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# Evaluation of Levels of Interleukins in GCF and Saliva in Patients with Periodontitis Over Healthy

*Anuj Singh Parihar[1], Tanu Priya Sonkar[2], Minal Awinashe[3], Rashmita Nayak[4], Murali PS[5], Amol Jain[6], T. Uma Arasu[7]*

[1]*Department of Periodontology, People's Dental Academy, Bhopal, MP, India.*

[2]*Department of Prosthodontics, Rural Dental College, Loni, Maharashtra, India.*

[3]*Department of Oral Surgery and Diagnostic Sciences, College of Dentistry, Qassim University, Buraidah, Kingdom of Saudi Arabia.*

[4] *Professor, Department of Periodontics, Institute of Dental Sciences, SOA University, Bhubaneswar, Odisha, India.*

[5]*Department of Orthodontics & Dentofacial Orthopedics, NITTE (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Derlakatte, Mangalore, Karnataka, India.*

[6]*Department of Oral Pathology and Microbiology, Sardar Patel Post graduate Institute of Dental and Medical Sciences, Lucknow, UP, India.*

[7]*Department of Microbiology, Swamy Vivekanandha Medical College Hospital & Research Institute, Elayampalayam, Namakkal District, Tamilnadu, India.*

**Abstract**

The objective of the current research was to evaluate interleukens levels in the saliva and GCF for patients with generalized periodontitis and healthy subjects.GCF and saliva samples were taken from 20 patients with generalised periodontitis and 20 healthy participants for this investigation. Interleukin levels were measured using the enzyme linked immune absorbent assay (ELISA).Patients with periodontitis had greater mean levels of IL6, IL 8, IL 12, and IL 17 in their saliva or GCF compared to healthy individuals. These findings imply that the pathogenesis of periodontitis may be influenced by the amounts of IL6, IL 8, IL 12, and IL 17 in GCF or saliva, and that measuring these cytokines may help identify patients who have the disease. Moreover, some periodontal disease biomarkers are more easily found in saliva, whereas others are more easily found in GCF. Compared to healthy individuals, patients with periodontitis reported higher mean levels of IL 6, IL 8, IL 12, and IL 17 in their GCF or saliva.

**Keywords:** Crevicular fluid, interleukin, saliva

**Full-length article** **Corresponding Author*, e-mail:tanupriya2627@gmail.com, anilkk44@gmail.com

## 1. Introduction

Periodontitis is a multifactorial, chronic infectious disease that is typified by the irreversible breakdown of matrix components of the gingiva, periodontal ligament, and alveolar bone around the teeth, including collagen fibres [1]. Periodontal pockets arise as a result of the irreversible deterioration of both soft and hard periodontal tissues caused by periodontitis [2].Clinical markers including as clinical attachment loss, bleeding scores, periodontal probing pocket depth, and radiographic evidence of bone loss are currently used by clinicians to diagnose and evaluate periodontal disease. It would therefore be extremely beneficial for clinical practise to identify biological markers at the patient level that might be utilised to assess health status, identify the evolution of an illness, highlight risk dynamics, and forecast treatment outcomes [3]. It is understood that the dynamic equilibrium of interactions between the host's immuno-inflammatory responses and the microbial assault determines the severity of periodontal disease. Membrane-associated lipopolysaccharide (LPS) activation of pro-inflammatory cytokines and inflammatory mediators from different host cells via a critical cell stimulation pathway is required for the recognition of gram-negative bacteria [1].The influx of T cells into the periodontal tissues, followed by their differentiation into diverse subsets including helper T cells, cytotoxic T cells, and regulatory T cells, is what defines the cellular immune response [4]. Pro- and anti-inflammatory cytokines, as well as certain cytokine receptors, are components of the intricate cytokine network that plays a role in the immune system [5]. The production of cytokines and chemokines by immune cells is crucial for the regulation of inflammation and the immune system. The subsets of T helper (Th) cells often determine the various cytokine profiles. Different cytokine patterns are associated with distinct subgroups of Th cells. Interleukin (IL) 1β, IL-2, IL-12, interferon gamma (IFN-γ), and tumour necrosis factor alpha (TNF-α) are the primary substances produced by Th1 cells, and they all trigger cellular immune responses. Th2 cells are the primary producers of IL-4, -5, -6, -10, and -13, which trigger the humoral immune response [6].Recent advancements in our understanding of the aetiology of

periodontal disorders have demonstrated the crucial roles that cytokines play in the onset and progression of periodontitis. In order to use biomarkers to stop the disease's progression as soon as possible, a "futuristic" periodontal diagnostic will be used in the near future to detect periodontal disease before it becomes clinically visible. Biomarkers, which are biological indicators of strong prognostic and predictive importance, can be used to determine the onset or course of a condition. They need to be highly predictive or prognostic [3]. Saliva is a complex system that includes invading pathogens, locally released chemicals, and systemic metabolites that reflect distant processes. Periodontology places a high value on the contribution of gingival crevicular fluid components, which may provide information about an individual's state of periodontal health. Saliva has therefore been proposed as a key resource for the identification of periodontal diagnostic biomarkers [7]. Both biological indicators of the inflammatory immune response to the plaque biofilm and indicators of the subsequent effects on the connective tissue attachment apparatus are present in the gingival crevicular fluid [3]. The bulk of studies examining the relationship between IL-1β and periodontitis have sampled using gingival crevicular fluid (GCF). GCF sampling, however, takes time and only captures periodontal inflammation at the individual sites examined [1].

In this study, the amounts of interleukins in GCF and saliva were examined between healthy individuals and patients with periodontitis.

## 2. Materials and Methods

### *2.1. Periodontal examination*

After receiving informed consent from participants and ethical clearance, this study was carried out at the Department of Periodontology. In this study, there were 40 participants: 20 patients with generalised periodontitis and 20 healthy persons who served as controls. Williams's periodontal probe was used to score six spots per tooth, and the third molar was not included.For the subjects to be classified as part of the periodontitis group, they had to meet the following requirements. With the exception of third molars, there needed to be a minimum of 18 teeth total (at least 12 of which needed to be posterior teeth), no systemic illness, moderate to severe periodontitis, no medication use within the previous six months, and no history of periodontal therapy within the previous three months. Pregnant women and smokers were excluded from the study. The periodontal status of each subject was assessed using the following criteria: subjects with periodontally healthy conditions should have <10% of sites with BOP, no PD >3 mm, and no clinical attachment loss; patients with generalised periodontitis should have >30% of the sites with PD and/or CAL 5 mm and BOP.

### *2.2. Collection of GCF*

After choosing a site and isolating it with cotton rolls, absorbent paper points of size 30 were used to gather GCF samples. These were inserted into the sulcus or pocket until a slight resistance was felt, and they were then held there for 30 seconds. Blood or saliva-contaminated paper points were not accepted. Every subject received four paper points. Immediately, each sterile polypropylene tube containing all the paper points with GCF was filled with 100 µl of phosphate buffer saline. The tubes were then gently shaken for an hour at ambient temperature and stored at -20°C until additional analysis was performed.

### *2.3. Collection of saliva*

Spit that was clean and hadn't been animated was gathered and kept in sterile research centre cups until it was brought to the lab for examination. For ten minutes, each sample was centrifuged at 3000 rpm in order to eliminate undesired free salivary particles. The clear salivary solution was then aspirated using micropipette tips for the last immunological examination inquiry utilising enzyme linked immunosorbent assay (ELISA) assays, and it was stored in a 1 ml sterile Eppendorf tube for freezing at (−20°C).

### *2.4. ELISA*

Using the Elabscience® ELISA kit, interleukins were measured in human saliva and gingival crevicular fluids. The ELISA kit protocol was followed in order to calculate the samples' mean absorbance.

## 3. Results

When comparing periodontitis patients with healthy people, the clinical periodontal indices (PS, BOP, PPD, and CAL) were considerably lower in the latter group (Table 1). Table 1 displays the clinical periodontal data for every tooth, including the sampling sites. Compared to healthy individuals, periodontitis patients had considerably greater levels of the periodontal markers PS, BOP, PPD, and CAL. Among healthy subjects, there was no discernible variation in the levels of interleukin in either saliva or GCF (Table 2). Table 2 displays the amounts of saliva and GCF IL 8, IL 6, IL 12, and IL 17 in patients with periodontitis. While there is no discernible difference between the levels of IL 8 in saliva and GCF, IL 6 levels were considerably greater in GCF than in saliva. Moreover, saliva had far higher concentrations of IL 12 and IL 17 than GCF.Table 3 clearly illustrates the difference in cytokine levels between periodontitis patients and healthy individuals in GCF and saliva. There is no discernible difference in the salivary levels of IL 8 and IL 6 between periodontitis patients and healthy individuals. On the other hand, salivary levels of IL 17 were considerably greater in healthy subjects, but CP patients' levels of IL 12 were significantly higher.In terms of GCF cytokine levels between healthy individuals and CP patients, CP patients had higher levels of IL 8, 6, and 17 than healthy individuals; however, this difference was not statistically significant. The levels of IL 12 in CP patients were notably greater than in healthy individuals.

## 4. Discussion

The current investigation shows the levels of interleukins in GCF and saliva in both healthy individuals and periodontitis patients. This study's primary goal was to compare the concentrations of biomarkers in saliva and gingival tissue in an effort to ascertain which biofluids provide a more accurate expression of biomarkers in patients with periodontal disease.

**Table 1.** Clinical periodontal parameters (plaque score, BOP, PPD, and CAL) for the periodontitis patients and control subjects

| **Clinical parameters** | **Patients with Generalized periodontitis** | **Control subjects** | p |
|---|---|---|---|
| PS (%) | 38.35±11.75 | 9.39±3.36 | 0.000* |
| BOP (%) | 32.28±6.26 | 6.1±1.1 | |
| PPD (mm) | 3.3±0.6 | 1.48±0.31 | |
| CAL (mm) | 3.28±0.5 | 0 | |

*$P<0.05$: significant difference, *t*-test

**Table 2.**The difference between interleukins levels in saliva and GCF among healthy participants and with periodontitis

| **In health participants** | | | **In participants with periodontitis** | | |
|---|---|---|---|---|---|
| **Interleukins** | **Mean±standarddeviation** | p | **Interleukins** | **Mean±standarddeviation** | p |
| IL-8 in saliva | 158.65±49.76 | 0.318 | IL-8 in saliva | 212.56±92.36 | |
| IL-8 in GCF | 168.76±23.56 | | IL-8 in GCF | 232.88±52.45 | 0.240 |
| IL-6 in saliva | 92.67±51.02 | 0.022 | IL-6 in saliva | 146.58±64.87 | |
| IL-6 in GCF | 138.21± 24.07 | | IL-6 in GCF | 176.58±38.87 | 0.002 |
| IL-12 in saliva | 201.12±69.57 | 0.076 | IL-12 in saliva | 416.86±187.76 | |
| IL-12 in GCF | 142.32±62.11 | | IL-12 in GCF | 212.28±51.86 | 0.000 |
| IL-17 in saliva | 42.75±21.25 | 0.457 | IL-17 in saliva | 75.48±32.26 | 0.000 |
| IL-17 in GCF | 39.22±11.58 | | IL-17 in GCF | 51.85±14.47 | |

**Table 3.** The difference between Interleukins levels in saliva and GCF among healthy subjects and patients with periodontitis

| **Interleukins** | **Mean±standard deviation** | p | **Interleukins** | **Mean±standarddeviation** | p |
|---|---|---|---|---|---|
| IL-8 in CP | 212.56±92.36 | 0.108 | IL-8 in CP | 232.88±52.45 | 0.131 |
| IL-8 in H | 158.65±49.76 | | IL-8 in H | 168.76±23.56 | |
| IL-6 in CP | 146.58±64.87 | 0.172 | IL-6 in CP | 176.58±38.87 | 0.012 |
| IL-6 in H | 92.67±51.02 | | IL-6 in H | 138.21± 24.07 | |
| IL-12 in CP | 416.86±187.76 | 0.000 | IL-12 in CP | 212.28±51.86 | 0.000 |
| IL-12 in H | 201.12±69.57 | | IL-12 in H | 142.32±62.11 | |
| IL-17 in CP | 75.48±32.26 | 0.000 | IL-17 in CP | 51.85±14.47 | 0.025 |
| IL-17 in H | 42.75±21.25 | | IL-17 in H | 39.22±11.58 | |

The primary objective of biomarker research in periodontology is the development of diagnostic techniques that have a significant influence on clinical decision-making by dentists and other healthcare practitioners. In the realm of periodontal diagnostics, GCF and saliva have been the primary subjects of oral fluid-based biomarker investigations [8].

The current study's findings demonstrated that every interleukin under investigation was found in every study sample. Furthermore, among healthy subjects, there was no discernible variation in the amounts of interleukin in either saliva or GCF. This may be explained by the absence of an inflammatory reaction to bacterial challenges, which are common when periodontal disorders are present.There is no discernible difference in the IL 8 levels in saliva and GCF among CP patients in the current investigation. The levels of several biomarkers in saliva and GCF among healthy individuals and patients with gingivitis and chronic periodontitis were shown by Afacan et al. in their study [9]. When CP patients' saliva and GCF were compared to those of healthy people, IL 12 levels were considerably greater in the former. In their investigation, Tsai et al. also discovered that, in comparison to healthy individuals, CP and gingivitis patients had greater amounts of IL 12 [10]. Before and after therapy, Yue et al. assessed the presence of cytokines in the saliva and gingival crevicular fluid (GCF) of patients suffering from aggressive periodontitis (AP). They found that there was a strong correlation between cytokine levels in GCF and saliva and clinical indicators as well as AP. Saliva cytokine measurements might be thought of as a rapid, non-invasive way to track the progression of periodontal disease [6].The proinflammatory cytokine IL-1β was measured in the saliva of individuals with and without periodontal disease, and its correlation with periodontal status was evaluated by Tobón-Arroyave et al. According to the study, one of the host-response factors linked to the clinical manifestations of periodontal disease may be the raised IL-1β content. It also emphasises the significance of using entire saliva as a sampling method for immunological purposes in periodontal disease [1].

In their comprehensive review, Finoti et al. gather data on the amounts of IL8 mRNA and protein in gingival tissue, saliva, and gingival crevicular fluid (GCF) that were examined in CP patients. Furthermore, two meta-analyses that examined the levels of IL-8 in GCF and saliva from patients with and without CP came to the conclusion that, in gingival tissues of CP patients, IL-8 gene expression and IL-8 protein levels were higher than in those in periodontally healthy persons [11].Majeed et al. assessed the levels of IL6, IL 8, IL 12, and IL 17 in the GCF and saliva of patients suffering from generalised periodontitis and compared them with those of healthy individuals. They propose that the pathogenesis of periodontitis may be influenced by the amounts of IL6, IL 8, IL 12, and IL 17 in GCF or saliva [3]. This complies with our financing sources. Sari et al. assessed the levels of IL-39 in gingival crevicular fluid (GCF) in both healthy and diseased gum tissue and associated them with GCF levels of periostin and IL-1β.They came to the conclusion that when periodontal disease was present, IL-39 levels were raised in tandem with an increase in IL-1β and periostin levels. The process of periodontal inflammation may involve IL-39 [5].

Enver et colleagues assessed the amounts of TNF-α, IL-1β, IL-10, and IL-17A in the saliva and GCF, as well as their correlations with the periodontal statuses of patients with UC, CD, and non-IBD. They also examined the connections between these cytokines, IBD disorders, and periodontal diseases. Researchers discovered that individuals with periodontal illnesses, such as ulcerative colitis (UC) and Crohn's disease (CD), expressed TNF-α, IL-1β, and IL-10 at distinct expression levels in their oral secretions as compared to people without inflammatory bowel diseases (IBD) [12].IL-1β levels and the clinical indicators of periodontal disease progression were associated by Chaudhari et al. They came to the conclusion that the quantity of GCF IL-1β and periodontal health are tightly related. Monitoring the progression of periodontal disease may benefit from this relationship [13]. When comparing aggressive periodontitis to chronic periodontitis, Batra et al. found that the former had greater levels of IL-34 [14]. It is possible to think of GCF's cytokines as indicators for the deterioration of periodontal tissue [4].According to Aleksandrowicz et al.'s conclusion, cytokine level analysis may provide light on the aetiology, early detection, and prognosis of peri-implantitis in high-risk individuals [15]. Yavuz and Çanakçı discovered that the group with uncontrolled diabetes had the greatest amounts of serum, saliva, and GCF visfatin [16].

As opposed to what our research revealed, Esfahrood et al. Comparable IL-18 levels across research groups indicated that GCF and salivary IL-18 levels cannot be relied upon as a reliable biomarker for the early detection of periodontal disease [17].The reduction of the bacterial count in supragingival and subgingival plaque samples is mostly dependent on IgY [18]. Additional research is required to verify the findings.

## 5. Conclusions

Compared to healthy individuals, patients with periodontitis reported higher mean levels of IL 6, IL 8, IL 12, and IL 17 in their GCF or saliva. These results suggest that the pathophysiology of periodontitis may be influenced by the concentrations of IL 6, IL 8, IL 12, and IL 17 in GCF or saliva.

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