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ORIGINAL ARTICLE

Effects of smoking on the salivary and GCF levels of IL-17 and IL-35 in periodontitis

Ezgi Sila Taskaldiran[1,2] · Gulay Tuter[2] · Aysegul Atak Yucel[3] · Melek Yaman[3]

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Abstract

Periodontitis progression is associated with a host response in which anti-inflammatory and pro-inflammatory cytokine networks play a key role. Smoking is involved in the production of various mediators. The study aims to evaluate the levels of IL-17 and IL-35 in saliva and gingival crevicular fluid (GCF), to investigate the effects of smoking on these cytokines in smoker and non-smoker periodontitis patients. 19 smokers with periodontitis, 20 non-smokers with periodontitis, and 18 periodontally healthy subjects were included in the study. Periodontal clinical indexes were recorded and the levels of IL-17 and IL-35 in saliva and GCF were analyzed. No significant difference was detected among the groups in terms of salivary IL-17 and IL-35 levels. GCF IL-17 and IL-35 concentration levels in the non-smoker periodontitis group were significantly lower than the others ($p < 0.05$). Total levels of GCF IL-17 were significantly higher in both periodontitis groups than the control group; and total levels of GCF IL-35 were significantly higher in non-smoker periodontitis group than the others ($p < 0.05$). A positive correlation was detected between the salivary IL-17 and IL-35 levels ($r = 0.884$), GCF IL-17 and IL-35 concentrations ($r = 0.854$), and total GCF IL-17 and IL-35 ($r = 0.973$) levels ($p < 0.01$). The present study revealed a positive correlation between the IL-35 and IL-17 levels both in saliva and GCF. IL-17 and IL-35 can be considered as one of the cytokines that play a role in periodontal health and periodontitis; and smoking may be among the factors that affect the levels of these cytokines in GCF and saliva.

Keywords Interleukins · Periodontitis · Smoking · Gingival crevicular fluid · Saliva

Introduction

Periodontitis is a destructive disease characterized by gingival inflammation and alveolar bone loss [1]. The pathological process from periodontal health to periodontitis and tooth loss is shaped by biofilm pathogens and host immune responses activated by these microorganisms. It has been reported that the difference between individuals in terms of sensitivity to periodontitis can be attributed to the fact that each individual's reaction to periodontal pathogens is unique [2]. In this regard, investigating the host immune responses to understand the pathogenesis of periodontitis constitutes the basis of many studies today.

Since cigarette contains more than 8400 chemicals, smoking is considered a modifiable risk factor for periodontal disease [1, 3]. Because of the dose-dependent effect of smoking on the periodontium, the incidence of periodontitis in smokers is 20 times higher than in non-smokers [4]. Smoking impairs microcirculation in gingiva. As a result, despite having the same level of biofilm as non-smokers, smokers have lower GCF and bleeding on probing scores [5]. One of the crucial effects of smoking during the pathogenesis of periodontitis occurs with the host response. It has been reported that smoking suppresses the defensive responses of

✉ Gulay Tuter
gulay@gazi.edu.tr

Ezgi Sila Taskaldiran
ezgisilataskaldiran@aydin.edu.tr

Aysegul Atak Yucel
aysegula@gazi.edu.tr

Melek Yaman
melek.yaman@gazi.edu.tr

1 Department of Periodontology, Faculty of Dentistry, Istanbul Aydin University, Istanbul, Turkey

2 Department of Periodontology, Faculty of Dentistry, Gazi University, Ankara, Turkey

3 Department of Immunology, Faculty of Medicine, Gazi University, Ankara, Turkey

the immune system but amplifies the pathological responses [6].

Intracellular signaling plays a considerable role in the initiation and progress of inflammatory processes such as periodontitis. Cytokines are soluble proteins produced by various cell types and play a crucial role in the aforementioned cell-to-cell pathways. Cytokines are classified as pro-inflammatory and anti-inflammatory depending on their functional properties. However, some cytokines have been shown to have both characteristics [7].

IL-17A, a member of IL-17 family and commonly recognized as IL-17, is a cytokine released upon stimulation of largely differentiated T helper (Th) 17 cells, and plays crucial roles in periodontal pathogenesis. While IL-17 induces production of pro-inflammatory mediators in inflammatory responses and promotes bone resorption, it also plays a role in the regulation of neutrophil migration, which is an important stage in periodontal tissue homeostasis, and provides a protective inflammatory response against pathogens in the mucosa. Considering these characteristics, it is believed that the pro-inflammatory role of IL-17, which contributes to tissue destruction during the advance of periodontitis, is superior to its antimicrobial roles [8].

IL-35 is a novel cytokine that was found in 2007, a member of the IL-12 family of cytokines, and is produced by the regulatory T cells (Treg) [9]. It has been reported that IL-35 inhibits the differentiation of the Th17 cells, and suppresses the production of IL-17 and the other pro-inflammatory cytokines induced by IL-17. Therefore, it plays an anti-inflammatory role in Th17-related diseases. [10].

The literature lacks information about the effects of smoking on IL-17 and IL-35 levels in GCF and saliva in periodontitis. Furthermore, the possible role of IL-35 in periodontal disease pathogenesis has been investigated in the limited number of studies. We hypothesized that IL-17 and IL-35 may play a role in the pathogenesis of periodontitis, that smoking may have an effect on these cytokines, and that these two cytokines may be negatively correlated in the study groups. Therefore, this study was aimed to evaluate the levels of IL-17 and IL-35 in saliva and GCF and to examine whether they are associated with smoking and periodontal health status in smoker and non-smoker patients of periodontitis.

Materials and methods

The ethical committee (Faculty of Dentistry, Gazi University, Ankara Turkey—19.10.2018–3970) issued a protocol approval for the study. The study was conducted in accordance with the 1975 Declaration of Helsinki as revised in 2000 and was registered in the Australian New Zealand Clinical Trials Registry (ANZCTR) (No:126180017382l3).

Study population and design

58 subjects took part in this study. The participants were grouped as follows: smokers with periodontitis (Group S, $n = 20$), non-smokers with periodontitis (Group NS, $n = 20$), and periodontally healthy controls (Group C, $n = 18$). All subjects were referred to Gazi University Faculty of Dentistry, Periodontology Department either for periodontal treatment or dental check-up between November 2018 and March 2019. All participants were informed about the clinical procedures including saliva and GCF sampling, and provided their informed written consent.

Participants were diagnosed as periodontally healthy or having periodontitis based on periodontal examination and dental radiographs. Subjects were assigned to the study groups according to the principles set in the 2017 International Workshop on Classification of Periodontal and Peri-implant Diseases and Conditions. The diagnosis of periodontitis was made on the basis of the following criteria: history of lost teeth due to periodontitis, ≥ 5 mm interdental clinical attachment loss (CAL) at site of the greatest loss, and radiographic bone loss extending to the middle or apical third of root. The control group consisted of periodontally healthy individuals with $< 10\%$ bleeding on probing (BOP), ≤ 3 mm probing depth (PD) and without swelling/edema or pus [1]. Subjects with stage III and IV, grade B and C periodontitis participated in the present study groups S and NS. Participants who (a) had received antibiotic medication or periodontal treatment during the past 6 months, (b) had any systemic disease that could affect the process of periodontal disease such as diabetes mellitus, (c) were on any medication that could affect the symptoms of periodontal disease such as cyclosporine, phenytoin, calcium channel blockers or anti-inflammatory drugs, and (d) were pregnant lactating were excluded from the study. Patients in Group S were smokers for more than 5 years and smoked more than 10 cigarettes per day. The subjects in Group NS and Group C had no history of smoking.

Periodontal examination

Periodontal clinical measurements were recorded by a single examiner (E.T.). Intra-examiner calibrations were performed in approximately 20% of cases randomly selected, with repeated measurements taken from the same pocket during the study. The clinical examination included recordings of the plaque index (PI) [11], gingival index (GI) [12], bleeding on probing (BOP), probing depth (PD) and CAL for both full mouth and sample sites. Periodontal clinical measurements performed at four sites on each

tooth (mesio-buccal, mid-buccal, disto-buccal, lingual) using a calibrated Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Village, IL, USA).

Saliva and GCF sampling

Unstimulated saliva samples were collected from the subjects in the morning to minimize the effects of diurnal variability on salivary composition. The subjects were asked not to eat or drink, use mouthwash or chew gum within 2 h before sampling. Before their saliva was collected, patients were kept seated at rest without talking for 5 min. Samples of resting saliva were collected in plastic tubes for 5 min, and then stored at –30 °C until the analysis.

GCF samples were collected using 8 periopaper strips (Periopaper® Oraflow Inc. Smithtown, NY, USA) from single-rooted teeth with the deepest pocket depth for each participant. Single rooted teeth were included in the collection of GCF samples since single-rooted teeth offers the most easy and convenient sites for the salivary isolation. Before the samples were collected, the sampling sites were isolated with cotton rolls from contamination of saliva, supragingival plaque was removed gently, and the sites were dried by gentle air flow. Paper strips were inserted into the crevice or pocket until a mild resistance was felt and left there for 30 s. Strips that were contaminated with saliva or blood were discarded. The strips were measured for fluid volume as described previously [13]. The sample strips were inserted in labeled plastic Eppendorf tubes and stored at − 30 °C for subsequent assays.

Salivary and GCF IL-17 and IL-35 analyses

The samples were analyzed for the levels of IL-17 and IL-35 using sandwich-enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory IL-17 and IL-35 ELISA kit, Shanghai Korain Biotech Co., Shanghai. Sensitivity detection ranges; IL-17 kit: 2.38 ng/L, IL-35 kit: 0.047 ng/ml) at Gazi University Faculty of Medicine, Department of Immunology, according to the instructions of the manufacturer. GCF samples were transferred to + 4 °C and 300 µl phosphate buffered saline solution (PBS) was added to the Eppendorf tubes. Saliva and GCF samples were transferred to room temperature after 24 h, vortexed for 3 min for homogenization, transferred to Eppendorf tubes, and then centrifuged at 800*g* for 10 min. All samples were analyzed in duplicate wells and the mean values of those duplicates were calculated for each sample. Optic densities of the wells in the plates were measured at 450 nm using a microplate reader (Synergy HT, Biotek Instruments, USA).

Salivary IL-17 and IL-35 levels were calculated as concentrations, and GCF cytokine levels were calculated as concentrations and the total amount.

Statistical analysis

Statistical analyses were performed using IBM SPSS for Windows Version 22.0 software package. Before the study, a power analysis was performed using reference articles to determine the number of individuals to be included in the groups (58 subjects in total), which was found 0.85 [14]. Numerical variables were presented as mean ± standard deviation or median values, while categorical variables were presented as numbers and percentages. Before the groups were compared in terms of numerical variables, parametric test assumptions (normality and homogeneity of variances) were checked. Whether the numerical variables had a normal distribution was analyzed by the Shapiro–Wilks test. Homogeneity of the variance of the compared groups was analyzed by Levene's test. Study groups were compared in terms of numerical variables using one-way analysis of variance if the variances were homogeneous, and using Welch ANOVA if the variances were not homogeneous. Where a difference was detected in these tests, a pairwise comparison was made by the Tukey or Games–Howell test. When parametric test assumptions were not met, the groups were compared in terms of variables using the Kruskal–Wallis test. Pairwise comparisons were performed by the Siegel Castellan test. The presence of any difference between the groups in terms of categorical variables was analyzed using the chi-square test. Presence of a correlation between numerical variables was analyzed using the Spearman correlation coefficient. A p-value < 0.05 was considered statistically significant.

Results

It was found after the ELISA test that the salivary IL-17 level of a subject included in Group S was below the detectable level. Therefore, the study continued with 57 participants: 19 in Group S, 20 in Group NS, and 18 in Group C.

Demographic characteristics of the participants

6 female and 13 male participants in Group S, 7 female and 13 male participants in Group NS, and 9 female and 9 male participants in Group C were included to the study. The mean age was 44.3 ± 9.6 years for Group S, 46 ± 9.74 years for Group NS, and 42.9 ± 12.8 years for Group C. No significant differences were found among the groups in terms of gender ($p = 0.474$) and age ($p = 0.674$).

Clinical recordings

Full mouth periodontal recordings (PI, GI, PD, BOP and CAL) were significantly lower for Group C than any of the Groups S and NS ($p < 0.05$). Out of all clinical indexes, only

GI scores were found significantly lower in Group S than NS ($p < 0.05$). None of the other recordings (PI, PD, BOP and CAL) showed any statistically significant difference in any of the periodontitis groups. According to our data, periodontal recordings of the GCF sampling sites showed similar statistical results to the full mouth recordings of the study groups. The highest GCF volume was detected in Group NS, which was followed by Groups S and C ($p < 0.5$) (Table 1).

Salivary and GCF IL-17 and IL-35 analyses

Salivary IL-17 and IL-35 levels did not show any statistically significant difference among our study groups. GCF IL-17 total levels were lower in Group C than the periodontitis groups S and NS. Also, the difference was found statistically significant ($p < 0.001$). Our results showed that the total levels of GCF IL-35 were significantly higher in Group NS compared to Groups S and C ($p < 0.05$). Both GCF IL-17 and IL-35 concentrations were significantly lower in Group NS than in Groups S and C ($p < 0.05$) (Table 2).

Correlations

Our data revealed a strong positive correlation between IL-17 and IL-35 cytokine levels in terms of salivary ($r = 0.884$), overall GCF levels ($r = 0.973$) and GCF concentration ($r = 0.854$) ($p < 0.01$) (Fig. 1).

Discussion

The progression of periodontitis, which is considered one of the most important causes of tooth loss, is associated with a complex and incompletely clarified host response in which pro-inflammatory and anti-inflammatory cytokine networks play a critical role [2]. Although smoking is known to be a risk indicator for periodontitis, the exact role of the contents of cigarette in the development of periodontitis continues to be a widely researched topic for current studies [15]. Despite these unknowns, one of the possible mechanisms by which smoking plays a role in the pathogenesis of periodontitis could be related to host cytokines [4]. Among the cytokines detected in the pathogenesis of periodontal diseases, IL-17 is considered a pro-inflammatory cytokine that promotes the destruction of connective tissue and alveolar bone, which marks a crucial stage of periodontitis. However, IL-17 also plays a protective role for the mucosal barrier integrity by forming the first line of the mucosal barrier defense and inducing the production of antimicrobial factors against pathogens [16]. IL-35, on the other hand, is a current anti-inflammatory cytokine that is thought to play a protective role in the pathogenesis of periodontal disease through Th17 and Th1 cell inhibition and stimulating Treg cell differentiation [17, 18]. In addition, it has been reported that IL-35

Table 1 Comparison of full-mouth and sample site periodontal clinical measurements among the groups

	S ($n=19$)	NS ($n=20$)	C ($n=18$)	p
Full mouth				
PI	2.54 ± 0.39*	2.32 ± 0.34*	$0.47 \pm 0.18^{\dagger\S}$	$<0.001^{a}$
GI	$1.35 \pm 0.27^{*\dagger}$	$1.77 \pm 0.19^{*\S}$	$0.47 \pm 0.17^{\dagger\S}$	$<0.001^{a}$
PD (mm)	3.8 ± 0.58*	3.36 ± 0.4*	$2.08 \pm 0.19^{\dagger\S}$	$<0.001^{a}$
BOP (%)	68.57 ± 19.57*	77.65 ± 19.63*	$5.1 \pm 1.96^{\dagger\S}$	$<0.001^{a}$
CAL (mm)	2.74 ± 1.52*	1.85 ± 1.01*	$0.04 \pm 0.18^{\dagger\S}$	$<0.001^{b}$
Sample site				
PI	2.53 ± 0.4*	2.45 ± 0.32*	$0.13 \pm 0.14^{\dagger\S}$	$<0.001^{a}$
GI	$1.26 \pm 0.28^{*\dagger}$	$1.81 \pm 0.23^{*\S}$	$0.15 \pm 0.12^{\dagger\S}$	$<0.001^{a}$
PD (mm)	4.21 ± 0.77*	3.68 ± 0.59*	$2.0 \pm 0.15^{\dagger\S}$	$<0.001^{a}$
BOP (%)	69.68 ± 20.48*	84.4 ± 14.73*	$0.5 \pm 1.29^{\dagger\S}$	$<0.001^{a}$
CAL (mm)	3.22 ± 1.49*	2.76 ± 1.3*	$0.0 \pm 0.0^{\dagger\S}$	$<0.001^{b}$
GCF Volume (µl)	$3.74 \pm 1.67^{*\dagger}$	$5.82 \pm 185^{*\S}$	$2.45 \pm 0.42^{\dagger\S}$	$<0.001^{b}$

Data presented as mean ± standart deviation

NS non-smoker periodontitis, *S* smoker periodontitis, *C* periodontally healthy control, *PI* Plaque index, *GI* Gingival index, *PD* probing depth, *BOP* bleeding on probing, *CAL* clinical attachment loss, *GCF* gingival crevicular fluid

*Significantly different from Group C ($p < 0.05$)

†Significantly different from Group NS ($p < 0.05$)

§Significantly different from Group S ($p < 0.05$)

[a]Obtained from Kruskal–Wallis test

[b]Obtained from Welch ANOVA test

Table 2 Salivary, GCF concentration levels and GCF total amounts of IL-17 and IL-35 in non-smoker periodontitis (NS), smoker periodontitis (S) and periodontally healthy control (C) groups

	S ($n=19$)	NS ($n=20$)	C ($n=18$)	p
Salivary IL-17 (pg/ml)	83.08 ± 42.92	63.34 ± 24.43	59.39 ± 26.81	0.064[a]
Salivary IL-35 (pg/µl)	3.25 ± 2.44	2.37 ± 1.17	1.93 ± 1.58	0.086[a]
GCF IL-17 concentration (pg/ml)	131.37 ± 52.76[†]	84.46 ± 28.92[*§]	136.94 ± 32.78[†]	0.000[b]
GCF IL-17 total amount (pg)	0.41 ± 0.029[*]	0.44 ± 0.065[*]	0.32 ± 0.035[†§]	0.000[a]
GCF IL-35 concentration (pg/µl)	4.24 ± 1.76[†]	2.83 ± 0.96[*§]	5.23 ± 1.19[†]	0.000[b]
GCF IL-35 total amount (pg)	13.36 ± 0.74[†]	15.01 ± 2.57[*§]	12.41 ± 1.00[†]	0.000[a]

Data presented as mean ± standart deviation

IL-17 interleukin-17, *IL-35* interleukin-35, *GCF* gingival crevicular fluid

*Significantly different from Group C ($p < 0.05$)

[†]Significantly different from Group NS ($p < 0.05$)

[§]Significantly different from Group S ($p < 0.05$)

[a]Obtained from ANOVA test

[b]Obtained from Welch ANOVA test

inhibits IL-17 expression by RORα and RORγ inhibition [19]. There are limited number of studies in the literature investigating the role of IL-35 and IL-17 in the pathogenesis of periodontitis in smokers. Furthermore, conflicting results were reported in those studies [20–23]. For this reason, we aimed to evaluate the effects of smoking on GCF and salivary IL-17 and IL-35 levels in patients with periodontitis in the present study.

In a previous study, it was reported that detectable salivary IL-17 levels in periodontitis were significantly higher than gingivitis or in healthy individuals [20, 21]. Another study showed that salivary IL-17 levels increased with the intensity of periodontitis [24]. However, other studies reported that salivary IL-17 levels were significantly higher in periodontally healthy individuals than subjects with periodontitis [22, 23]. A limited number of studies in the literature investigated the effects of smoking on salivary IL-17 levels. Javed et al. detected statistically higher salivary IL-17 levels in heavy smokers than non-smokers with periodontitis [25]. Contrary to these studies, Kanmaz et al. reported that there were higher but statistically insignificant salivary IL-17 levels in smokers with periodontitis compared to non-smoker periodontitis group, which is consistent with our results [26]. Also, our results showed that salivary IL-17 levels were insignificantly higher in both smoker and non-smoker periodontitis groups compared to the control group. In this regard, our results may corroborate the information that IL-17 is a pro-inflammatory cytokine that could play a role in the pathogenesis of periodontitis [16]. In addition to the studies in which IL-17 in saliva and GCF samples was analyzed, there are studies in which different samples such as gingival biopsies were studied. In the majority of these studies, significantly higher levels of IL-17 were detected in gingival samples with periodontitis compared to healthy tissue samples [27]. On the other hand, some other studies failed to find any statistically significant result [28]. Moreover, studies have reported contradictory results regarding GCF IL-17 levels as well as salivary IL-17 levels [29, 30]. Many studies reported significantly higher GCF IL-17 concentrations in healthy individuals than in periodontitis subjects [29, 31]. Similarly, in our study, GCF IL-17 concentration levels were found significantly lower in non-smokers with periodontitis than in the smokers with periodontitis and the healthy group. However, there are other studies presenting contrary results, which is attributable to the differences between research methods, such as whether or not the GCF volume is included [21]. As proposed before by Takahashi et al. [32], it can be suggested that the GCF IL-17 concentration levels in the present study could be associated with the fact that IL-17 in GCF binds to cells with IL-17 receptor or IL-17 degrades at the gingival crevice. In addition, we suggest that the GCF volume, which increases due to inflammation in periodontitis, may also be effective in the contradicting results. There is a limited number of studies examining the effects of smoking on GCF IL-17 levels in patients with periodontitis. In parallel to a previous study [33], our data showed that the GCF IL-17 concentration in group S was significantly higher than that of group NS. Also, salivary IL-17 levels were found to be higher in smokers than non-smokers in our study. However, the difference between the groups was statistically insignificant. Interestingly, in a study, authors reported that increased IL-17 levels in peripheral circulation and lung tissues were associated with smoking [6]. Although their samples are different than ours, it can be suggested that our results may support their data.

The number of studies evaluating the role of IL-35 in periodontal disease pathogenesis is limited. In vitro and animal studies have shown that IL-35 suppresses the production of Th17-related cytokines including IL-17, and inhibits the alveolar bone resorption [19, 34]. However, clinical studies using different samples have shown conflicting results

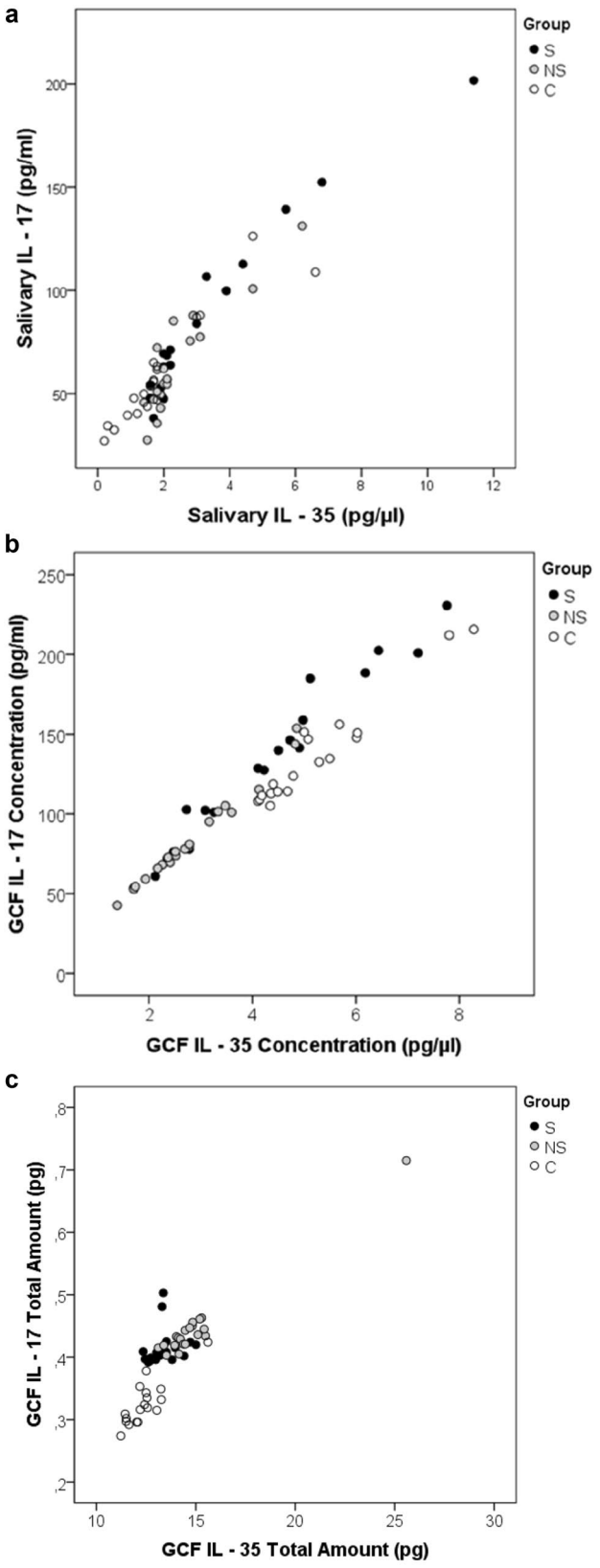

Fig. 1 **a** Correlation between salivary IL-17 and IL-35 levels ($p < 0.05$, $r = 0.884$). **b** Correlation between GCF IL-17 and IL-35 concentration levels ($p < 0.05$, $r = 0.854$). **c** Correlation between GCF IL-17 and IL-35 total amounts ($p < 0.05$, $r = 0.973$). *S* smoker with periodontitis group, *NS* nonsmoker with periodontitis group, *C* control group, *GCF* gingival crevicular fluid, *IL-17* interleukin-17, *IL-35* interleukin-35

concerning the role of IL-35 in periodontal disease. Among these, a limited number of studies examining saliva samples revealed different results and reported that IL-35 may play an anti-inflammatory or key role in the pathogenesis of periodontal disease [35]. According to our results, salivary IL-35 levels were higher in the periodontitis groups than the control group, which was statistically insignificant. This could be explained by the increase in Treg cells, resulting in elevated IL-35 levels, to regulate tissue destruction, as suggested by Kalburgi et al. [36]. There are also few studies evaluating the association between the GCF IL-35 levels and periodontal health status. One of those studies used the microcapillary tube method to obtain GCF unlike our study, and reported that the GCF IL-35 concentration was significantly higher in periodontitis patients than in periodontally healthy individuals [37]. On the other hand, Koseoglu et al. reported a significantly lower GCF IL-35 concentration in the periodontitis group than in the periodontally healthy group, which is consistent with our results [35]. Different results of these studies support the idea that the method of obtaining GCF may have an effect on the IL-35 concentration levels. In addition, we believe that the increased concentration of GCF IL-35 may be one of the factors involved in maintaining periodontal health. Sample size may also be a factor of different results. Although some of the studies were conducted with the lower sample size than ours [38], the number of subjects included in the groups may be a limitation on our study. However, to avoid this limitation, a data pool was made by collecting GCF samples from 8 different deep pocket sites for each subject in our study. To the best of our knowledge, the present study is the first to evaluate the effects of smoking on salivary and GCF IL-35 levels in periodontitis patients. Therefore, it was not possible to compare the data in the present study to the results of other studies. However, a previous study reported increased IL-35 levels in the lungs of the rats exposed to cigarettes [39]. In fact, in parallel with their results, we found higher, but statistically insignificant, salivary IL-35 and GCF IL-35 concentrations in group S than group NS. The insignificance of these differences between the groups in terms of salivary IL-17 and IL-35 levels may be due to the fact that the effect of periodontitis and smoking on the levels of those samples may not have been yet reflected at the time of collection.

One of the most remarkable results of our study was the strong positive correlation between the levels of IL-17 and IL-35 in saliva and GCF. To our knowledge, only two studies

have been conducted examining the relationship between IL-17 and IL-35 in the patients with periodontitis. One of those studies found a moderate negative correlation between IL-17 and IL-35 levels in 12 GCF samples in which both cytokines could be detected [38]. In the other study, no significant correlation was found between IL-17 and IL-35 in saliva samples [40]. The correlations found in our study support the assumption that IL-17 and IL-35 are highly related cytokines. This may be due to several reasons: the inhibitory effect of IL-35 on IL-17, which was proven in vitro, might not be observed in vivo due to complex inflammatory characteristics [19]. Moreover, IL-17 and IL-35 levels might have been in a state of equilibrium or the IL-35 level inadequate to control increased IL-17 levels when the samples were collected from the subjects [41]. In addition, it can be suggested that IL-35 levels may also increase by a feedback mechanism in response to the increased IL-17 levels during the inflammation observed in the course of periodontitis. Future studies conducted with different sample types such as gingival biopsies might clarify the association between these cytokines and also the effects of smoking on IL-17 and IL-35 levels in periodontitis.

The present study revealed a positive correlation between IL-35 and IL-17 unlike their in-vitro characteristics. The results of the study support the idea that IL-17 and IL-35 play a role in the pathogenesis of periodontitis. Also, the components of cigarette may cause an increasing effect on the levels of IL-17 and IL-35 in saliva and GCF. The effects of different cytokines and chemokines on IL-17 and IL-35 should be investigated by further in vitro and clinical studies due to the complex nature of inflammation and the components of cigarette.

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Declarations

Conflict of interest The authors declare that they have no potential conflicts of interest in this study.

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