However, cytokine quantification in the current study was performed using ELISA, which requires laboratory infrastructure, trained personnel, and associated costs, limiting its feasibility for routine chairside use at present. Therefore, these findings should be interpreted primarily as preliminary and hypothesis-generating, supporting the biological relevance of IL-1β as an adjunctive marker rather than a replacement for clinical indices. As biochemical diagnostic techniques become more accessible and simplified in the future (*e.g.*, point-of-care or rapid immunoassay platforms), approaches such as those explored here may help inform subsequent translational research. Overall, integrating clinical evaluation with accessible molecular markers may contribute to improved periodontal surveillance during childhood and guide preventive care frameworks.

ABBREVIATIONS

AUC, Area Under the Curve; BOP, Bleeding on Probing; CI, Confidence Interval; EG, Experimental Gingivitis; ELISA, Enzyme-Linked Immunosorbent Assay; GCF, Gingival Crevicular Fluid; GI, Gingival Index; ICC, Intraclass Correlation Coefficient; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IL-8, Interleukin-8; NPV, Negative Predictive Value; PPD, Probing Pocket Depth; PI, Plaque Index; PPV, Positive Predictive Value; ROC, Receiver Operating Characteristic; rpm, Revolutions Per Minute; SD, Standard Deviation; SPSS, Statistical Package for the Social Sciences; TNF-α, Tumor Necrosis Factor-α; μL, Microliter; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

MU—prepared the initial draft of the manuscript. ZUG—critically revised and refined the text, provided overall study supervision and coordination. Both authors reviewed and approved the final version of the manuscript, contributed to data collection, formal analysis, and statistical evaluation; conceptualized the study, and developed the methodological framework.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This cross-sectional clinical study was approved by the Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University (Protocol No: 2025/119). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all parents or legal guardians prior to their children's participation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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