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An Evaluation of Periodontal Status and Cytokine Levels in Saliva and Gingival Crevicular Fluid of Patients with Inflammatory Bowel Diseases

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Running title: Cytokines in oral secretions in IBD patients.

One-sentence summary: In the presence of periodontal diseases in UC and CD, altered cytokine responses may be observed.

Abstract

Aims Periodontal diseases and inflammatory bowel diseases (IBD, ulcerative colitis [UC] and Crohn's disease [CD]) have been reported to present with increased salivary and gingival crevicular fluid (GCF) concentrations of cytokines. The aim of this study was to evaluate the salivary and GCF levels of TNF-α, IL-1β, IL-10, and IL-17A and their associations with the periodontal statuses of UC, CD and non-IBD patients, and to analyze the interrelationships among these cytokines, IBD conditions, and periodontal diseases.

Materials and Methods This cross-sectional study was performed with a total of 131 patients (62 women and 69 men, mean age 42.96±13.02 years). Patients were divided into three groups: UC, CD,

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and non-IBD. Periodontal status was defined according to the 2017 World Workshop Disease Classification. Salivary and GCF cytokine levels were analyzed using ELISA.

Results UC and CD patients diagnosed as having periodontitis and gingivitis presented with significantly higher levels of TNF-α and lower levels of IL-10 as compared with non-IBD patients ($p<0.05$). UC patients diagnosed with periodontitis exhibited significantly higher scores of bleeding on probing ($p=0.011$) and increased salivary and GCF IL-1β levels as compared with CD patients ($p=0.005$, and 0.012 respectively). Considering the active and remission status of IBD, salivary IL-1β was found to be correlated with the parameters representing the severity of periodontal diseases in active UC and CD patients.

Conclusion(s) In the presence of periodontal diseases, UC and CD patients showed different expression levels of TNF-α, IL-1β, and IL-10 in oral secretions as compared with non-IBD patients.

Keywords gingival crevicular fluid; periodontal diseases; cytokines; saliva, Crohn's disease, Ulcerative colitis.

1 INTRODUCTION

Inflammatory bowel diseases (IBD) represent one group of immune-mediated inflammatory diseases categorized into two major forms: ulcerative colitis (UC) and Crohn’s disease (CD). IBD are chronic, idiopathic, and relapsing inflammatory disease of the gastrointestinal tract characterized by complex interactions among genetic susceptibility, environmental factors, microbiota, and host immune response[1, 2].

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It has been well-documented that an increased prevalence of periodontal diseases has been found in patients with IBD as compared with systemically healthy patients[3-6]. It has also been reported that the prevalence was more pronounced in UC-patients than in CD-patients and more severe forms of periodontitis were observed in UC-patients as compared with CD-patients[4, 7]. However, the pathological interactions between periodontitis and IBD have not been established.

Increased salivary and gingival crevicular fluid (GCF) concentrations of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-18, as well as anti-inflammatory cytokines such as IL-4 and IL-10, and matrix metalloproteinases (MMPs) including MMP-8, have been shown in UC and CD-patients[7-9]. It is important to highlight that IBD-patients exhibit different dysregulations of immunity because they are distinct pathologies due to different involvements of the gastrointestinal track[10]. This difference could also be explained by T helper (Th) cell differentiation. UC has been shown to be a Th2 disease, whereas CD is considered to be a Th1 disease[2]. In a previous study, the levels of IL-1β, immunoglobulin (Ig) A, and LL37 were found to be significantly higher in UC and CD-patients as compared with healthy controls. Additionally the UC-patients demonstrated significantly higher salivary IL-6, IL-8, and MCP-1 levels, whereas the level of TNF-α was significantly higher in the CD-patients[11].

Th17 cell-associated cytokines, such as TNF-α, IL-1β, and IL-17A, have been shown to stimulate neutrophil infiltration in periodontitis and IBD, and to regulate the development of Th1/Th17 lymphocytes that aggravate inflammatory responses[7, 12, 13]. Therefore, the present study aimed to evaluate the salivary and GCF levels of TNF-α, IL-1β, IL-10 and IL-17A in association with periodontal status of UC, CD, and non-IBD patients, and to analyze the interrelationship among these cytokines, IBD conditions, and periodontal diseases.

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2 MATERIALS AND METHODS

The present study was carried out as a cross-sectional study with a total of 131 patients; UC (*n*=46, CD (*n*=48), and non-IBD (*n*=37) (62 women and 69 men, mean age 42.96±13.02 years), who were outpatients at Faculty of Medicine, Department of Gastroenterology, Ankara University, from July 2018 to July 2019. Forty-six patients were diagnosed with UC and 48 patients with CD. Non-IBD patients were recruited among patients who came for routine medical check-ups, with the suspicion of IBD but not diagnosed with UC or CD. The diagnoses of UC and CD were established according to clinical, endoscopic, and histopathological analyses. According to their periodontal status, patients with UC, CD, and non-IBD were diagnosed as having periodontitis, gingivitis, or healthy periodontium. The diagnosis of periodontitis was performed based on criteria that included pocket depth (PD)≥4 mm and clinical attachment loss of ≥3 mm in at least two non-adjacent teeth[14], whereas gingivitis was diagnosed by the presence of bleeding on probing (BOP)≥10% and PD≤3 mm[15]. Healthy periodontium was identified as the presence of BOP≤10% and PD≤3 mm[15]. The current study protocol was approved by Clinical Investigation Ethics Committee of Ankara University's Faculty of Dentistry (ID 36290600/92). All participants gave their written informed consent. The study was conducted in accordance with the Helsinki Declaration of Principles.

The inclusion criteria for the patients were as follows: aged between 18 and 70 years, being cooperative, not having any uncontrolled chronic condition other than IBD, and presence of at least 20 teeth. The exclusion criteria comprised smoking>10 cigarettes/day, having active infectious diseases, having received periodontal treatment or antibiotic medication in the last 3 months, and being pregnant or lactating.

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The Colitis Activity index (CAI) and the Harvey–Bradshaw index (HBI) were used to identify disease activity in the UC and CD-patients, respectively. Disease activity was determined as CAI≥5 for UC-patients, and HBI≥10 for CD-patients[16].

2.1 Clinical Examinations

A calibrated examiner performed a comprehensive periodontal examination from six different aspects of each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distobuccal) using a Williams periodontal probe*. The following measurements were recorded: Plaque Index[17] (PI), Gingival Index[18] (GI), BOP[19], PD, clinical attachment level (CAL), and gingival recession (GR). To perform the intra-examiner calibration, PD and CAL were measured twice at six sites of each tooth within 24 hours. Intra-examiner agreement was at least 90% for both PD and CAL within 1 mm.

The presence of oral soft-tissue lesions was also evaluated during the oral examinations.

2.2 Collection of Saliva Samples

Saliva sampling was performed before the periodontal examination and GCF collection. For each patient, unstimulated whole mixed saliva was collected using a modified method[20]. participants were asked to rinse her/his mouth with water for a minute, 10 minutes before the sampling. The saliva samples were collected into a sterile plastic container for 5 minutes. Samples were centrifuged for 10 minutes at 15,000x g at 4°C and the supernatant was frozen at -20 °C until analyses.

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2.3 Collection of Gingival Crevicular Fluid (GCF)

GCF specimens were collected by standardized filter paper strips[*]. A notch was placed at 1 mm part of periopaper which was inserted sub-gingivally for 30 sec. Filter papers contaminated with blood and saliva were excluded from the evaluation. Two GCF samples were obtained from the deepest PD if a pathological pocket was present, otherwise, the samples were taken from the mesio-buccal site of the first molar. Since all patients diagnosed with gingivitis were observed with BOP in molar teeth, samples were taken from the areas of inflammation. The strips were pooled in a sterile Eppendorf tube stored at -80 °C. GCF samples were measured by using a Periotron 8000®[†]. The values were measured using software[‡]. Before biochemical analyses, paper strips were placed in 300 µL of phosphate buffered saline solution containing 0.5% bovine serum albumin in Eppendorf tubes, and GCF was eluted from the strips by centrifugation for 6 minutes at 5000x g at 4 °C.

2.4 Determination of TNF-α, IL-1β, IL-10, and IL-17A Levels in GCF and Saliva Samples

The concentrations of IL-17A and IL-10 in GCF and saliva were measured with enzyme-linked immunosorbent assay (ELISA) using human IL-17A[§] and IL-10[**] ELISA kits. The absorbance was

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[*] Periopaper® Proflow Inc. NY, USA

[†] Oraflow Inc., Plainview, NY, USA

[‡] MLCONVERT.exe software version 2.52, Oraflow, Amityville, NY, USA

[§] Sunred Biological Technology Co., Shanghai, China, Catalog no: 201-12-0048

[**] Sunred Biological Technology Co., Shanghai, China, Catalog no: 201-12-0103

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measured at a wavelength of 450 nm using a microplate reader. IL-17A levels were expressed as ng/L and IL-10 levels were expressed as pg/mL. The concentrations of IL-1β and TNF-α in GCF and saliva were measured with ELISA using human IL-1β[*] and TNF-α[†] ELISA kits. The absorbance was measured at a wavelength of 450 nm using a microplate reader. IL-1β and TNF-α levels were expressed as pg/mL.

A ChemWell 2900 ELISA Autoanalyzer[‡] was used during the analyses and absorbance measurements. measurements. The sensitivity of Human IL-17A was 0,05 ng/L, detection range was 0,1 – 20 ng/L.; for Human IL-10 sensitivity was 9,012 pg/mL, detection range was 10-3000 pg/mL.; for Human IL-1β the detection limit was 0.35 pg/mL and intra assay CV% was 2,3%, inter assay CV% was 4,9%; for Human TNF-α the detection limit was 0,7 pg/mL and intra assay CV% was 6,6%, inter assay CV% was 4,5%. ELISA tests for each cytokine had been duplicated.

2.5 Statistical Methods

The sample size was determined as a total of at least 90 individuals, with at least 10 individuals in each group by using "Factorial Measurements Analysis of Variance" method, in which one of the factors was repeated in the factorial order. According to this analysis, the power was 81.19%.

[*] DIAsource ImmunoAssays Co., Louvain-la-Neuve, Belgium, Catalog No: KAP1211 – Lot: 180314/1

[†] DIAsource ImmunoAssays Co., Louvain-la-Neuve, Belgium, Catalog No: KAP1751 – Lot: 180309/1

[‡] ChemWell Awareness Technology, Inc., USA

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SPPS 25 statistical software[*] was used to evaluate the data. Descriptive statistics (means, standard deviations, median values, minimum and maximum numbers, and percentiles) were given for categorical and continuous variables. Additionally, the homogeneity of the variances, was checked with Levene test. Normality assumption was checked with Shapiro–Wilk test. As for the evaluation of the differences between two groups, Student's *t*-test was used when the parametric test prerequisites were met, and Mann–Whitney U test was used when they were not met.

For multiple comparisons, one-way analysis of variance and Tukey’s HSD test were used for a comparison of three or more groups, and when necessary, non-parametric Kruskal–Wallis and Bonferroni–Dunn tests were performed. The relationship between two continuous variables was evaluated with Pearson’s correlation coefficient, and if the parametric test prerequisites were not met, Spearman’s correlation coefficient was used. Univariate logistic regression analysis was used to determine the risk factors. The levels $p<0.05$ and $p<0.01$ were considered to be statistically significant.

3 RESULTS

Forty-one patients were with active IBD and 43 with remission. The mean age at the time of IBD diagnosis was 32.64±11.26 years. Depending on disease localization, patients with CD were diagnosed as ileal (n=6), ileocecal (n=14), colonic (n=8), perianal (n=2), ileocecal and colonic (n=5), ileocecal and perianal (n=5), or colonic and perianal (n=4).

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[*] IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY, IBM Corp.

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Among UC-patients, 16 patients were diagnosed with periodontitis (*n*=7 Stage II, *n*=6 Stage III, and *n*=3 Stage IV); 16 patients had gingivitis; and 14 had healthy periodontium. Among CD-patients, 12 patients were diagnosed with periodontitis (*n*=6 Stage II, *n*=4 Stage III, and *n*=2 Stage IV); 23 patients had gingivitis; and 13 had healthy periodontium. Among non-IBD patients, 12 patients were diagnosed with periodontitis (*n*=5 Stage II, *n*=4 Stage III, and *n*=3 Stage IV); 12 patients had gingivitis; and 13 had healthy periodontium.

The demographic data, oral hygiene habits, frequency of dental visit, presence of oral lesions, periodontitis and IBD status, and characteristics are presented in Table 1. In patients with periodontitis, there were significant differences in smoking consumption (<10cigarettes/day) and the presence of other systemic diseases among the patients with UC, CD, and non-IBD (p=0.018, P=0.024). Besides, there was a significant difference seen in periodontitis group regarding medications used in IBD treatment among UC and CD patients (p=0.013). None of the patients in the UC group were smokers. All non-IBD-periodontitis group patients had other systemic diseases; (*n*=4/66.66%) had diabetes mellitus, and (*n*=2/33.33%) had hypertension. Furthermore, (*n*=2/20.0%) of non-IBD-healthy periodontium group patients had hypertension. While none of the CD-periodontitis patients were taking immunomodulators, UC-periodontitis patients were detected as (n=5/31.3%).

Patients with gingivitis and healthy periodontium were detected with the presence of oral lesions among the groups (p<0.044, and p=0.033, respectively). Besides, aphthous ulcer was the only lesion that detected among the participants. In healthy periodontium patients, the highest frequency of brushing as once a day was seen in CD group as compared with the other groups (p=0.010) (Table 1).

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3.1 Comparisons According to the Periodontal Statuses of Patients with UC and CD and Non-IBD Patients

3.1.1 Clinical Periodontal Parameters

For the patients with periodontitis, only the mean BOP values differed between UC and CD groups, depicting a higher value for UC group ($p<0.05$). While the mean BOP value was higher in UC group than in non-IBD group, this value was observed to be lower in CD group as compared with non-IBD group ($p<0.05$). Among the patients with gingivitis, the mean PD, CAL, and GR values were higher for non-IBD group as compared with UC and CD groups ($p<0.05$). In healthy periodontium, the mean PD and CAL values differed between UC and non-IBD groups and between CD and non-IBD groups, indicating the highest mean value for non-IBD group in all comparisons ($p<0.05$) (Table 2).

3.1.2 Cytokine Levels in Saliva Samples

In patients with periodontitis and gingivitis, the mean levels of TNF-α were higher for UC group as compared with the other groups, whereas the mean IL-1β and IL-10 levels were found to be lower for CD group ($p<0.05$). For periodontitis patients, the salivary IL-10 levels were higher in non-IBD group as compared with the other groups ($p<0.05$). Regarding healthy periodontium patients, no significant differences were found for the salivary biomarkers between the groups (Figure 1) (see Table S1 in online Journal of Periodontology).

3.1.3 Cytokine Levels in GCF Samples

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In patients with periodontitis and gingivitis, the mean levels of TNF-α were found to be higher in CD group as compared with the other groups ($p<0.05$). For periodontitis patients, the highest GCF levels of IL-1β were found in UC group ($p<0.05$). The mean IL-10 and IL-17A levels were both higher in non-IBD group as compared with UC and CD groups for patients with periodontitis and gingivitis ($p<0.05$). Regarding the patients with healthy periodontium, the mean IL-10 level was lower in UC group ($p<0.05$) (Figure 2) (see Table S2 in online Journal of Periodontology).

3.2 Correlations among Clinical Periodontal Parameters and the Salivary and GCF levels of Cytokines in Patients with UC and CD

In UC-patients, significant correlations were found between salivary TNF-α levels and PI and GI values (p=0.049 and p=0.03, respectively), and between salivary TNF-α and IL-10 levels (p=0.001). Positive correlations were observed between salivary IL-1β levels and BOP and CAL values (p=0.006 and p=0.032), as well as between salivary IL-1β and TNF-α levels and between salivary IL-1β and IL-10 levels (p=0.001 and p=0.006, respectively). Salivary IL-17A levels were strongly positively correlated with salivary IL-10 levels (p=0.004). GCF IL-1β levels were positively correlated with the CAL, salivary IL-17A levels, and GCF TNF-α levels (p=0.047, p=0.012, and p=0.021, respectively). A strongly positive correlation was found between GCF IL-17A and GCF IL-10 levels (p=0.001) (see Table S3 in online Journal of Periodontology).

In CD-patients, there was a positive correlation between salivary IL-1β levels and PI, PD, BOP and CAL values (P=0.016, P= 0.048, p=0.003 and p=0.017, respectively). Salivary IL-17A levels were strongly positively correlated with salivary IL-10 levels in CD-patients (p=0.001). GCF IL-1β levels were correlated with GI and BOP values (p=0.033 and p=0.040). GCF IL-10 and IL-17A levels

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were both negatively correlated with PI values (p=0.019 and p=0.024). A strong positive correlation was found between GCF IL-17A and GCF IL-10 levels (p=0.001) (see Table S4 in online Journal of Periodontology).

In non-IBD-patients, there was a strong positive correlation between salivary IL-17A levels and salivary IL-10 levels (p=0.001). GCF IL-17A levels were negatively correlated with BOP and CAL values (p=0.021 and p=0.017, respectively). Similar to salivary concentrations, positive correlations were also found between GCF IL-1β levels and GCF TNF-α levels (p=0.035), and between GCF IL-17A and GCF IL-10 levels (p<0.000) (see Table S5 in online Journal of Periodontology).

When the correlation analyses were performed according to the activity status of IBD-patients, correlations were shown between salivary IL-1β levels and GI, and BOP values (p=0.008, and p=0.031, respectively), and between salivary IL-17A levels and PD and CAL values (p=0.017 and p=0.024, respectively) in active UC-patients. Additionally, there was an association between GCF IL-1β levels and GR values (p=0.044). For UC-patients in remission period, no significant correlations were identified between clinical periodontal parameters and the salivary and GCF levels of biomarkers. In active CD-patients, salivary TNF-α levels were correlated with PI values (p=0.018), and salivary IL-1β levels were correlated with PD and CAL values (p=0.016 and p=0.023). Regarding GCF biomarkers, a significant correlation was only found between IL-17A levels and GI values (p=0.044). For CD-patients in remission period, GCF IL-1β and IL-10 levels correlated with BOP values (p=0.016 and p=0.046) (Figure 3).

The correlation analyses were performed according to medications used (see Table S6 and Table S7 in online Journal of Periodontology). Regarding to immunomodulators, significant positive correlations were found between salivary IL-1β level with GI value and between salivary IL-17A level

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with PI and GI values. Besides, a significant negative correlation was detected between GCF TNF-α level with GR value (see Table S7 in online Journal of Periodontology).

3.3 Multilevel Logistic Regression Analysis

To identify the effects of determinant variables related to patients' demographics, dental health behaviors, presence of oral lesions, disease characteristics of IBD, and medication usage with respect to the periodontal status, univariate logistic regression analyses were performed. A lack of interdental cleaning was only found to be a risk indicator for periodontitis (p=0.041). The risk of periodontitis in patients without interdental cleaning was 3.2 times higher than in patients with interdental cleaning. The medication used for IBD treatment was found to be a risk factor for gingivitis (p=0.027). The risk of gingivitis in patients using medications was 3.5 times higher than that of patients not using medications. Since only one variable was statistically significant in the univariate logistic regression analyses, a multivariate logistic regression analysis could not be performed (Table 3).

4 DISCUSSION

This study investigated the salivary and GCF levels of inflammatory cytokines (TNF-α, IL-1β, IL-10, and IL-17A), in patients with UC, and CD and non-IBD, with respect to their periodontal status for further understanding of the relationships among the selected cytokines, IBD, and periodontal diseases.

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Previous investigations have indicated an association of severity of periodontal diseases for UC and CD-patients as compared with healthy control.[3, 16, 21-24]. A recent cross-sectional study demonstrated that UC and CD-patients showed significantly higher percentages of sites with PD≥5 mm, and CAL≥4 mm as compared with controls[23]. In contrast, other studies have revealed an inverse relationship between IBD and periodontal diseases or the absence of a difference in periodontal disease susceptibility between IBD and non-IBD patients[25-27].

In the present study, molar teeth were selected for GCF sampling as the amount of production of GCF is quite small and changes regarding to the size of gingival sulcus. Moreover, larger GCF volumes have been detected at the posterior sites compared to anterior sites, at mandibular sites compared to maxillary sites, and at interproximal sites of posterior teeth than anterior labial sites[28-32]. In a study conducted by Challacombe et al.[33], authors reported that the mean GCF volume ranged from 0.43 to 1.56 μL in the proximal site of molar teeth[33]. In our study, non-IBD group presented statistically significant higher mean values of PD as compared with IBD groups in both gingivitis and healthy periodontium patients. This finding was in line with a previous study conducted by Grossner-Schreiber et al.[26], which demonstrated deeper PD values in the healthy control group as compared with patients with IBD[26]. In agreement, a previous cross-sectional study reported that periodontal diseases in patients with IBD was more generalized but less severe than in the general population[34]. In the present study, the highest mean value of BOP was observed in the UC group, while the lowest value was observed in the CD group in periodontitis patients. Moreover, our study exhibited lower values of PD and CAL for CD group as compared with UC and non-IBD groups in gingivitis patients. In contrast, Bucbender et al.[27], reported that the mean BOP and PD values were increased significantly in CD-patients as compared with healthy controls and no significant difference was observed between UC-patients and healthy controls[27]. It was emphasized in a recent retrospective cohort

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study that some medications for IBD could show a protective effect against periodontitis development in CD-patients[35]. Another study highlighted that CD-patients needed markedly more immunomodulators in their treatment than UC-patients and, therefore, CD-patients might be less vulnerable to attachment loss[21]. Another possible explanation for having higher means of clinical periodontal parameters in UC-patients may be due to a significantly low number of smokers in the group. In fact, smoking was reported to play a protective role for UC, while it has been shown as an evident risk indicator for periodontal diseases in CD-patients[6].

The majority of previous studies have indicated that IBD and periodontal diseases share similar cytokine expression patterns due to having some common pathogenic inflammatory processes[36, 37]. Prior studies have reported elevated levels of proinflammatory and anti-inflammatory mediators and MMPs, such as IL-4, -10, -17A, -18, -21, IFN-γ, MMP-7, and MMP-8, in the periodontal tissues of IBD patients[12, 27, 38]. To the best of our knowledge, the present study was the first to examine the selected inflammatory mediators in both saliva and GCF in UC, CD, and non-IBD patients according to their periodontal status. In this study, UC group of patients had the highest salivary concentrations of TNF-α as compared with CD and non-IBD groups for both periodontitis and gingivitis patients. The salivary levels of TNF-α, IL-1β, and IL-10 exhibited the lowest concentrations for CD group in periodontitis and gingivitis patients. These findings could be attributed to the fact that anti-TNF-α therapy has been demonstrated to induce neutrophil apoptosis and, moreover, to suppress neutrophils to produce proinflammatory cytokines and chemokines[39]. However, this medication was reported to have a slight effect on UC-patients and healthy controls. Interestingly, in our study, all the salivary biomarkers were not significantly different among UC, CD, and non-IBD groups for the patients with healthy periodontium. This result highlighted the importance of having a healthy periodontium in IBD-patients, which may have prevented the

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excessive inflammatory response evident in the saliva of IBD-patients. In agreement with previous studies, in GCF of periodontitis sites, CD-patients had significantly increased levels of TNF-α as compared with UC and non-IBD patients, which was consistent with CD being considered to be driven by a Th1 response[40].

IL-10 plays a key role in regulating the physiological state of tissue and in downregulating the production of proinflammatory cytokines, however, its contribution to IBD is not fully understood. Elevated serum concentrations of IL-10 in active UC and CD-patients as compared with healthy controls have been previously reported[41, 42]. Overexpression of IL-10 in subgingival biofilm samples for UC and CD-patients as compared with healthy controls have also been demonstrated[27]. In contrast, other studies have shown similar or lower IL-10 levels in the serum of patients with UC and CD as compared with non-IBD patients[43, 44]. The present study exhibited higher levels of IL-10 in both saliva and GCF in non-IBD group as compared with UC and CD groups, in periodontitis and gingivitis patients. One possible reason for the increased levels of IL-10 in non-IBD patients might be that these patients did not use medications such as anti-TNF-α therapy, which have been reported to reduce the expressions of IL-17 A, IFNγ, and IL-10 in IBD-patients[45].

The present study indicated significant correlations between the clinical periodontal parameters and salivary and GCF cytokine levels according to the activity status of IBD. In active UC-patients, there were positive correlations between salivary IL-17A levels and CAL values. Therefore, it seems that overexpression of salivary levels of IL-17A may also increase the severity of periodontal diseases. Additionally, in active CD-patients, while there were positive relationships between salivary IL-1β levels and PD and CAL values, no relationship was found between salivary IL-17A and any clinical parameters. In line with these findings, in a previous study by Figueredo et al.[12], a trend towards increased levels of IL-1β and TNF-α in gingival and intestinal tissues were demonstrated as

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potent markers for differentiating active and remission status in IBD-patients[12]. Furthermore, in the present study, significant positive correlations between IL-17A and IL-10 in GCF and the saliva of both UC and CD-patients were identified. T-cell-specific blockade of IL-10 signaling using a dominant-negative IL-10 receptor was suggested to lead to increased Th17 cells in an anti-CD3 antibody-induced model of small intestinal inflammation[46]. Some limitations of the current study must be mentioned. First, there was an absence of completely systemically healthy individuals, which could possibly affect the interpretation of the results. Additionally, immune cells interactions and other cytokines functions could have been investigated as they may play an important role between both diseases.

5 CONCLUSIONS

In the presence of periodontal diseases, UC and CD-patients presented with higher levels of TNF-α and lower levels of IL-10 as compared with non-IBD patients. In addition, UC-patients had higher scores of BOP and increased levels of IL-1β in the presence of periodontal disease, whereas in the absence of periodontal disease, IL-10 levels were lower in UC as compared with CD-patients. On the other hand, non-IBD patients with gingivitis or healthy periodontium seemed to have higher means of clinical periodontal parameters as compared with UC and CD-patients. While no relationship between periodontal clinical parameters and cytokine levels in oral secretions were identified in IBD-patients in remission status, salivary IL-1β seemed to be a key inflammatory cytokine of UC and CD activity for severity of periodontal inflammation. Additionally, salivary IL-17A could potentially be a biomarker of increasing severity of periodontal disease in active UC-patients.

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AUTHOR CONTRIBUTIONS

A.E. worked on data collection; N.O. worked on interpretation of data, conception and design of the study, as well as analysis and approval of the version to be published; S.C.I. worked on interpretation of data and drafting the article; M.T. worked on data collection; S.E.U., G.D., and C.F. performed the biochemical analyses of cytokines; A.P. worked on interpretation of data, conception and design of the study, analysis, and approval of the version to be published.

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FIGURES AND TABLES LEGENDS

Figure 1. Graphical descriptions of salivary cytokines levels in relation to periodontal health status and bowel health status.

A. In patients with periodontitis and gingivitis, the mean levels of TNF-α were significantly higher for the UC group of patients as compared with the other groups.

B. In patients with periodontitis and gingivitis, the mean levels of IL-1β were significantly lower for the CD group of patients as compared with the other groups.

C. In patients with periodontitis and gingivitis, the mean levels of IL-10 were significantly lower for the CD group of patients as compared with the other groups.

Figure 2. Graphical descriptions of GCF cytokines levels in relation to periodontal health status and bowel health status.

A. In patients with periodontitis and gingivitis, the mean levels of TNF-α were significantly higher for the CD group of patients as compared with the other groups.

B. In patients with periodontitis, the mean level of IL-1β was significantly higher for the UC group of patients as compared with the other groups.

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C. In patients with periodontitis and gingivitis, the mean levels of IL-10 were significantly higher for the non-IBD group of patients as compared with the other groups, and in patients with healthy periodontium, the mean level of IL-10 was significantly lower for the UC group of patients as compared with the other groups.

D. In patients with periodontitis and gingivitis, the mean levels of IL-17A were significantly higher for the non-IBD group of patients as compared with the other groups.

Figure 3. Significant correlations between clinical measurements (PI, GI, BOP, PD, CAL, and GR) and both GCF and salivary cytokines in IBD patients in relation to the activity status of the diseases.

A. In active UC, positive correlations between the mean salivary IL-1β and (GI, and BOP) values, and IL-17A levels and (PD, and CAL) values.

B. In active UC, a positive correlation between the mean GCF IL-1β levels and GR value.

C. In active CD, a negative correlation between the mean salivary TNF-α levels and PI value, and positive correlations between salivary IL-1β levels and (PD, and CAL) values.

D. In active CD, a negative correlation between the mean GCF IL-17A levels and GI value.

E. In remission CD, a positive correlation between the mean GCF IL-1β and BOP, and negative correlation between mean GCF IL-17A levels and BOP value.

Table 1. Relationships among IBD status (UC, CD, and non-IBD), demographic, and other variables for patients with periodontal health statuses as periodontitis, gingivitis, and healthy periodontium.

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Table 2. Comparison of clinical measurements (PI, GI, PD, BOP CAL, and GR) in periodontitis, gingivitis, and healthy periodontium categories according to bowel health status.

Table 3. Univariate logistic regression analysis for risk factors influencing periodontal diseases (periodontitis and gingivitis).

Supplemental Table 1. Statistical data of saliva regarding figure 1.

Supplemental Table 2. Statistical data of GCF regarding figure 2.

Supplemental Table 3. Relationship between clinical indexes (PI, GI, PD, BOP, CAL, and GR) with GCF and salivary cytokines levels in UC patients.

Supplemental Table 4. Relationship between clinical indexes (PI, GI, PD, BOP, CAL, and GR) with GCF and salivary cytokines levels in CD patients.

Supplemental Table 5. Relationship between clinical indexes (PI, GI, PD, BOP, CAL, and GR) with GCF and salivary cytokines levels in non-IBD patients.

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Supplemental Table 6. Relationship between clinical indexes (PI, GI, PD, BOP, CAL, and GR) with GCF and salivary cytokines regarding medications used in IBD treatment (TNF-inhibitors and Anti-integrins).

Supplemental Table 7. Relationship between clinical indexes (PI, GI, PD, BOP, CAL, and GR) with GCF and salivary cytokines regarding medications used in IBD treatment (Immunomodulators and 5-ASA).

Tables

Table 1. Relationships among IBD status (UC, CD, and non-IBD), demographic, and other variables for patients with periodontal health status as periodontitis, gingivitis, and healthy periodontium.

Variables		Periodontitis							Gingivitis							Healthy periodontium						
		UC		CD		non-		P	UC		CD		non-		P	UC		CD		non-		P
		n	%	n	%	n	%	val	n	%	n	%	n	%	val	n	%	n	%	n	%	val
Gende	Male	1	75	7	58	5	41	0.2	7	4	1	6	5	41	0.1	6	42	8	6	3	23	0.1
r	Female	4	25	5	41	7	58	02	9	5	7	3	7	58	61[a]	8	57	5	3	1	76	40[a]
Smoki	Yes	0	0.	4	33	5	41	**0.0**	2	1	1	4.	1	8.	0.8	0	0.	1	7.	2	15	0.3
ng	No	1	10	8	66	7	58	**18**	1	8	2	9	1	91	06[b]	1	10	1	9	1	84	00[b]
Dental	Yes	4	25	1	8.	5	41	0.1	6	3	9	3	3	25	0.6	4	28	5	3	7	53	0.4
visit	No	1	75	1	91	7	58	69	1	6	1	6	9	75	91[a]	1	71	8	6	6	46	04[a]
Brushi	Yes	1	62	1	83	9	75	0.4	1	9	1	8	1	83	0.6	1	85	1	9	1	10	0.7
ng	No	6	37	2	16	3	25	62	1	6.	4	1	2	16	66[b]	2	14	1	7.	0	0.	61[b]
Brushi	Once /day	4	40	5	50	3	33	0.6	9	6	1	5	6	60	0.5	2	16	8	6	2	16	**0.0**
ng	Twice	5	50	5	50	4	44	22	6	4	6	3	4	40	91[b]	1	83	3	2	7	58	**10**
Frequ	More than	1	10	0	0.	2	22		0	0.	3	1	0	0.		0	0.	1	8.	3	25	**b ***
Inter-	Yes	3	18	3	25	1	8.	0.5	7	4	9	3	3	25	0.5	7	50	6	4	6	46	0.9
dental	No	1	81	9	75	1	91	53	9	5	1	6	9	75	79[a]	7	50	7	5	7	53	37[a]
Oral	Yes	2	12	0	0.	1	8.	0.4	4	2	1	4.	0	0.	**0.0**	2	14	5	3	0	0.	**0.0**
lesion	No	1	87	1	10	1	91	58	1	7	2	9	1	10	**44[b]**	1	85	8	6	1	10	**33[b]**
Other	Yes	6	40	4	36	6	10	**0.0**	2	2	2	1	0	0.	0.7	4	40	2	2	2	20	0.5
syste	No	9	60	7	63	0	0.	**24**	8	8	9	8	2	10	89[b]	6	60	6	7	8	80	92[b]
IBD	Active	9	64	4	36	-	-	0.1	7	5	1	5	-	-	0.9	5	35	5	4	-	-	0.7

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status	Remission	5	35	7	63	-	-	65[a]	6	4	9	4	-	-	48[a]	9	64	7	5	-	-	56[a]
Time	0-5 Years	3	27	4	36	-	-		3	3	9	4	-	-		6	46	8	8	-	-	0.2
since	6-10	4	36	3	27	-	-		3	3	6	3	-	-		3	23	0	0.	-	-	44[b]
IBD	11 Years	4	36	4	36	-	-	1.0	3	3	4	2	-	-	0.8	4	30	2	2	-	-	
Medic	TNF-	3	18	7	58	-	-		2	1	7	3	-	-		3	21	5	3	-	-	0.6
ations	Anti-	2	12	4	33	-	-		2	1	3	1	-	-		-	-	-	-	-	-	38[b]
used	Immunom	5	31	0	0.	-	-		5	3	9	4	-	-		9	64	7	5	-	-	
in	5-ASA	6	37	1	8.	-	-		6	4	2	9.	-	-		2	14	1	7.	-	-	

*p<0.05

a; Chi-square test, b; Fisher-exact test

UC; Ulcerative Colitis. CD; Crohn's Disease. IBD; Inflammatory Bowel Diseases. mm; millimeter. %; Percent. UC; Ulcerative Colitis. CD; Crohn's Disease. IBD; Inflammatory Bowel Diseases. n; number. %; Percent.

Table 2. Comparison of clinical measurements (PI, GI, PD, BOP CAL, and GR) in periodontitis, gingivitis, and healthy periodontium categories according to bowel health status.

Periodontal health status	Measurement name	UC ($\bar{x}$±SD)	CD ($\bar{x}$±SD)	non-IBD ($\bar{x}$±SD)	P value
Periodontitis	PI	0.89±0.60	0.60±0.46	0.67±0.83	0.249
	GI	0.51±0.71	0.16±0.33	0.43±0.52	0.149
	PD (mm)	2.46±0.52	2.43±0.57	2.74±0.91	0.516
	BOP %	36.72±24.22	19.10±16.00	27.04±13.70	**0.011** $^{\Psi}$ *
	CAL (mm)	2.47±0.52	2.43±0.57	2.74±0.91	0.239
	GR (mm)	0.18±0.30	0.13±0.19	0.15±0.15	0.868
Gingivitis	PI	0.56±0.55	0.57±0.40	0.48±0.40	0.674
	GI	0.26±0.55	0.04±0.08	0.14±0.25	0.969
	PD (mm)	1.79±0.26	1.74±0.30	2.14±0.20	**0.001** $^{\Delta}$ **
	BOP %	24.49±14.20	22.89±12.77	28.36±13.29	0.341
	CAL (mm)	1.85±0.30	1.75±0.31	2.29±0.39	**0.001** $^{\Psi}$ **
	GR (mm)	0.06±0.13[a]	0.01±0.01	0.16±0.33	**0.032** $^{\Psi}$ *
Healthy periodontium	PI	0.24±0.27	0.20±0.32	0.12±0.16	0.136
	GI	0.08±0.20	0.01±0.01	0.00±0.01	0.821
	PD (mm)	1.58±0.27	1.74±0.35	1.99±0.31	**0.009** $^{\Psi}$ **
	BOP %	5.43±3.13	6.37±1.93	5.33±3.55	0.516

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	CAL (mm)	1.65±0.31	1.76±0.34	2.05±0.32	**0.008** $^{\Delta}$ **
	GR (mm)	0.07±0.10	0.02±0.03	0.06±0.08	0.507

**p<0.01 *p<0.05

Δ One way variance analysis (ANOVA); ψ Kruskal Wallis Test.

UC; Ulcerative Colitis. CD; Crohn's Disease. IBD; Inflammatory Bowel Diseases. PI; Plaque Index. GI; Gingival Index. PD; Pocket Depth. BOP; Bleeding on Probing. CAL; Clinical Attachment Level. GR; Gingival Recession. SD; Standard Deviation. mm; millimeter. %; Percent.

Table 3. Univariate logistic regression analysis for risk factors influencing periodontal diseases (periodontitis and gingivitis).

Variables (Reference)		Periodontal Diseases											
		Periodontitis						Gingivitis					
		B	S.E.	P value	OR	95% C.I for OR		B	S.E.	P value	OR	95% C.I for OR	
						Lower limit	Upper limit					Lower limit	Upper limit
IBD status (Remission)	Active	0.550	0.568	0.333	1.733	0.569	5.278	0.652	0.534	0.222	1.920	0.675	5.464
Medications used in IBD treatment (No)	Yes	0.560	0.627	0.372	1.750	0.133	2.040	1.253	0.567	**0.027** *	3.500	0.089	1.081
Smoking (No)	Yes	1.466	1.153	0.204	4.333	0.452	41.545	0.773	1.183	0.513	2.167	0.213	22.019
Oral lesion (Yes)	No	1.515	0.855	0.076	4.550	0.851	24.318	0.867	0.650	0.182	2.380	0.666	8.506
Brushing Habits (Yes)	No	1.163	0.742	0.117	3.200	0.748	13.690	0.163	0.777	0.834	1.176	0.256	5.399
Inter-dental cleaning habits (Yes)	No	1.225	0.600	**0.041** *	3.405	1.050	11.044	0.289	0.504	0.567	1.335	0.497	3.587
Dental visit (Yes)	No	0.833	0.640	0.193	2.300	0.656	8.070	-0.223	0.524	0.670	0.800	0.286	2.236

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Other systemic diseases (No)	Yes	0.223	0.642	0.728	1.250	0.355	4.402	0.754	0.748	0.313	2.125	0.491	9.198

*p<0.05

IBD; Inflammatory Bowel Diseases. B; Beta coefficient. S.E.; Standard Error. OR; Odds Ratio. C.I.; Confidence Interval. %; Percent.

Figure 1. Graphical descriptions of salivary cytokines levels in relation to periodontal health status and bowel health status.

A. In patients with periodontitis and gingivitis, the mean levels of TNF-α were significantly higher for the UC group of patients as compared with the other groups. B. In patients with periodontitis and gingivitis, the mean levels of IL-1β were significantly lower for the CD group of patients as compared with the other groups. C. In patients with periodontitis and gingivitis, the mean levels of IL-10 were significantly lower for the CD group of patients as compared with the other groups.

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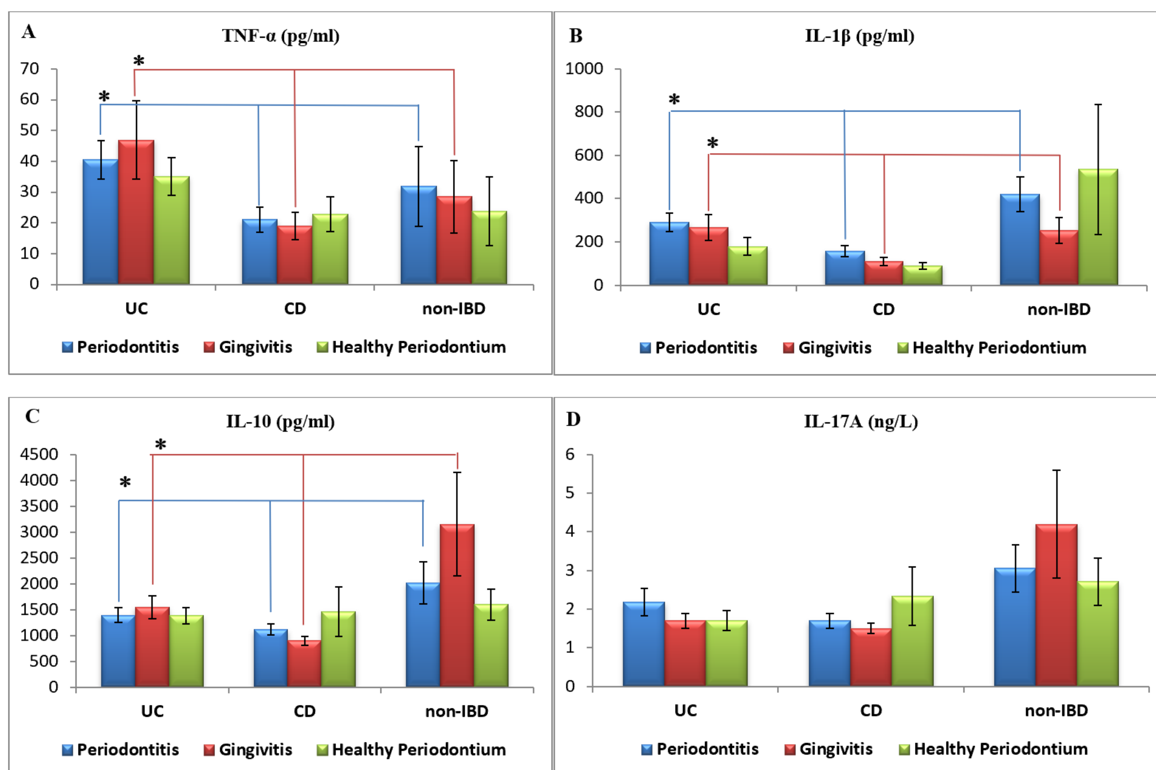

* Represents statistically significant difference between groups (P<0.05). Kruskal Wallis H test
UC; Ulcerative Colitis. CD; Crohn's Disease. IBD; Inflammatory Bowel Diseases. TNF-α; Tumor Necrosis Factor-alpha. IL-1β; Interlukin-1beta. IL-10; Interlukin-10. IL-17A; Interlukin-17A. pg/ml; picogram/milliliter. ng/L; nanogram/Liter.

Figure 2. Graphical descriptions of GCF cytokines levels in relation to periodontal health status and bowel health status.

A. In patients with periodontitis and gingivitis, the mean levels of TNF-α were significantly higher for the CD group of patients as compared with the other groups. B. In patients with periodontitis, the mean level of IL-1β was significantly higher for the UC group of patients as compared with the other groups. C. In patients with periodontitis and gingivitis, the mean levels of IL-10 were significantly higher for the non-IBD group of patients as compared with the other groups, and in patients with

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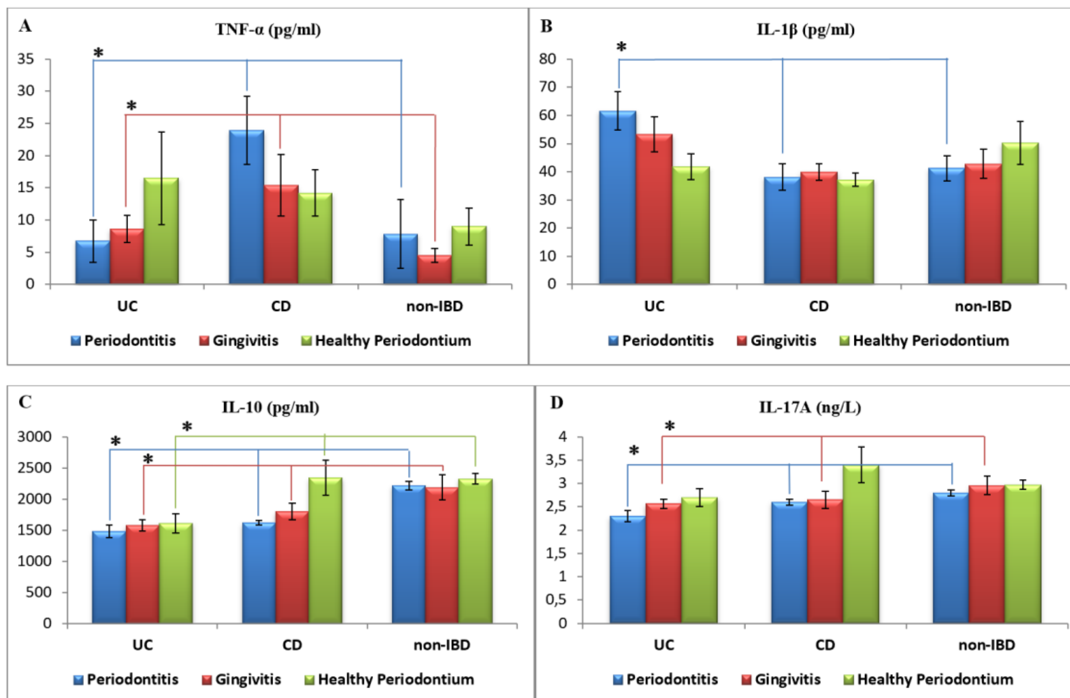
healthy periodontium, the mean level of IL-10 was significantly lower for the UC group of patients as compared with the other groups. D. In patients with periodontitis and gingivitis, the mean levels of IL-17A were significantly higher for the non-IBD group of patients as compared with the other groups.



* Represents statistically significant difference between groups ($P<0.05$).

One Way ANOVA test. Kruskal Wallis H test

GCF; Gingival Crevicular Fluid. UC; Ulcerative Colitis. CD; Crohn's Disease. IBD; Inflammatory Bowel Diseases. TNF-α; Tumor Necrosis Factor-alpha. IL-1β; Interlukin-1beta. IL-10; Interlukin-10. IL-17A; Interlukin-17A. pg/ml; picogram/milliliter. ng/L; nanogram/Liter.

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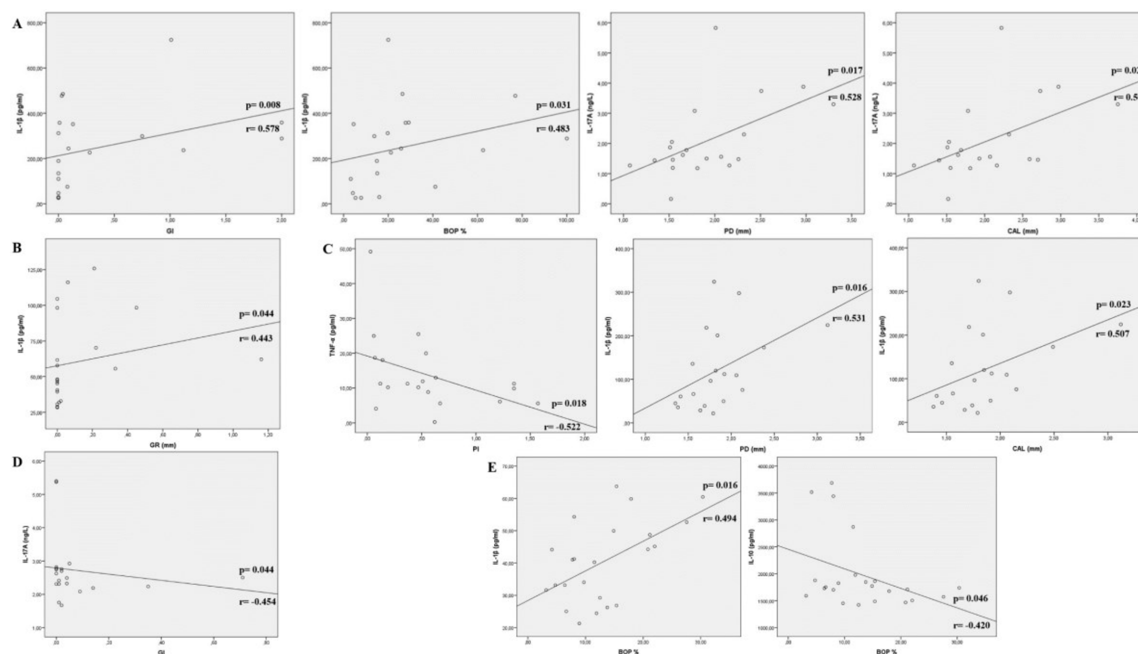
Figure 3. Significant correlations between clinical measurements (PI, GI, BOP, PD, CAL, and GR) and both GCF and salivary cytokines in IBD patients in relation to the activity status of the diseases. A. In active UC, positive correlations between the mean salivary IL-1β and (GI, and BOP) values, and IL-17A levels and (PD, and CAL) values. B. In active UC, a positive correlation between the mean GCF IL-1β levels and GR value. C. In active CD, a negative correlation between the mean salivary TNF-α levels and PI value, and positive correlations between salivary IL-1β levels and (PD, and CAL) values. D. In active CD, a negative correlation between the mean GCF IL-17A levels and GI value. E. In remission CD, a positive correlation between the mean GCF IL-1β and BOP, and negative correlation between mean GCF IL-17A levels and BOP value.



p; p value r: Correlation coefficient

UC; Ulcerative Colitis. CD; Crohn's Disease. GCF; Gingival Crevicular Fluid. PI; Plaque Index. GI; Gingival Index. BOP; Bleeding on Probing. PD; Pocket Depth. CAL; Clinical Attachment Level. GR; Gingival Recession. TNF-α; Tumor Necrosis Factor-alpha. IL-1β; Interleukin-1beta. IL-17A; Interleukin-17A. IL-10; Interlukin-10. pg/ml; picogram/milliliter. ng/L; nanogram/Liter. mm; millimeter. %; Percent. p; p value<0.05.

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