

## Value of Gingival Crevicular Fluid Levels of Biomarkers IL-1 $\beta$ , IL-22 and IL-34 for the Prediction of Severity of Periodontal Diseases and Outcome of Non-Surgical Periodontal Treatment

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**Abstract:** Interleukins (IL) -1 $\beta$ , -22 and -34 play a crucial role in osteoclastogenesis and bone resorption through modulating inflammatory processes and osteoclastogenesis. To date, there is no study investigating both gingival crevicular fluid (GCF) ILs -22 and -34 levels before and after non-surgical periodontal treatment in patients with aggressive periodontitis (AgP) and chronic periodontitis (CP). We aimed to examine the relationship of GCF levels of biomarkers IL-1  $\beta$ , IL-22 and IL-34 with the clinical evidence of periodontal tissue breakdown and alveolar bone resorption and to determine the value of these biomarkers for the prediction of severity of periodontal diseases and outcome of non-surgical periodontal treatment. Thirty-five AgP patients, 30 CP patients and 30 periodontally healthy volunteers (C) were included in this research. The AgP and CP patients underwent scaling and root planning interventions, performed with periodontal hand instruments. Clinical findings and GCF samples were collected at baseline and 6 weeks later. The GCF ILs were measured by ELISA. The AgP and CP groups exhibited significant improvement in clinical parameters. The GCF ILs -1 $\beta$ , -22, and -34 levels were significantly higher in the CP group compared to the C group at baseline. The GCF levels of these parameters were decreased in CP group after treatment. The ILs -22 and -34 levels were lower in AgP patients at baseline, they increased after treatment. In accordance with clinical improvement of CP patients, the ILs -22 and -34 levels in GCF decreased meaningfully; however, ILs -22 and -34 were lower in AgP patients at baseline and later there were increased after treatment although IL-1 $\beta$  was found higher at first measurement and later it decreased. These findings require further studies to elucidate the mechanisms involved in the regulation of ILs -22 and -34 in the pathogenesis of AgP.

### INTRODUCTION

Periodontitis is a complex inflammatory process and with its long-term effects, it can lead to destruction of the soft and hard components of periodontal tissues including alveolar bone supporting teeth. Local stimulation of osteoclast activity is the main component of alveolar bone loss induced in periodontitis patients. The regulation of osteoclastic cells and their activity are modulated by several cytokines, growth factors, and systemic hormones according to need of tissue homeostasis <sup>1</sup>. In addition to these beneficial roles, when inflammatory events including periodontitis develops, cytokines can play harmful roles determined by the strength, nature, and duration of immune responses <sup>2</sup>. At the end, the interplay of many cytokines causes tissue response changing from remaining stable to tissue destruction and in long-term condition to the progression of disease <sup>3</sup>. In affected tissues, the progression of local inflammation contributes to the clinical outcomes of the disease.

Proinflammatory cytokines, especially IL-1 $\beta$ , are very important in initiating and regulating immune responses in the periodontium by stimulating osteoclastic activity and inflammation-induced bone resorption in patients with periodontal disease <sup>1</sup>. IL-1 $\beta$  levels are found according to the involvement of periodontal inflammation in the gingival tissue as well as crevicular fluid <sup>4</sup>. That after successful treatment, a decrease in the IL-1 $\beta$  levels in gingival crevicular fluid (GCF) also supports this relationship <sup>5,6</sup>.

Both IL-22 and IL-34 were associated with inflammatory disease such as rheumatoid arthritis <sup>7-9</sup>. There is considerable relatedness regarding the pathophysiological features of periodontal disease and rheumatoid arthritis. This may be related to the persistent inflammatory reactions presented in these diseases as areas of bone and connective tissues with the involvement of several components of the inflammatory machinery including secretion of inflammatory cell products consisting mainly cytokines

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modulating inflammatory events<sup>10</sup>. Periodontitis had considerably similar cytokine secretions to those of rheumatoid arthritis<sup>11</sup>. Again, These have been reported as having prominent roles in osteoclastogenesis and bone resorption through furthering inflammation<sup>12,13</sup>. IL-22 and IL-34 can function with the involvement of cross-talk of other cytokines and this condition results in an increase in cytokines such as IL-1, IL-6, and TNF- $\alpha$ , on the other hand, their increases result in augmented secretion of IL-22 and IL-34<sup>14,15</sup>.

There is a need to develop novel biomarkers for screening and diagnosis of periodontal disease. Especially, activity status of periodontal disease should be elucidated instead of simple assessment of the cumulative effects of historical tissue destruction as by measured conventional tools used in periodontal practice.

To date, there is no study investigating both GCF IL-22, and IL-34 levels after periodontal treatment performed non-surgically in patients diagnosed with aggressive periodontitis (AgP) and chronic periodontitis (CP). It was hypothesized that GCF ILs-1 $\beta$ , -22, and -34 levels decrease after non-surgical periodontal treatment in AgP and CP patients. The aim of current study was to investigate the relationship of GCF levels of biomarkers IL-1  $\beta$ , IL-22, and IL-34 with the clinical evidence of periodontal tissue breakdown and alveolar bone resorption and to determine the value of these biomarkers for the prediction of severity of periodontal diseases and outcome of non-surgical periodontal treatment.

## METHOD

### *Study population*

Patients were enrolled in the current research by collecting the whole mouth clinical periodontal data. This research was designed as a case-control study. The research protocol was confirmed by the Medical Research Ethics Committee of Cumhuriyet University with regard to the Declaration of Helsinki (2016-02/05), and all participants gave informed. The total number of subjects in the current research was 95, consisting of 35 AgP patients (24 female, 11 male), 30 CP patients (17 females, 13 males), and 30 periodontally healthy controls (C) (19 females, 11 males).

Patients over the age of 35 years, having attachment loss equal to or higher than 5 mm at multiple tooth sites and having >3 sites of probing depth >6 mm including >1 tooth distributed in every quadrant were assessed as CP. According to "Classification of Periodontal and Peri-Implant Diseases and Conditions 2017", our CP patients can be included in the 'Stage 3, Generalized, Grade B' group, based on the clinical parameters including disease course and affected age<sup>16</sup>. According to "Classification of Periodontal and Peri-Implant Diseases and Conditions 2017", our AgP patients can be included in the 'Stage 4, Generalized, Grade C' and our G-CP patients can be included in the 'Stage 3, Generalized, Grade B' group, based on the clinical parameters including disease course and affected age. Patients without the evidence of attachment loss at multiple sites or with the pocket depth equal to or less than 3 mm were diagnosed as being periodontally healthy and utilized as controls.

Exclusion criteria were using antibiotics or received periodontal therapy within 6 months before the study examination, conservative or prosthetic restorations in the anterior region, periodontal destruction caused by poor restorations, begin pregnant and performing breastfeeding, a history of systemic inflammatory disorder or antiinflammatory medication that might influence the periodontal condition, and subjects lacking capacity to consent for themselves. Furthermore, smoking may constitute the main risk factor in periodontal diseases. Therefore, we have excluded smokers from the study.

### *Clinical measurement and periodontal therapy*

Clinical examinations assessing the gingival index (GI) and plaque index (PI), clinical attachment loss (CAL), and probing depth (PD) were performed, and panoramic radiographs were taken to measure interproximal bone loss from the cemento-enamel junction of the tooth to the bone crest in order to diagnose AgP and CP as well as having no periodontal disease. The diagnostic evaluation was performed according to the criteria for periodontal diseases as accepted at the World Workshop for Periodontics and the American Academy of Periodontology (1999)<sup>17</sup>. In short, to diagnose AgP, the following findings need to be obtained: having >8 teeth with the attachment loss equal  $\geq 5$  mm and the probing depth  $\geq 6$  mm, and  $\geq 3$  affected teeth, being not first molars or incisors. A Williams periodontal probe (Hu-Friedy, Chicago, IL, USA) was utilized to obtain PD and CAL measures.

The full-mouth measurements of PD and CAL were obtained at six points per tooth. The PI was utilized for scoring the presence of supragingival plaque<sup>18</sup>, while the GI was used for scoring gingival inflammation<sup>18</sup>. In all subjects, individual acrylic stents were formed using grooves as reference points to measure CAL. A single specialist performed the collection of clinical data and specimens (EPG). The intra-examiner reproducibility of PD was evaluated, and the intra-examiner reliability was found to be high ( $\geq 98\%$ ). After the baseline measurements were recorded, phase 1 therapy, consisting of oral hygiene instructions, scaling, and root planing (SRP), was implemented on patients having periodontitis<sup>19</sup>. The SRP procedure was carried out within 10 days by utilizing Gracey curettes (Hu-Friedy, Chicago, IL, USA). No antibiotics were prescribed. Recording of the clinical measurements and GCF sampling from the exemplification sites was performed at baseline and six weeks later.

### *GCF collection and analysis*

Before starting to collect GCF samples, the area surrounding the tooth was isolated using cotton rolls, dried, and the removal of the supragingival plaque was performed by means of a sterile curette. The collection of GCF specimens was carried out, and they were pooled from 3 non-adjacent proximal sites with a moderate probing pocket depth. The collection of GCF samples was performed using Periopaper® strips, and they were stored at a temperature of -80°C until the analysis was conducted. 300 $\mu$ L of phosphate buffer solution was added to Eppendorf tubes to extract the collected GCF specimens by impregnating Periopaper. The vial was mixed by the vortex mixer for 1 minute to allow the passage of the GCF liquid to the solution. The obtained contents were used for analyzing IL-1 $\beta$ , IL-22 and IL-34. An enzyme-linked immunosorbent assay (ELISA) kit (FineTest, Wuhan, China) was utilized for measuring the cytokine levels, following the instructions of the manufacturer.

The calculation of GCF cytokine levels was performed from the standard curves, and they were defined as picogram/site for the total amount of cytokine levels. The score of 0 was given to sites having cytokine levels lower than the limits of the detectability of the assay.

### *Statistical analysis*

For data analyses, a statistical package (IBM SPSS v22.0, IBM, USA) was used. After the Kolmogorov-Smirnov test, comparisons of numeric variables of the study groups were evaluated by the Kruskal-Wallis ANOVA test with post hoc Mann-Whitney test for pairwise comparisons. The Wilcoxon test was conducted for assessing differences between baseline and 6-week values in the AgP and CP groups. A p value <0.05 was used to decide statistical significances. The calculation of the sample size was performed under a 5% error considering a required sample size of 30 in every group, with a statistical power of 80%, considering an effect size of 300 and a SD value of 350 for IL-22.

## FINDINGS

### Demographic features and clinical periodontal measurements.

A total of 35 AgP patients, 30 CP patients, and 30 healthy controls took part in the research. The mean ages (mean±SD) of the AgP, CP, and C groups were 28.9±5.8 (range 18-39), 35.2±5.9 (range 25-48 years), and 34.4±6.3 (range 26-44 years), respectively.

Figure 1 presents the PIs, GIs, PDs, and CALs measured at baseline examination and at examination after treatment. In the AgP and CP groups, the PIs, GIs, PDs, and CALs measured at baseline examination were significantly higher compared to the controls ( $p < 0.05$ ); however, regarding these parameters, there was no significant difference between the AgP and CP groups ( $p > 0.05$ ). In the AgP and CP groups, the PIs, GIs, and PDs measured at examination after treatment were significantly lower compared to those measured at baseline examination ( $p < 0.05$ ); however, these study groups were similar to the controls regarding these parameters ( $p > 0.05$ ). The CALs of the AgP and CP groups were found similar regarding measurements performed at baseline and after treatment ( $p > 0.05$ ).

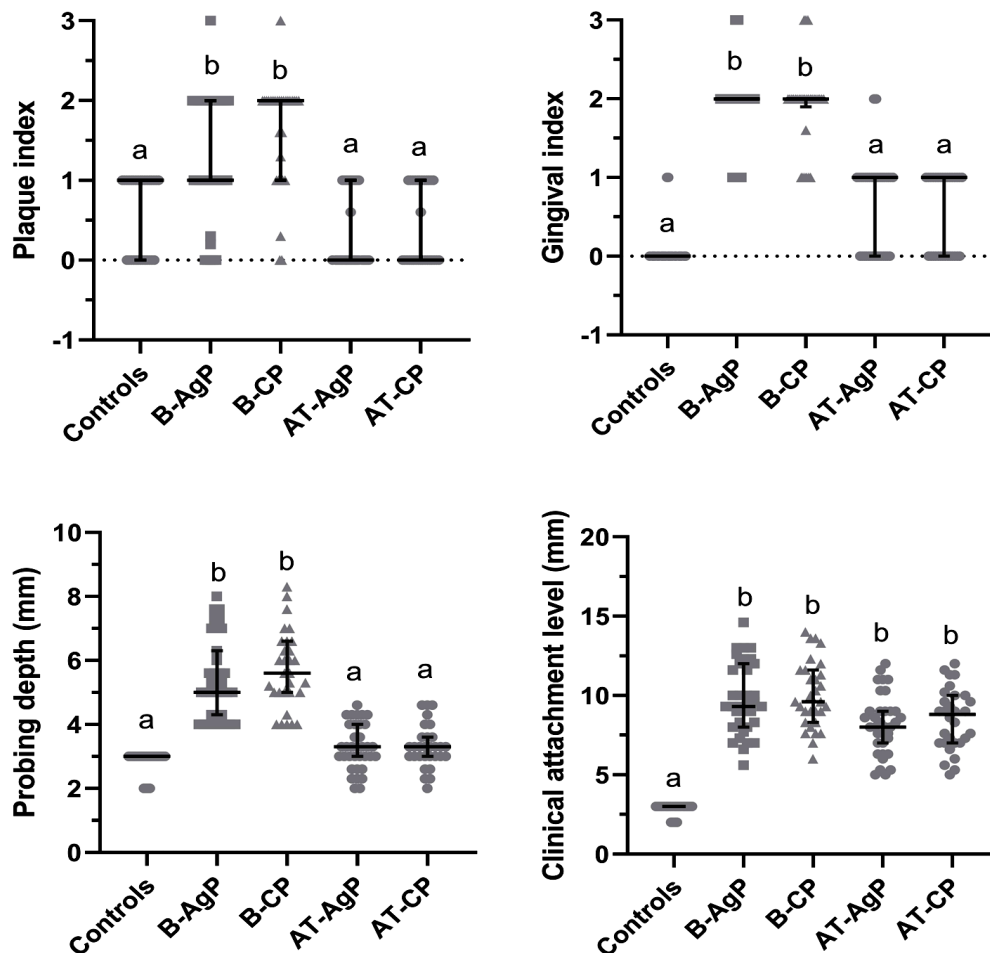
### Biochemical findings

Figure 2 presents the median levels of GCF IL-1 $\beta$  in the AgP, CP and C groups. At the baseline sampling, the median level of GCF IL-1 $\beta$  in the AgP group was significantly higher compared to the other groups ( $p < 0.05$ ); however, there was no significant difference between the CP and control groups with regard to the median level of GCF

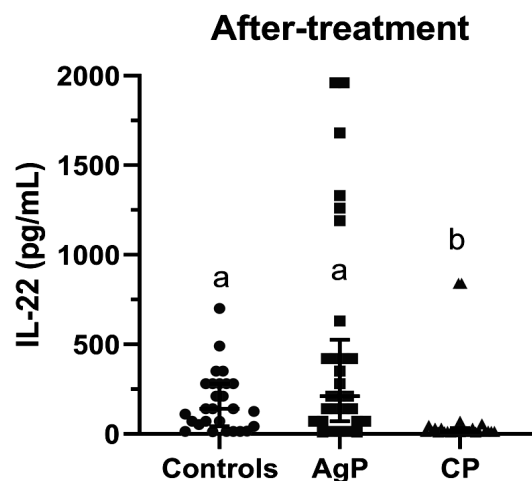
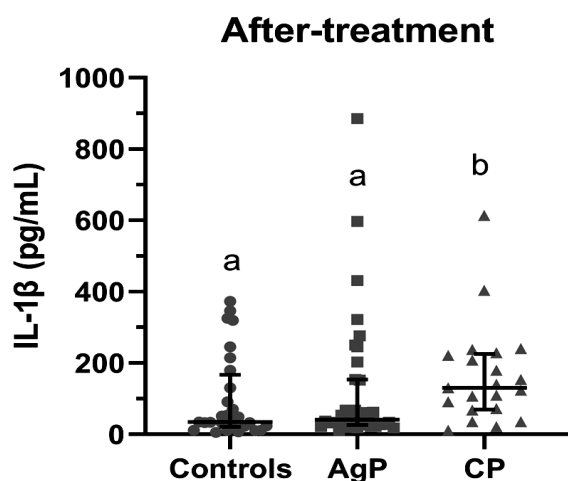
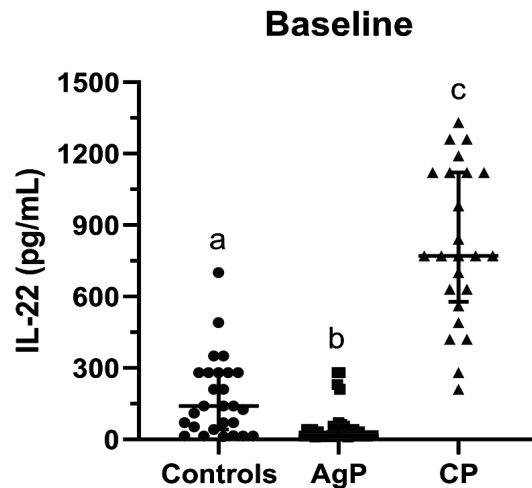
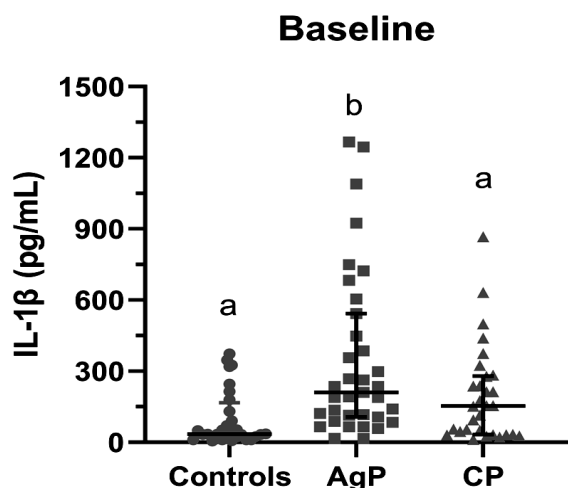
IL-1 $\beta$  ( $p > 0.05$ ). At the sampling after treatment, the median level of GCF IL-1 $\beta$  in the CP group was significantly higher than those of the AgP and control groups ( $p < 0.05$ ); however, there was no significant difference between the AgP and control groups with regard to the median level of GCF IL-1 $\beta$  ( $p > 0.05$ ).

Figure 3 presents the median levels of GCF IL-22 in the AgP, CP and control groups. At the baseline sampling, the median level of GCF IL-22 in the CP group was significantly higher than those of the AgP and control groups ( $p < 0.05$ ); and the median level of GCF IL-22 in the control group was significantly higher than that of the AgP group ( $p < 0.05$ ). At the sampling after treatment, the median level of GCF IL-22 in the CP group was significantly lower than those of the AgP and control groups ( $p < 0.05$ ); however, there was no significant difference between the AgP and control groups with regard to the median level of GCF IL-22 ( $p > 0.05$ ).

Figure 4 presents the median levels of GCF IL-34 in the AgP, CP and control groups. At the baseline sampling, the median level of GCF IL-34 in the CP group was significantly higher than those of the AgP and control groups ( $p < 0.05$ ); and the median level of GCF IL-34 in the control group was significantly higher than that of the AgP group ( $p < 0.05$ ). At the sampling after treatment, the median level of GCF IL-34 in the CP group was significantly lower than those of the AgP and control groups ( $p < 0.05$ ); however, there was no significant difference between the AgP and control groups with regard to the median level of GCF IL-34 ( $p > 0.05$ ).



**Figure 1.** Median levels of plaque index, gingival index, probing depth, clinical attachment level in the aggressive periodontitis measured baseline and after treatment, chronic periodontitis measured baseline and after treatment and control groups. Data are expressed as median with interquartile range and analyzed with Kruskal-Wallis and post hoc Mann-Whitney tests. Significances are shown as marked with different letters ( $p < 0.05$ ), when coded with same letters, there is no significant difference ( $p > 0.05$ ). B-AgP, aggressive periodontitis measured at baseline; AT-AgP, aggressive periodontitis measured after treatment; B-CP, chronic periodontitis measured at baseline; AT-CP, chronic periodontitis measured after treatment.



**Figure 2.** Median levels of gingival crevicular fluid interleukin-1 $\beta$  (IL-1 $\beta$ ) in the aggressive periodontitis, chronic periodontitis and control groups. Data are expressed as median with interquartile range and analyzed with Kruskal-Wallis and post hoc Mann-Whitney tests. Significances are marked with different letters ( $p < 0.05$ ), when coded with same letters, there is no significant difference ( $p > 0.05$ ). AgP, aggressive periodontitis; CP, chronic periodontitis.

**Figure 3.** Median levels of gingival crevicular fluid interleukin-22 (IL-22) in the aggressive periodontitis, chronic periodontitis and control groups. Data are expressed as median with interquartile range and analyzed with Kruskal-Wallis and post hoc Mann-Whitney tests. Significances are marked with different letters ( $p < 0.05$ ), when coded with same letters, there is no significant difference ( $p > 0.05$ ). AgP, aggressive periodontitis; CP, chronic periodontitis.

## DISCUSSION

In the current study we investigated that firstly the predictive value of biomarkers IL-1  $\beta$ , IL-22, and IL-34 measured in the GCF of AgP and CP patients during the assessment of severity of periodontal diseases and outcome of non-surgical periodontal treatment.

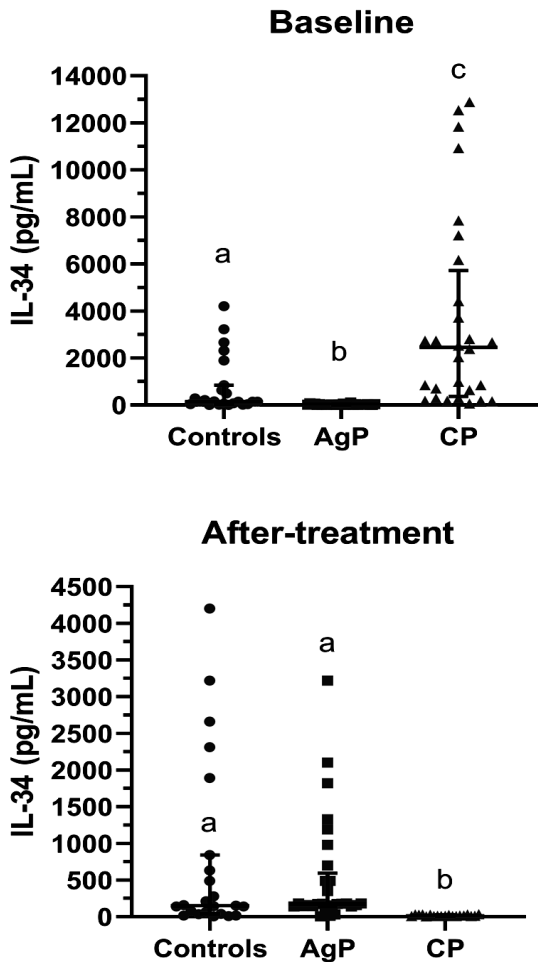
When researched periodontitis related literature, this investigation is firstly assessed the GCF levels of cytokines IL-22 and IL-34 and their possible relationship with GCF IL-1 $\beta$  level before and after periodontal treatment performed non-surgically in the AgP and CP patients. In our study, along with the improved clinical parameters after periodontal treatment, the IL-22 and IL-34 GCF levels were elevated in CP patients and significantly decreased following periodontal treatment, nevertheless they were decreased in AgP patients and significantly increased after periodontal treatment. To increase reliability of our results, as mentioned previously, smoking subjects were not included in this study, since it has been reported that host cytokine levels are affected by smoking<sup>20-22</sup>.

On comparison of PI, GI, PD and CAL values among the groups, the mean PI, GI, PD and CAL was found to be statistically highly significant between groups. All clinical periodontal parameters were highly in periodontitis groups.

Cytokines are produced locally in the periodontal tissues and

secreted into the GCF. The GCF is composed of an inflammatory exudate as collected in the gingival sulcus and consists of components of host derived inflammatory molecules. This unique composition makes the GCF as a valuable specimen that can be used as a non-invasive approach to investigate periodontal inflammatory events with the measurement of several potential inflammatory biomarkers<sup>23</sup>. This research was designed primarily to assess the cytokine content of total GCF. It is important to note that the individual GCF samples (three for each subject) used in this research were later combined. This approach was preferred to make it easier to identify all of the biochemical signs present in all of the subjects. It has been suggested that the total amounts of cytokines in the GCF samples per sampling time is a better marker of relative GCF constituent activity<sup>24,25</sup>.

In the present study, in accordance with the results of studies mentioned below, GCF IL-1 $\beta$  level decreased after treatments of AgP patients. In the AgP patients, as usual, IL-1 $\beta$  was higher compared to the CP patients in accordance with the severity of periodontitis. However, there was no significant difference in CP group before and after treatment. IL-1 $\beta$  prominently mediates the inflammatory response and takes part in cell proliferation, differentiation, apoptosis, and in the periodontitis pathophysiology. Furthermore, IL-1 $\beta$  and TNF- $\alpha$  effectively induce bone resorption and inhibit bone formation<sup>26</sup>. A number of studies indicated that an association



**Figure 4.** Median levels of gingival crevicular fluid interleukin-34 (IL-34) in the aggressive periodontitis, chronic periodontitis and control groups. Data are expressed as median with interquartile range and analyzed with Kruskal-Wallis and post hoc Mann-Whitney tests. Significances are shown as marked with different letters ( $p < 0.05$ ), when coded with same letters, there is no significant difference ( $p > 0.05$ ). AgP, aggressive periodontitis; CP, chronic periodontitis.

between IL-1 $\beta$  levels in gingival tissues and GCF and the inflammatory status of periodontal disease<sup>27</sup>. Hyun et al. reported that IL-1 $\beta$  level decreased at shallow and deep sites 2 and 4 months later after onset of study in the periodontitis patients<sup>27</sup>. Toker et al. reported that periodontal treatment meaningfully healed the clinical complaints and this positive effects were in accordance with the decreased IL-1 $\beta$  in GCF<sup>28</sup>.

IL-22 is one of the prominent cytokines and modulates inflammatory reactions of tissues. In the present study, at the baseline sampling, GCF IL-22 level in the CP group was significantly higher compared to the other groups. At the sampling after treatment, the GCF level of IL-22 in the CP group was significantly decreased and lower than the other groups. Similar to our finding, Sidharthan et al. reported the GCF IL-22 level was significantly greater in CP group than healthy controls and gingivitis group. They also demonstrated significantly elevated levels of IL-22 and a positive correlation with clinical parameters in CP subjects<sup>29</sup>. In a study demonstrates the increase of IL-22-expressing CD4<sup>+</sup> AhR<sup>+</sup> T lymphocytes in periodontitis-affected tissues and shows a positive correlation between IL-22, RANKL expression, and alveolar bone resorption. Increased expression of IL-22 has been reported in periapical granulomas compared with healthy samples<sup>30</sup>. T-cell phenotype is defined as a risk factor for periodontal disease as a supporting finding the complex nature of harmful effects of periodontitis to the connective and bone

tissues<sup>31</sup>.

In rheumatoid arthritis, as parallel to the increased IL-22, RANKL production increases and this leads to osteoclastogenesis and bone resorption in affected articular tissues<sup>9,32</sup>. In the present study the total amount of IL-22 and IL-34 is significantly lower than controls at baseline and after treatment they were increased in AgP patients. That might be attributable to its degradation in GCF, a relatively lower level of IL-22 and IL-34 in GCF, a heterogeneous response because of racial variation, or a combination of some or all of these factors. In the present study we can report that GCF IL-22 and IL-34 act differently in AgP and CP patients. CP differs from AgP in many respects, such as genetic factors, progression rate, and susceptibility factors. In addition, inflammatory mediators seem to play a dual role in the disease, contributing to the control of periodontal infection while triggering tissue destructive pathways.

IL-34 is one of the cytokines that stimulate monocyte and macrophage differentiation and known as a factor stimulating macrophage colonies to release pro-inflammatory cytokines. It plays important roles in the pathogenesis of chronic inflammations such as developed in rheumatoid arthritis and systemic lupus erythematosus<sup>7</sup>. It is known as associated with several chronic conditions such as obesity, chronic inflammation, and insulin resistance<sup>8</sup>. IL-34 contributes to the increase in IL-1, IL-6, and TNF- $\alpha$  levels that can augment its secretion reciprocally<sup>14</sup>. Boström et al. reported that gingival fibroblasts express IL-34 and the pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  regulate IL-34 expression in gingival fibroblasts. IL-34 plays a crucial role in bone destruction through promoting inflammation and osteoclastogenesis, as seen in RA that stimulates osteoclastogenesis<sup>12</sup>.

Chemel et al. reported a positive association between synovial IL-34 expression and disease severity in synovitis patients<sup>33</sup>. Synovial fibroblasts do, as gingival fibroblasts, produce IL-34 as modulated by TNF- $\alpha$  and IL-1 $\beta$ . IL-34 can entirely substitute M-CSF in RANKL-induced osteoclastogenesis and this reveals the pivotal role of IL-34 in inflammation-driven bone resorption<sup>34</sup>. In the present study, at the baseline sampling, the GCF IL-34 level in the CP group was higher compared to the other groups. At the sampling after periodontal treatment, the GCF level of IL-34 in the CP group was decreased and found lower compared to the other groups. Similar to our findings, in three different study that evaluated CP and healthy groups together with obesity, smoking and type-2 diabetes mellitus reported that IL-34 levels were highest in CP with obesity, CP with smoking and CP with type-2 diabetes mellitus. Than only CP groups followed this order<sup>35-37</sup>. In another study, Guruprasad et al. evaluated the IL-34 levels in GCF and plasma in subjects with CP and the effect of nonsurgical periodontal therapy on the GCF and plasma IL-34 levels. They reported that IL-34 levels decreased after periodontal therapy performed non-surgically<sup>38</sup>. Opposite to our result, Parichaya et al. demonstrated highest levels of IL-34 in AgP when compared with CP and periodontally healthy groups<sup>39</sup>.

### Conclusion

In the current study, the relationship of IL-1 $\beta$ , IL-22, and IL-34 in the GCF of CP and AgP patients were evaluated to obtain laboratory evidence of periodontal tissue breakdown and alveolar bone resorption and to determine the value of these biomarkers for the prediction of severity of periodontal diseases and outcome of non-surgical periodontal treatment. In accordance with clinical improvement of CP patients, the ILs -22 and -34 levels in GCF decreased meaningfully; however, ILs -22 and -34 were lower in AgP patients at baseline and later there were increased after treatment although IL-1 $\beta$  was found higher at first measurement and later it decreased. Thus, the level of GCF ILs-1beta, -22 and -34 might be a valuable means of

identifying the current status of periodontitis. Evaluating the GCF levels of these cytokines might help monitor the response to periodontal therapy and the progression of periodontal inflammation. Further studies investigating GCF ILs-1beta, -22 and -34 levels combined with molecular microbiological analysis are needed to support these findings and better understand their role in the development of periodontitis-related inflammation.

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#### Conflict of interest statement

There is no conflict of interest related to the content of this manuscript.

#### Ethics committee approval

All procedures carried out in the research including human participants were performed according to the ethical standards of the institutional and/or rational research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was acquired from all the subjects enrolled in this research.

#### REFERENCES

- Gilowski L, Wiench R, Plocica I, Krzemiński TF. Amount of interleukin-1 $\beta$  and interleukin-1 receptor antagonist in periodontitis and healthy patients. *Archives of Oral Biology*. 2014;59:729-734.
- Garlet G. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. *Journal of Dental Research*. 2010;89:1349-1363.
- Seymour GJ, Gemmell E. Cytokines in periodontal disease: where to from here? *Acta Odontologica Scandinavica*. 2001;59:167-173.
- Faizuddin M, Bharathi S, Rohini N. Estimation of interleukin 1 $\beta$  levels in the gingival crevicular fluid in health and in inflammatory periodontal disease. *Journal of Periodontal Research*. 2003;38:111-114.
- Rosalem W, Rescala B, Teles R, Fischer R, Gustafsson A, Figueredo C. Effect of non-surgical treatment on chronic and aggressive periodontitis: clinical, immunologic, and microbiologic findings. *Journal of Periodontology*. 2011;82:979-989.
- Al Shammari KF, Giannobile WV, Aldredge WA, et al. Effect of non-surgical periodontal therapy on C-telopeptide pyridinoline cross-links (ICTP) and interleukin-1 levels. *Journal of Periodontology*. 2001;72:1045-1051.
- Chang E-J, Lee SK, Song YS, et al. IL-34 is associated with obesity, chronic inflammation, and insulin resistance. *The Journal of Clinical Endocrinology & Metabolism*. 2014;99:E1263-E1271.
- Masteller EL, Wong BR. Targeting IL-34 in chronic inflammation. *Drug Discovery Today*. 2014;19:1212-1216.
- Kim KW, Kim HR, Park JY, et al. Interleukin-22 promotes osteoclastogenesis in rheumatoid arthritis through induction of RANKL in human synovial fibroblasts. *Arthritis & Rheumatism*. 2012;64:1015-1023.
- Cetinkaya B, Guzeldemir E, Ogus E, Bulut S. Proinflammatory and anti-inflammatory cytokines in gingival crevicular fluid and serum of patients with rheumatoid arthritis and patients with chronic periodontitis. *Journal of Periodontology*. 2013;84:84-93.
- Mercado F, Marc hall R, Bartold PM: Inter-relationship between RA and periodontal disease a review. *J Clin Periodontol*. 2003;30:761-772.
- Clavel G, Thiolat A, Boissier M-C. Interleukin newcomers creating new numbers in rheumatology: IL-34 to IL-38. *Joint Bone Spine*. 2013;80:449-453.
- Chen Z, Buki K, Vääräniemi J, Gu G, Väänänen HK. The critical role of IL-34 in osteoclastogenesis. *PLoS one*. 2011;6:e18689.
- Zorena K, Jachimowicz-Duda O, Wąz P. The cut-off value for interleukin 34 as an additional potential inflammatory biomarker for the prediction of the risk of diabetic complications. *Biomarkers*. 2016;21:276-282.
- Shabgah AG, Navashenaq JG, Shabgah OG, Mohammadi H, Sahebkar A. Interleukin-22 in human inflammatory diseases and viral infections. *Autoimmunity Reviews*. 2017;16:1209-1218.
- Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions—Introduction and key changes from the 1999 classification. *Journal of Periodontology*. 2018;89:S1-S8.
- Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4:1-6.
- Loe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol*. 1967;38:Suppl:610-616.
- Lindhe J, Nyman S, Lang N. Treatment planning. *Clinical Periodontology and Implant Dentistry*. 2003;3:420-437.
- Boström L, Linder LE, Bergström J. Smoking and crevicular fluid levels of IL-6 and TNF- $\alpha$  in periodontal disease. *Journal of Clinical Periodontology*. 1999;26:352-357.
- Buduneli N, Bıyıkoğlu B, Sherrabeh S, Lappin DF. Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. *Journal of Clinical Periodontology*. 2008;35:846-852.
- Özçaka Ö, Bıçakçı N, Pussinen P, Sorsa T, Köse T, Buduneli N. Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. *Oral Diseases*. 2011;17:68-76.
- Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontology 2000*. 2003;31:32-42.
- Figueredo C, Ribeiro M, Fischer R, Gustafsson A. Increased interleukin-1 $\beta$  concentration in gingival crevicular fluid as a characteristic of periodontitis. *Journal of Periodontology*. 1999;70:1457-1463.
- Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1 $\beta$ , IL-8, and IL-10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *Journal of Periodontology*. 2000;71:1535-1545.
- Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD. Interleukin-1 $\beta$  is a potent inhibitor of bone formation in vitro. *Journal of Bone and Mineral Research*. 1987;2:559-565.
- Oh H, Hirano J, Takai H, Ogata Y. Effects of initial periodontal therapy on interleukin-1 $\beta$  level in gingival crevicular fluid and clinical periodontal parameters. *Journal of Oral Science*. 2015;57:67-71.
- Toker H, Poyraz O, Eren K. Effect of periodontal treatment on IL1 $\beta$ , IL1ra, and IL10 levels in gingival crevicular fluid in patients with aggressive periodontitis. *Journal of Clinical Periodontology*. 2008;35:507-513.
- Sidharthan S, Dharmarajan G, Kulloli A. Gingival crevicular fluid levels of Interleukin-22 (IL-22) and human  $\beta$  Defensin-2 (hBD-2) in periodontal health and disease: A correlative study. *Journal of Oral Biology and Craniofacial Research*. 2020;10:498-503.
- Aranha AMF, Repeke CE, Garlet TP, et al. Evidence supporting a protective role for th9 and th22 cytokines in human and experimental periapical lesions. *Journal of Endodontics*. 2013;39:83-87.
- Diaz Zúñiga J, Melgar Rodriguez S, Rojas L, et al. Increased levels of the T-helper 22-associated cytokine (interleukin-22) and transcription factor (aryl hydrocarbon receptor) in patients with periodontitis are associated with osteoclast resorptive activity and severity of the disease. *Journal of Periodontal Research*. 2017;52:893-902.
- Zhao L, Jiang Z, Jiang Y, et al. IL-22+ CD4+ T-cells in patients with active systemic lupus erythematosus. *Experimental biology and medicine*. 2013;238:193-199.
- Chemel M, Brion R, Segaliny A-I, et al. Bone morphogenetic protein 2 and transforming growth factor  $\beta$ 1 inhibit the expression of the proinflammatory cytokine IL-34 in rheumatoid arthritis synovial fibroblasts. *The American Journal of Pathology*. 2017;187:156-162.
- Boström EA, Lundberg P. The newly discovered cytokine IL-34 is expressed in gingival fibroblasts, shows enhanced expression by pro-inflammatory cytokines, and stimulates osteoclast differentiation. *PLoS one*. 2013;8:e81665.
- Guruprasad C, Pradeep A. Interleukin-34 levels in gingival crevicular fluid and plasma in periodontal health and disease with and without type-2 diabetes mellitus. *Journal of Investigative and Clinical Dentistry*. 2018;9:e12317.
- AR P. Interleukin-34 levels in gingival crevicular fluid and plasma in healthy and diseased periodontal tissue in presence or absence of obesity: A clinico-biochemical study. *The Bulletin of Tokyo Dental College*. 2018;59:79-86.

37. AR P. Influence of Smoking on Interleukin-34 Levels in Gingival Crevicular Fluid and Plasma in Periodontal Health and Disease: A Clinico-biochemical Study. *The Bulletin of Tokyo Dental College*. 2018;2017-0051.
38. Guruprasad C, Pradeep A. Effect of nonsurgical periodontal therapy on interleukin-34 levels in periodontal health and disease. *Indian Journal of Dental Research*. 2018;29:280.
39. Batra P, Das S, Patel P. Comparative evaluation of Gingival Crevicular Fluid (GCF) levels of Interleukin-34 levels in periodontally healthy and in patients with chronic and aggressive periodontitis-A cross-sectional study. *The Saudi Dental Journal*. 2019;31:316-321.