

**How to Cite:**

Mittal, S., Waghmare, P., Dodwad, V., Mariam, S., Bhosale, N., & Gaikwad, I. (2022). Comparison of serum interleukin 34 levels in chronic periodontitis patients before and after periodontal flap surgery. *International Journal of Health Sciences*, 6(S5), 2231–2240.  
<https://doi.org/10.53730/ijhs.v6nS5.9130>

## **Comparison of serum interleukin 34 levels in chronic periodontitis patients before and after periodontal flap surgery**

**Dr. Sonal Mittal**

Post Graduate, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

Corresponding author Email: [sonalmittal008@gmail.com](mailto:sonalmittal008@gmail.com)

**Dr. Pramod Waghmare**

Associate Professor, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

**Dr. Vidya Dodwad**

HOD, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

**Dr. Sarah Mariam**

Assistant Professor, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

**Dr. Nishita Bhosale**

Assistant Professor, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

**Dr. Ishani Gaikwad**

Post Graduate, Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India

**Abstract**--Background: Interleukin-34 (IL-34) is a recently identified alternative ligand for colony-stimulating factor-1 receptor which plays an important role in osteoclastogenesis. The aim of this study was to evaluate the IL-34 levels in serum in subjects with chronic periodontitis and to evaluate the effect of surgical periodontal therapy on the serum IL-34 levels. Materials and Methods: Thirty individuals (age range: above 20 years) with chronic periodontitis were selected and divided into groups: Group I (chronic periodontitis patients before periodontal flap surgery), while Group II patients after periodontal flap surgery Ramfjord periodontal diseases index and sulcus bleeding index (Muhlemman and Sons 1971) were examined. Serum samples

were collected to estimate the levels of IL-34 using enzyme-linked immunosorbent assay kit. Results: The mean IL-34 concentration in serum was highest for Group I and it significantly decreased after periodontal flap surgery. The difference between them was statistically significant ( $P < 0.05$ ). Conclusion: IL-34 can be considered as an “inflammatory marker” of periodontal disease and can be explored in the future as a potential therapeutic target in the treatment of periodontal disease.

**Keywords**---Interleukin 34, proinflammatory cytokines, chronic periodontitis, therapeutic agent.

## Introduction

Inflammation is the physiological response to a variety of injuries or insults, including heat, chemical agents or bacterial infection [1]. Periodontitis is inflammation of the periodontium that extends beyond the gingiva and produces destruction of the connective tissue attachment of the teeth. It is the extension of the inflammatory process initiated in the gingiva to the supporting periodontal tissues. Periodontitis results in the destruction of the supporting connective and bony tissues of the teeth. Human periodontal ligament (HPDL) cells are exposed to periopathogenic factors and inflammatory cytokines [2,3]. An imbalance between the plaque biofilm and the host immune system results in overexpression of an array of Proinflammatory Cytokines [4], propagation of inflammation through gingival tissues leading to destruction of connective tissue subsequent destruction of alveolar bone. This is the hallmark of periodontal disease. Proinflammatory cytokines increases the bactericidal activity of phagocytes, recruit additional innate cell population to site of infection and play a central role in periodontal tissue degradation. IL-34 is a novel cytokine that was identified in 2008 in a comprehensive proteomic analysis as a tissue-specific ligand of CSF-1 receptor (CSF-1R). IL-34 has been identified as a novel and moderate inflammatory cytokine that significantly induces macrophage activation and migration and plays an important role in inflammation, as it increases IL-6 and chemokine levels in human whole blood [5],[6] Furthermore, IL-34 is produced by synovial and gingival fibroblasts in response to TNF- $\alpha$  and IL-1 $\beta$  through NF- $\kappa$ B and c-JunN-terminal kinase pathways.

Hence, IL-34 can be considered as an “inflammatory marker” of periodontal disease and can be explored in the future as a potential therapeutic target in the treatment of periodontal disease. Until today, no studies have reported the levels of IL-34 in chronic periodontitis affected. Individuals before and after the surgical periodontal treatment. In the light of the above facts, present study was designed to estimate the levels of IL-34 levels in serum in chronic generalized periodontitis-affected individuals after periodontal flap surgery.

Patients with inflammatory periodontal disease have elevated serum levels of proinflammatory cytokines. Depending on the pathological conditions, increased formation of IL-34 amplifies inflammatory cascades. The level of IL-34 increases with an increase in amount of periodontal destruction It can be postulated that

IL-34 level decreases with periodontal destruction. Thus IL-34 can be considered as potential inflammatory marker of periodontal destruction and also act as therapeutic target in management of illness.

### **Material and Methods**

A total of 30 subjects were selected from those visiting Out Patient Department of Periodontology, Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune.

#### **Inclusion criteria**

1. Systematically healthy individuals
2. Age above 20 years
3. Individuals with chronic periodontitis
4. Individuals with periodontal pocket depth of more than 5mm

#### **Exclusion criteria**

1. Individuals with systemic diseases.
2. Individuals who have received surgical periodontal treatment in last 6 months.
3. Pregnant or lactating women.
4. Individuals with antibiotic therapy in the last 3months.
5. Smokers

#### **Study Protocol**

The research proposal was approved by the institutional ethics committee (IEC) of the University to carry out the research project. Subject's coming to Out Patient Department in Post graduate clinic of Department of Periodontology, Bharati Vidyapeeth Deemed to be University Dental College and Hospital Pune were selected. A total of 30 subjects who are willing to participate were included in the study. A special performa was designed for the present study so as to have a systematic and methodical recording of all the observations and information. The relevant data comprising of chief complaint, preliminary history, oral hygiene habit etc. were recorded in the special performa. All subjects were explained about the treatment procedure and an informed written consent was obtained from each subject. All clinical parameters were recorded at baseline before and after the treatment.

Following parameters were recorded at baseline before and after the treatment-

1. Periodontal Disease index (S.Ramfjord,1959)
2. Sulcus bleeding Index (Muhlemman and Sons,1971)

30 subjects are included in the study and divided into 2 group

Group 1 (chronic periodontitis patients before periodontal flap surgery) and

Group 2 (patients after periodontal flap surgery)

Recording of clinical data:

Clinical examination were made on a dental chair, under standard conditions of light, using a mouth mirror, explorer, graduated periodontal probe (UNC) and tweezers and assessment of clinical parameters were carried out.

At baseline a detailed case history of all 30 individuals will be obtained. Also Ramfjord Periodontal Index and Sulcus Bleeding Index were recorded by using UNC15 probe calibrated with millimetre markings.

### Periodontal Therapy

30 subjects having chronic periodontitis (probing depth more than 5 mm) were included in the study.

For 30 subjects with chronic periodontitis haematological investigation for analysis of serum Interleukin 34 level was carried out and 2 ml of blood from antecubital vein was withdrawn. A detailed case history, clinical examination and radiographic examination was recorded for the same. Interleukin 34 levels were determined using a commercially available ELISA kit according to the manufactures instructions.

Through full mouth scaling, root planing and polishing was carried out and oral hygiene instructions were given. Patient was recalled 4 weeks after completion of scaling and root planing. Periodontal flap surgery was performed after checking various parameters like periodontal pocket depth, bleeding on probing and rechecking the periodontal index and sulcus bleeding index. Postoperative instructions were given and antibiotics were prescribed postoperatively. Patient recalled 7 days postoperative for suture removal. Patient was recalled again after 1 month for Hematological investigation for evaluation of serum Interleukin 34 level postoperatively.

### Recall Visits

Recall visits were scheduled after 7 days for suture removal and after 4 weeks for measuring the clinical parameters Interleukin 34 serum samples were again collected on the 28 days.

### Statistical analysis

Data analysis was performed using SPSS (Statistical Package For social sceinces) Version 25:0 Qualitative data variables express by using mean and SD etc. Qualitative data variables express by using frequency and percentage. Paired t-test was used to compare the mean IL-34 before and after periodontal flap surgery. P-value <0.05 considered as significant.

## Results

Table 1: Demographic details of study participants

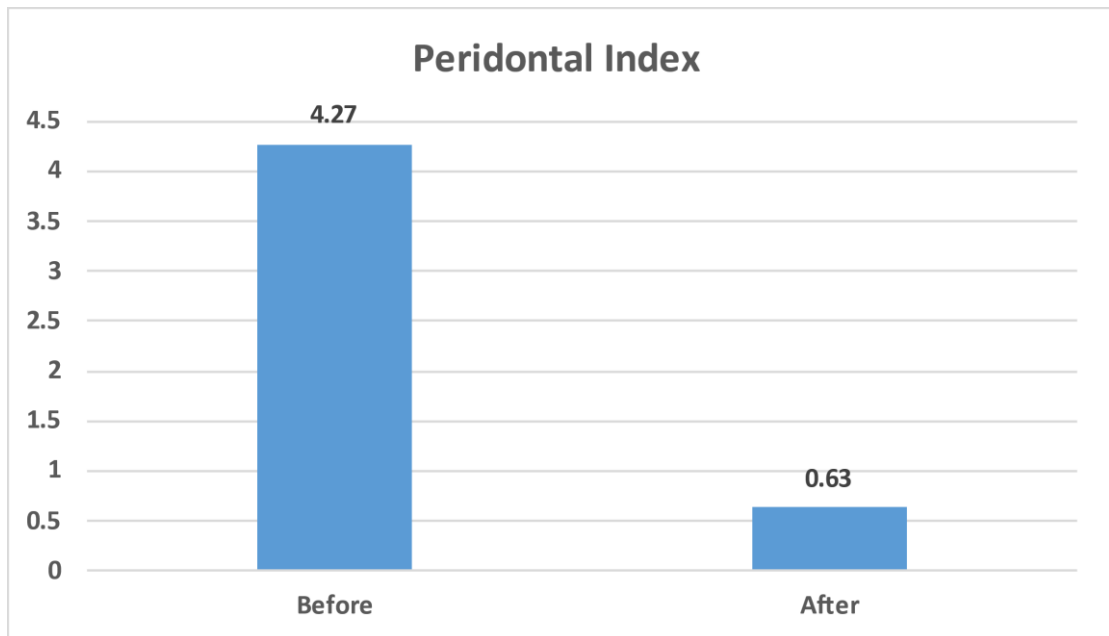
<b>Variable</b>	<b>Category</b>	<b>Mean ± SD/n(%)</b>
Age	Above 20 yrs	48.07 ± 11.93
Gender	Male	22 (73.3%)
	Female	8 (26.7%)

The mean value for Ramfjord Periodontal Index levels at baseline was 4.27 with a standard deviation of 1.11 and 4 weeks after periodontal flap surgery was 0.63 with a standard deviation of 0.67. Using Paired t test, P- value was found to be 0.001 indicating that there was a statistically highly significant decrease in the Periodontal Index levels from baseline to 4 weeks after periodontal flap surgery in subjects with chronic periodontitis.[ table 2 and graph 1]

TABLE 2: Comparison of Ramfjord Periodontal Disease Index levels at baseline and 4 weeks after periodontal flap surgery

Interval	Mean	SD	Difference	p value
Before	4.27	1.11	3.64	0.001*
After	0.63	0.67		

Paired t test; \* indicates significant difference at  $p \leq 0.05$



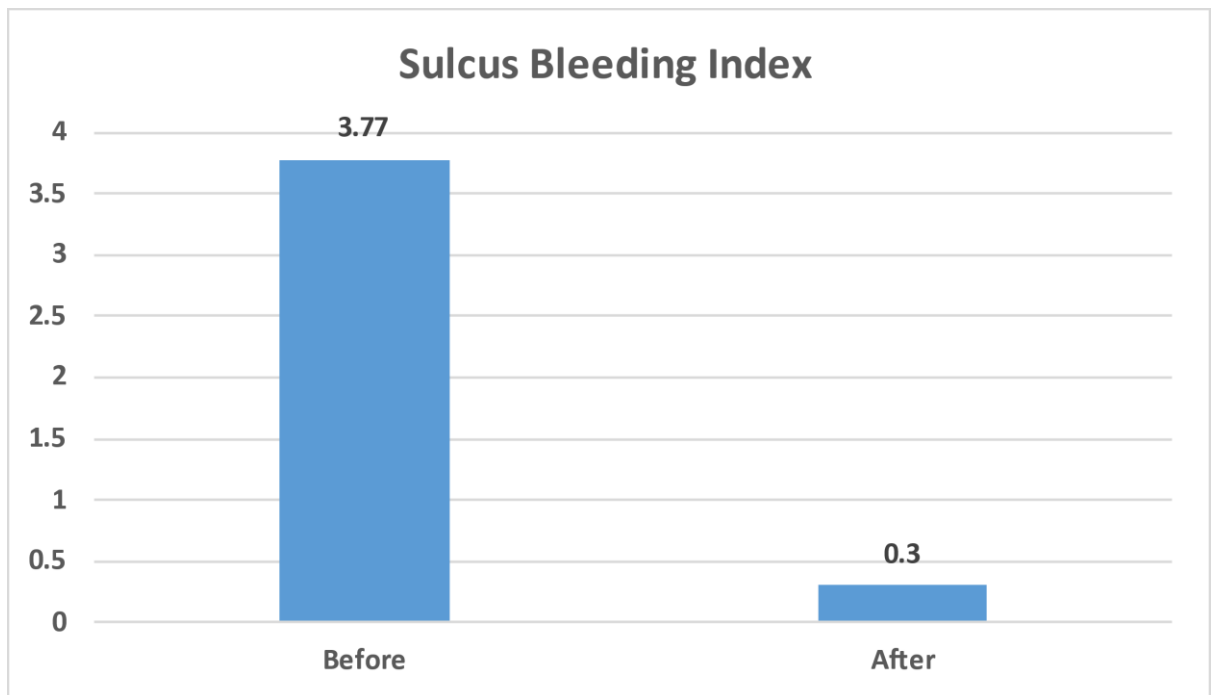
GRAPH 1-Bar graph showing Comparison of Ramfjord Periodontal Disease Index levels at Baseline and 4 weeks after periodontal flap surgery.

The mean value for sulcus bleeding index at baseline was 3.77 with a standard deviation of 1.31 and 4 weeks after periodontal flap surgery was 0.30 with a standard deviation of 0.47. Using Paired t test, P value was found to be 0.001 indicating that there was a statistically highly significant decrease in the Sulcus bleeding index levels from baseline to 4 weeks after periodontal flap surgery in subjects with chronic periodontitis. (table 3 and graph 2)

TABLE 3 -Comparison of Sulcus Bleeding Index levels in Chronic periodontitis patients at baseline and 4 weeks after periodontal flap surgery

Interval	Mean	SD	Difference	p value
Before	3.77	1.31	3.47	0.001*
After	0.30	0.47		

Paired t test; \* indicates significant difference at  $p \leq 0.05$



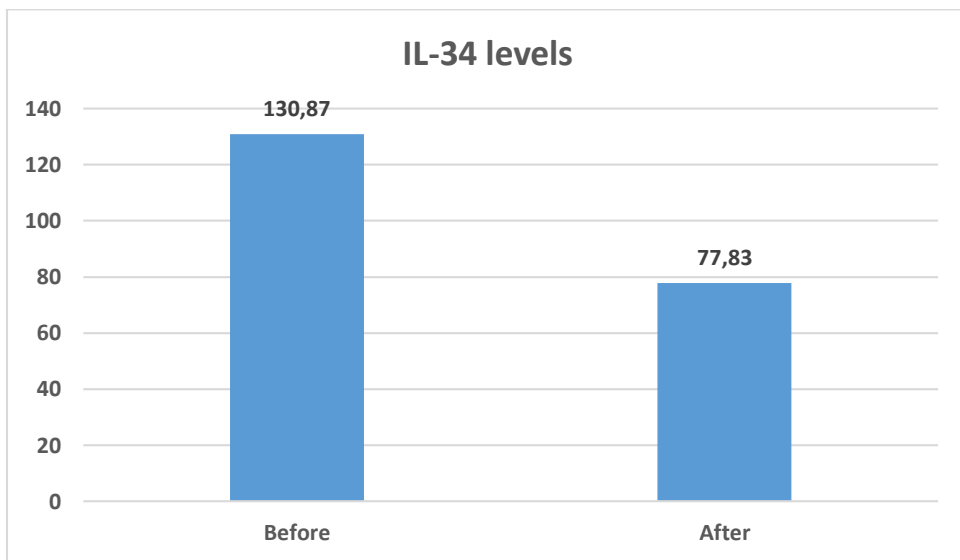
GRAPH 2. Bar graph showing Comparison of Sulcus Bleeding Index levels at baseline and 4 weeks after periodontal flap surgery.

The mean value for Serum IL-34 levels at baseline was 130.87 with a standard deviation of 18.15 and 4 weeks after periodontal flap surgery was 77.83 with a standard deviation of 8.32. Using Paired t test, P value was found to be at  $p \leq 0.05$  indicating that there was a statistically highly significant decrease in the Serum Interleukin 34 levels from baseline to 4 weeks after periodontal flap surgery in subjects with chronic periodontitis (table 4 and graph 3)

TABLE 4-Comparison of serum Interleukin 34 levels in Chronic periodontitis patients at baseline and 4 weeks after periodontal flap surgery

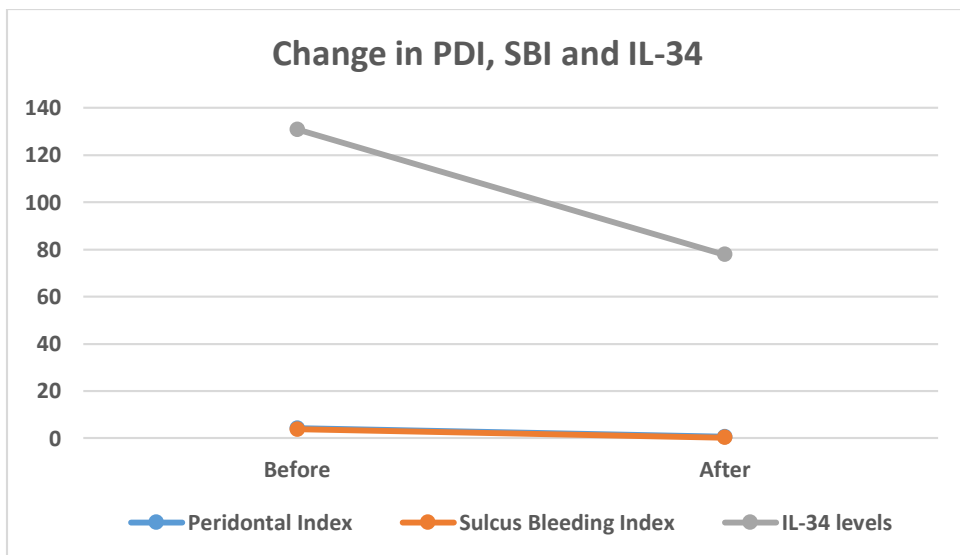
Interval	Mean	SD	Difference	p value
Before	130.87	18.15	53.03	0.001*
After	77.83	8.32		

- Paired t test; \* indicates significant difference at  $p \leq 0.05$

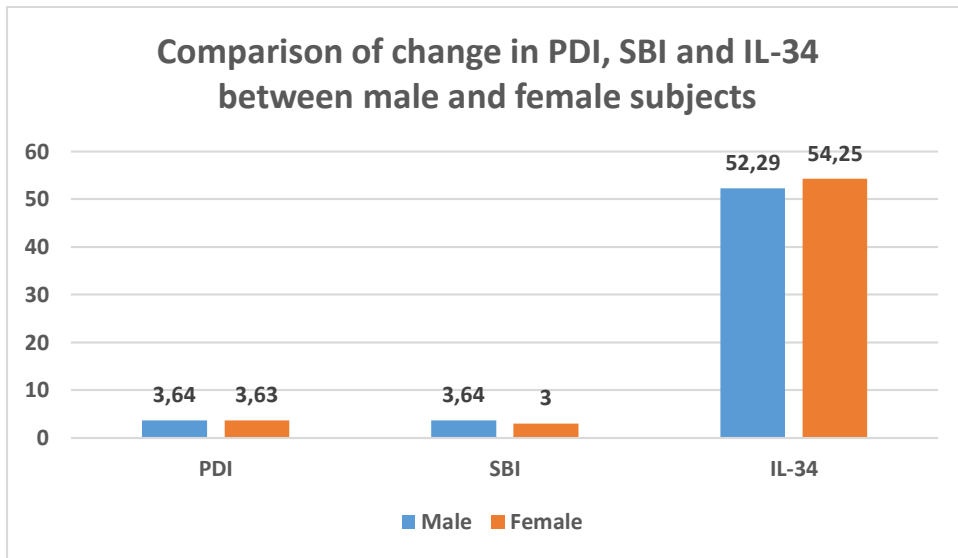


GRAPH 3. Bar graph showing Comparison of Serum Interleukin 34 levels at baseline and 4 weeks after periodontal flap surgery.

There was a statistically significant difference change in Ramjford Periodontal Disease Index, Sulcus Bleeding Index and Serum Interleukin 34 levels at baseline and 4 weeks after periodontal flap surgery when compared together ( Graph 4)



GRAPH 4. Change in Ramjford Periodontal Disease Index, Sulcus Bleeding Index and Serum Interleukin 34 levels at baseline and 4 weeks after periodontal flap surgery



GRAPH 5. Bar graph showing Comparison of change in Ramfjord Periodontal Index, Sulcus Bleeding Index and Serum Interleukin 34 levels at baseline in males and females subjects.

Therefore the **results of our study** shown that the levels of Interleukin 34 increases with periodontal inflammation and as soon as the inflammation subsided after periodontal flap surgery the levels reduced and came back to normal .As reviewed from literature, it is understood that Interleukin 34 is responsible for various systemic diseases. Increased levels of Interleukin 34 **acts as a risk factor** for systemic ailments. If IL-34 levels are found to be elevated in periodontitis subject is at higher risk of getting systemic diseases.

- In this study, we are evaluating the serum levels of IL-34 before and after periodontal flap surgery .If these levels decrease we can consider that the periodontal health of individual has improved.
- Thereby, we can conclude that our subject is at reduced risk of getting the any systemic conditions.

## Discussion

Interleukin-34 (IL-34) was functionally identified as a cytokine by comprehensive proteomic analyses and was shown to act as an alternative ligand of CSF-1 receptor (CSF-1R, c-fms) although it shares no sequence homology with CSF-1. IL-34 mRNA is expressed in various tissues, including heart, brain, lung, liver, kidney, spleen, thymus, testes, ovary, small intestine, prostate, and colon, and it is most abundant in the spleen.<sup>[7],[8]</sup> IL-34 plays an important role in RANKL-induced osteoclastogenesis, as it can substitute for M-CSF and support osteoclast differentiation in the same way that M-CSF does.<sup>[9]</sup>IL-34 plays an important role in RANKL-induced osteoclastogenesis, as it can substitute for M-CSF and support osteoclast differentiation in the same way that M-CSF does.

To the best of our knowledge, this is the first study evaluating IL-34 levels in serum in chronic periodontitis patients, and effect of surgical periodontal therapy



on IL-34 levels in serum. Further, multicenter, longitudinal, and prospective studies can be carried out along with other proinflammatory cytokines like IL-1  $\beta$ , TNF- $\alpha$ , IL-6, and regulators of osteoclastogenesis such as RANK/RANKL/Osteoprotegerin to confirm the findings of the study and better understand the role of IL-34 as an inflammatory marker in periodontal health and disease

## Conclusion

IL-34 has been identified as a novel and moderate inflammatory cytokine that significantly induces macrophage activation and migration and plays an important role in inflammation, it increases IL-6 and chemokine levels in human whole blood. Results of our study have shown that with an increase in the amount of periodontal destruction there is a substantial increase in the concentration of serum IL-34 levels.

- As reviewed from literature Interleukin 34 is responsible for various systemic diseases like neurological disorders, skin lesions, transplant rejection and infection.<sup>[10,11,12]</sup> If IL-34 levels are found to be elevated in periodontitis, the subject is at a higher risk of getting systemic diseases.
- In addition, treatment of periodontal disease leads to a proportional reduction in serum levels of IL-34. Thus, IL-34 can be considered as an “inflammatory marker” of periodontal disease and can be explored in the future as a potential therapeutic target in the treatment of periodontal disease.
- Moreover, with reduction of levels of serum Interleukin 34, our subject is at a reduced risk of getting various systemic diseases. Hence it can be concluded that our study has potential benefits.
- Within the limitations of the present study, it can be postulated that with an increase in the amount of periodontal destruction there is a substantial increase in the concentration of IL-34 in serum. In addition, treatment of periodontal disease leads to a proportional reduction in serum levels of IL-34. Thus, IL-34 can be considered as a potential inflammatory marker of periodontal disease. However, further multicenter, longitudinal, and prospective studies with a larger sample size and long-term follow-up are needed to validate IL-34 as an inflammatory marker in periodontal disease as well as its possible therapeutic applications in periodontal health and disease.

## References

1. Trowbridge HO, Emling RC. *Inflammation: a review of the process*. Illinois: Quintessence Publishing Co, Inc.; 1997.
2. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodontol Res* 1993;
3. Nakaya H, Oates TW, Hoang AM, Kamoi K, Cochran DL. Effects of interleukin-1 beta on matrix metalloproteinase-3 levels in human periodontal ligament cells. *J Periodontol* 1997;
4. Nishikawa M, Yamaguchi Y, Yoshitake K, Saeki Y. Effects of TNF $\alpha$  and prostaglandin E2 on the expression of MMPs in human periodontal ligament fibroblasts. *J Periodontol Res* 2002;37:167-76.

5. Wang Y, Colonna M. Interleukin-34, a cytokine crucial for the differentiation and maintenance of tissue resident macrophages and langerhans cells. *Eur J Immunol* 2014;
6. Eda H, Zhang J, Keith RH, Michener M, Beidler DR, Monahan JB, et al. Macrophage-colony stimulating factor and interleukin-34 induce chemokines in human whole blood. *Cytokine* 2010
7. Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 2008;320:807-11.
8. Liu H, Leo C, Chen X, Wong BR, Williams LT, Lin H, et al. The mechanism of shared but distinct CSF-1R signaling by the non-homologous cytokines IL-34 and CSF-1. *BiochemBiophys Acta* 2012;1824:938-45.
9. Wang Y, Colonna M. Interleukin-34, a cytokine crucial for the differentiation and maintenance of tissue resident macrophages and langerhans cells. *Eur J Immunol* 2014;44:1575-81
10. Franzè E, Monteleone I, Cupi ML, et al. Interleukin-34 sustains inflammatory pathways in the gut. *Clin Sci*. 2015;129:271–280. 52. Zwicker S, Martinez GL, Bosma M, et al. Interleukin 34
11. Ma N, Qu L, Xu LY, Yu YQ, Qiu LH. Expression of IL-34 in chronic periapical lesions and its clinical significance. *Shanghai Kou Qiang Yi Xue*. 2016;25:53–57.
12. San Segundo D, Ruiz P, Irure J, et al. Serum levels of interleukin34 during acute rejection in liver transplantation. *Transplant Proc*. 2016;48:2977–29
13. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, 6(1), i-v. <https://doi.org/10.53730/ijhs.v6n1.5949>