

# Level of Interleukin-17 in Gingival Crevicular Fluid of Patients with Chronic Periodontitis

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## ABSTRACT

**Aim:** Interleukin-17 (IL-17) is a proinflammatory cytokine that plays an important role in inflammation and tissue destruction in periodontal disease. This study aimed to assess the level of IL-17 in gingival crevicular fluid (GCF) of patients with chronic periodontitis (CP) and healthy controls.

**Materials and methods:** This case-control study was performed on 30 patients with CP (53% males, 47% females, mean age of  $37.2 \pm 5.95$  years) and 30 healthy controls (53% males, 47% females, mean age of  $30.63 \pm 5.22$  years). The GCF was collected using paper points. A paper point was inserted into the pocket and remained there for 30 seconds. It was then placed in a sterile tube containing 300  $\mu$ L of phosphate buffered saline and stored at  $-70^{\circ}$  C. Level of IL-17 was measured using enzyme-linked immune sorbent assay (ELISA). The level of IL-17 was compared between the two groups using independent sample t-test at 0.05 level of significance.

**Results:** The mean GCF level of IL-17 was 53.46 pg/L in CP patients and 38.1 pg/L in healthy controls. This difference was statistically significant ( $p = 0.025$ ).

**Conclusion:** The CP patients had significantly higher GCF level of IL-17 compared to healthy controls.

**Clinical significance:** The finding of this study highlight the role of IL-17 in the pathogenesis of periodontal disease. Within the limitations of the present study, it may be suggested that measurement of GCF level of IL-17 can serve as a bioindicator of periodontal destruction and gingival inflammation.

**Keywords:** Chronic periodontitis, Gingival crevicular fluid, Interleukin-17.

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## INTRODUCTION

Periodontal disease is defined as the inflammation of the supporting tooth structures. It is caused by periopathogenic microorganisms and leads to the progressive breakdown of the periodontal ligament and alveolar bone resulting in pocket formation, gingival recession or both.<sup>1</sup> The interaction of bacteria and the immune system leads to the progression of periodontal disease.<sup>1-3</sup>

Cytokines and chemokines control these interactions. These mediators are released from macrophages/monocytes, dendritic cells, lymphocytes, neutrophils, endothelial cells, and fibroblasts.<sup>4</sup> Production and release of some cytokines promote the immune system while other types result in cell destruction and progression of the disease.<sup>5</sup>

Assessment of the host response is an advanced diagnostic method for the detection of periodontal disease. This includes an assessment of the level of specific and non-specific mediators, as part of the individual response to periodontal infection by biochemical and immunologic methods. Saliva, GCF, and serum can be used for assessment of the level of these cytokines.<sup>6</sup> Cytokines are proteins released from the cells of the innate and acquired immunity in response to microorganisms and antigens to control the activity of immune cells. Cytokines stimulate the response of cells involved in immunity and inflammation. In this regard, a complex mixture of cytokines is produced in response to microorganisms, playing a major role in periodontal disease development and progression.<sup>7</sup>

Interleukin-17 (IL-17) is a pro-inflammatory cytokine mainly produced by Th17 cells. It affects the epithelium, endothelium, and fibroblast cells and leads to secretion of IL-6 and IL-8. In the past, periodontal disease used to be diagnosed based on radiographic and clinical evidence including pocket depth, attachment loss, and degree of inflammation, microbial accumulation and presence of exudate and treated accordingly. However, evidence shows that these methods were not efficient for detection of periodontal disease and its appropriate management because periodontal pockets may be present in the active or passive state of disease<sup>8</sup> and old techniques could not differentiate between the active and passive periodontal pockets. Assessment of the composition of GCF may be considered as a sensitive technique for detection of periodontitis. This has been investigated in

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a few previous studies and more research is still needed to prove this relationship a develop future chair-side tests. Pradeep et al.<sup>4</sup> evaluated the presence of IL-17 and IL-18 in GCF of patients with periodontitis and healthy controls and according to their results, the concentration of IL-17 was equal (0) in both groups while there was a greater concentration of IL-18 in the periodontitis group. Al-Hassan et al.<sup>9</sup> evaluated the possible role of IL-17 in periodontal disease and observed that the level of IL-17 in the serum of periodontal patients was higher compared to healthy controls.

These findings highlight the role of IL-17 in the pathogenesis of periodontal disease. Considering the gap of information and the existing controversy in the role of IL-17 in the pathogenesis of periodontal disease and its GCF level in patients and the need for further investigation, this study aimed to comparatively access the GCF level of IL-17 in CP patients and healthy controls.

## MATERIALS AND METHODS

This case control study was conducted on 60 individuals including 30 patients with CP) presenting to the Periodontology Clinic of Zahedan University of Medical Sciences, School of Dentistry and 30 healthy controls.

The minimum sample size was calculated to be 40 according to a previous study.<sup>10</sup> To increase the accuracy of the findings, 60 individuals were enrolled using convenience sampling ( $Z_1 = 1.96$ ,  $Z_2 = 1.64$ ,  $S_1 = 1.02$ ,  $S_2 = 0.98$ ).

The inclusion criteria for the patient group was a clinical diagnosis of untreated CP by a periodontist with probing depth (PD)  $\geq 5.0$  mm, CAL  $\geq 3.0$  mm, the presence of bleeding on probing and gingival discoloration.

The exclusion criteria were pregnancy, menopause, patients with less than 22 permanent teeth, systemic diseases affecting the periodontal tissue such as diabetes mellitus, immune disorders, AIDS, conditions requiring antibiotic therapy such as history of cardiovascular diseases and joint replacement, history of antibiotic therapy in the past 3 months, history of periodontal treatment in the past year, no history of scaling in the past six months, smoking, alcohol consumption, severe caries, and chronic inflammatory conditions of the skin and oral mucosa such as lichen planus, pemphigus, psoriasis, aphthous ulcers and estrogen therapy.

The controls were healthy and had no signs/symptoms of gingivitis or periodontitis. Healthy controls had the probing depth of less than 3 mm (measured by a

Williams probe) in all teeth and had no clinical symptoms of gingival inflammation. They were recruited among age and sex-matched patient companions. This study was approved by the Ethical Committee of Zahedan University of Medical Sciences and all patients signed an informed consent form before the study.

For a collection of GCF, the tooth with the deepest pocket was first cleaned and isolated with cotton rolls. A paper point was inserted into the pocket and remained there for 30 seconds. It was then placed in a sterile tube containing 300  $\mu$ L of phosphate buffered saline and stored at  $-70^\circ$  C. Level of IL-17 was measured using ELISA.

The mean and standard deviation of the level of IL-17 were calculated and reported. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc., IL, USA), and the two groups were compared using independent sample t-test. Level of significance was set at 0.05.

## RESULTS

There were 16 (53%) males and 14 (47%) females in the control group and 16 males (53%) and 14 (47%) females in the patient group. The mean age of the participants was  $33.92 \pm 6.46$  years. The mean age was  $30.63 \pm 5.22$  years (range 25–42 years) in healthy controls and  $37.2 \pm 5.95$  years (range 25–45 years) in the patient group (Table 1).

The mean GCF level of IL-17 was 53.46–45 pg/mL (range 23.8–87.1 pg/mL) in patients and  $38.18 \pm 11.23$  (range 26.2–73.1 pg/mL) in healthy controls (Table 2)

According to the t-test, the mean GCF level of IL-17 in CP patients was significantly higher than healthy controls,  $p = 0.025$  (Fig. 1 and Table 3).

## DISCUSSION

Periodontitis is a multifactorial disease caused by microbial plaque. However, its extension and severity depend on environmental factors, acquired diseases and genetics. Destruction of tooth-supporting structures, tooth mobility, and eventual tooth loss are among the most important complications of periodontitis.<sup>11,12</sup>

The IL-17 is considered a proinflammatory cytokine released from T-cells.<sup>13</sup> It plays a fundamental role in inflammation and auto-immune diseases and has a synergistic effect with tumor necrosis factor alpha.<sup>14</sup> It plays a significant role in inflammation and periodontal destruction in periodontitis.<sup>15</sup> IL-17 seems to have a

**Table 1:** Mean and standard deviation, minimum and maximum age of healthy and patient's groups

	No.	Mean	Standard deviation	Minimum age	Maximum age
Healthy control	30	30.63	5.22	25	42
Patient	30	37.2	5.95	25	45
Total	60	33.92	6.46	25	45

**Table 2:** Mean and standard deviation of IL-17 in healthy and patient's groups

Group	No.	Mean	Standard deviation
Healthy controls	30	38.18	11.23
Patients	30	53.46	45

serious role in the bone loss, and this cytokine stimulates cell differentiation in bone metabolism.<sup>16</sup> It has been also shown that IL-17 family may play an important role in tissue homeostasis.<sup>17</sup>

The GCF is an important physiological fluid that contains high levels of biological markers indicative of systemic conditions. It is an inflammatory exudate that contains bacteria, leukocytes, electrolytes such as sodium and potassium, calcium, carbohydrates, proteins, metabolic compounds such as hydroxyl proline, lactic acid, hydrogen sulfide and enzymes such as acid phosphatase, cathepsin, alkaline phosphatase, and transaminase.<sup>18</sup> It also has protective and antibacterial properties.<sup>19</sup> Researchers have reported a correlation between the amount and composition of GCF and severity of gingival inflammation.<sup>20</sup>

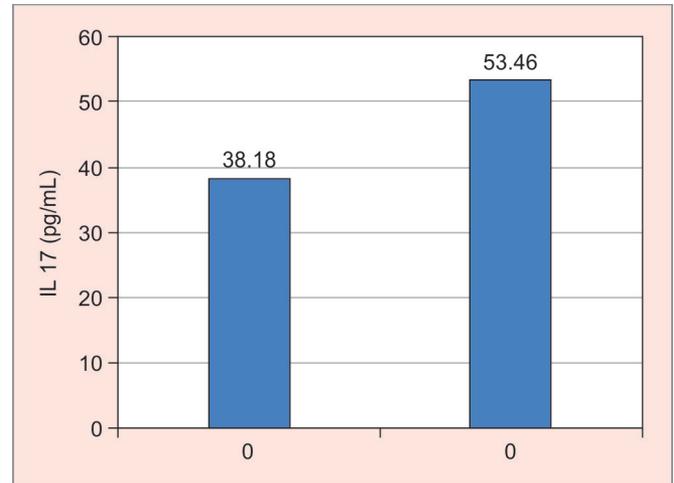
The results of the present study showed that CP patients had significantly higher GCF level of IL-17 compared to healthy controls.

In a similar study, Vernal et al.<sup>21</sup> also indicated that IL-17 levels showed a statistically significant difference with greater levels in periodontal patients than controls (45.9 pg/mL versus 35.6 pg/mL), which was in agreement with our findings.

Shaker and Ghallab,<sup>8</sup> Al-Hassan et al.,<sup>9</sup> Ohyama et al.,<sup>22</sup> and Takahashi et al.<sup>23</sup> have also previously reported the correlation of IL-17 with periodontal disease.

Nagireddy et al.<sup>24</sup> stated statistically meaningful higher amounts of IL-17 in the gingival crevicular fluid of CP patients in their study compared to healthy controls. Moreover, they pointed to the significant correlation of the level of IL-17 with the degree of periodontal destruction and stated that GCF level of IL-17 can serve as an efficient indicator for periodontal disease. It was also recently demonstrated by Sunandhakumari et al. that plasma levels of IL-17 positively correlated with the periodontal condition and HbA1C levels, and nonsurgical periodontal therapy reduced IL-17 in well-controlled diabetic and systematically healthy patients with chronic periodontitis.<sup>25</sup>

Zhao et al. have also found that IL-17, IL-21 decreases in chronic periodontitis patient's GCF after nonsurgical

**Fig. 1:** Comparison of GCF levels of IL-17 in CP patients and healthy groups

therapy.<sup>26</sup> Indicating the destructive role of this cytokine in the immunological balance of periodontitis.

Cardoso et al. have also demonstrated expression of IL-17 in diseased alveolar bone and presence of Th 17 cell in gingiva from Patients with chronic periodontitis.<sup>27</sup> They reported that IL-17 is produced in Periodontal lesion, and it has a potential role in the pathogenesis of periodontal disease which is consistent with the findings of the current study. Rohaninasab et al. demonstrated the mean IL-17 Saturation in GCF significantly decrease after periodontal therapy.<sup>28</sup> Lester et al.<sup>29</sup> have also found an increased concentration of IL-17 in gingival tissue supernatants at sites of severe attachment loss they have reported a positive correlation of tissue level of this cytokine and clinical attachment loss and destruction. Increased level of IL-17 has also been shown to reflect its potential role in the etiopathogenesis of aggressive periodontitis.<sup>22</sup>

However, Pradeep et al.<sup>4</sup> reported results different from the present study and the previously mentioned reports. They indicated that the GCF level of IL-17 is not suitable as a biological indicator of periodontitis in the Indian population. But, they reported that IL-18 can be used as an efficient indicator of inflammation. Difference between these results may be due to a different method of sampling since micro-capillary pipettes were used instead of paper cones for the collection of GCF. Also, it was not reported to what degree they diluted the samples for the conduction of ELISA. Avani et al. have also indicated an absence of IL-17 in GCF of Indian patients with periodontal disease.<sup>4</sup>

**Table 3:** T-test \* for comparison of mean IL-17 in healthy and patient's groups

High limit	Low limit	Difference in averages	p value	Degree of freedom	t value	95% confidence for difference
-0.99256	-14.44744	-7.72000	0.025	56	-2.299	IL-17

\*Independent sample t-test

In the search for finding simple methods of detection of the destruction of the periodontium in patients with periodontitis and their immunological susceptibility to more severe forms of this disease researchers have also investigated cytokine levels in saliva. In Ozcaka et al. study, whole saliva and blood sample from healthy untreated nonsmokers with chronic periodontitis and systemically and periodontal healthy control subjects were compared to determine IL-17 and IL-18 concentration. Their finding showed that salivary concentration of IL-17 in chronic periodontitis subjects was lower than healthy subjects but IL-18 saliva levels were significantly higher, in patients from the chronic periodontitis plasma IL-17 concentration was similar in both groups.<sup>30</sup>

Their results may be due to the fact that increased gingival crevicular fluid levels of IL-17 observed in previous studies indicate a local event adjacent to areas of bone resorption and periodontal pockets, while there is still no systemic change in the levels of this cytokine and therefore, saliva or plasma samples may not reveal significant effects of changes in IL-17 content in periodontal destruction.

It still seems that future studies are required to assess and compare the level of IL-17 before and after treatment to better elucidate its role in the course of the periodontal disease.

## CONCLUSION

The GCF level of IL-17 in patients with CP was significantly higher than that in healthy controls. Thus, Within the limits of the present study, it may be suggested that GCF level of IL-17 can serve as a bioindicator of periodontal destruction and gingival inflammation.

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