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Expression of tight junction proteins in smokers and non-smokers with generalized Stage III periodontitis

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Abstract

Objective: This study aims to evaluate the gingival crevicular fluid (GCF) levels of tumor necrosis factor-α (TNF-α), zonula occludens-1 (ZO-1), occludin (Occ), and tricellulin (Tric) in periodontitis, as well as their alterations due to smoking.

Background: Tight junctions (TJ), which consist of transmembrane and cytoplasmic scaffolding proteins, connect the epithelial cells of the periodontium. Occ, claudins, junctional adhesion molecules, and Tric are transmembrane TJ proteins found at the cell membrane. The transmembrane TJ proteins and the intracellular cytoskeleton are directly linked by cytoplasmic scaffolding proteins such as ZO-1. Although the functions and locations of these molecules have been defined, their behavior in periodontal inflammation is unknown.

Methods: The study included four groups: individuals with periodontal health without smoking (C; $n=31$), individuals with generalized Stage III periodontitis without smoking (P; $n=28$), individuals with periodontal health while smoking (CS; $n=22$), and individuals with generalized Stage III periodontitis while smoking (PS; $n=18$). Clinical periodontal parameters were recorded, and enzyme-linked immunosorbent assay (ELISA) was used to examine ZO-1, Occ, Tric, and TNF-α levels in GCF.

Results: In the periodontitis groups, clinical parameters were significantly higher ($p<.001$). The site-specific levels of TNF-α, ZO-1, Tric, and Occ in the P group were statistically higher than those in the other groups ($p<.05$). TNF-α, probing pocket depth (PPD), and bleeding on probing (BOP) exhibited positive correlations with all TJ proteins ($p<.005$).

Conclusions: Smoking could potentially affect the levels of epithelial TJ proteins in the GCF, thereby potentially playing a significant role in the pathogenesis of the periodontal disease.

KEYWORDS
barrier function, periodontitis, smoking, tight junction proteins

1 | INTRODUCTION

The molecular structure of tissues has evolved to serve specific purposes. The gingival epithelium, a specialized tissue, primarily protects the underlying structures while facilitating selective exchange with the oral environment.[1] Tight junctions (TJ) serve as the primary component of the epithelial barrier, which seals the paracellular space and regulates the flow of gingival crevicular fluid (GCF), as well as lipopolysaccharide (LPS) deposition under normal conditions.[2] However, inflammatory conditions can lead to TJ damage by allowing paracellular transport of macromolecules. Previous studies have shown that together with proinflammatory cytokines, pathogenic bacteria can increase the permeability of intestinal epithelial cells by modulating TJ regulation.[3] Although pioneering studies aimed to unravel the barrier function of the pocket epithelium in periodontitis,[4,5] the underlying mechanism has not been fully elucidated yet.

TJs play a major role in maintaining the integrity and impermeability of the epithelial barrier; thus, they serve as ideal targets for pathogens to promote their translocation through the mucosa by damaging or redistributing TJs.[2,6] For example, *Aggregatibacter actinomycetemcomitans* increases the expression of E-cadherin and detachment of basal cells from the basement membrane,[7] *Porphyromonas gingivalis* hydrolyzes of interconnecting adherens junction E-cadherin molecules,[8] and *Treponema denticola* reduces the expression of F-actin and junctional proteins like desmoplakin II.[9] Furthermore, proinflammatory factors such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), reactive oxygen species, and various inflammatory mediators can increase TJ-mediated permeability.[10]

The cytoskeleton and transmembrane TJ proteins are directly connected by zonula occludens-1 (ZO-1).[11] Occludin (Occ), an integral membrane protein, was the first protein identified in TJ and is expressed in almost all cells.[12] Tricellulin (Tric), initially observed at the junction of three cells, plays a crucial role in barrier function by regulating the passage of macromolecules.[4,13] In inflammatory conditions, barrier dysfunction, characterized by increased paracellular permeability, results an increased flow through tight junctions, and increased apoptosis can lead to epithelial damage.[14] Additionally, inflammatory stimuli have been shown to effect ZO-1, Occ, and Tric.[15] In an animal study, it was reported that Tric accumulation can be considered normal despite impaired Occ.[14] Furthermore, overexpression of Occ has been shown to enhance barrier function.[16] Moreover, TNF-α inhibits the promoter activity of Occ[17] and causes the redistribution of Occ and ZO-1.[18]

It has been shown that smoking affects Occ and ZO-1 gene expression in different epithelial models such as airway epithelium[19] and the intestinal barrier.[20] However, to our knowledge, there are currently no studies evaluating comparable potential changes in the gingival epithelium. Epithelial cell-to-cell adhesion disorder, known as "leaky gum," is closely associated with the onset and progression of periodontal diseases. Based on these findings, we hypothesized that the periodontal inflammatory response, combined with smoking, may lead to changes in TJ formation in the gingival epithelium. Therefore, our aim is to assess the levels of TJ proteins in GCF among individuals with periodontitis and to investigate how smoking influences these protein levels.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was conducted at the Department of Periodontology, Ankara University Faculty of Dentistry, between November 2020 and November 2021. A total of 286 patients who visited the department were initially examined. Among them, 192 patients were diagnosed with periodontitis. Out of the 192 patients, 17 individuals declined to participate in the study. Additionally, 71 patients were excluded due to recent antibiotic use, 18 patients were excluded as they had recently received periodontal therapy, and 40 patients were excluded due to systemic diseases. Consequently, 94 patients were classified as periodontally healthy. Among the 94 participants, 24 individuals declined to participate in the study, 15 individuals were excluded due to recent antibiotic use, and 11 individuals were excluded due to systemic disorders. The final study groups consisted of 28 periodontitis patients without smoking (P group; 17 females, 11 males; mean age: 39.3 ± 8.8 years), 18 periodontitis patients who smoked (PS group; 5 females, 13 males; mean age: 38.1 ± 8.3 years), 31 periodontally healthy individuals without smoking (C group; 14 females, 17 males; mean age: 34.4 ± 6.3 years), and 22 periodontally healthy control individuals who smoked (CS group; 10 females, 12 males; mean age: 36.8 ± 6.1 years). All participants completed all study procedures. The study received ethical approval from the Ethics Committee of Ankara University (No. 12/03, 04/11/2020) and was conducted in accordance with the principles outlined in the Helsinki Declaration. Written informed consent was obtained from all participants at the beginning of the trial.

The clinical diagnosis was established based on previously described clinical and radiographic criteria.[21] Clinical periodontal parameters, including plaque index (PI), probing pocket depth (PD), bleeding on probing (BOP), and clinical attachment loss (CAL), were measured and recorded by a single investigator (ZG). Patients with interdental CAL at the site of greatest loss ≥5 mm or radiographic bone loss extending to the middle or apical third of the root were diagnosed with Stage III periodontitis. Regarding smoking status, participants who smoked more than 10 cigarettes per day for at least five years were considered smokers, while those who had never smoked in their lifetime were classified as non-smokers.[22] The grading of periodontitis was determined using the % bone loss/age value of the tooth with the highest attachment loss, along with the smoking habit. Grade B was assigned to individuals with % bone loss/age ranging from 0.25 to 1 without smoking, while Grade C was assigned to individuals with % bone loss/age ranging from 0.25 to 1 and a history of smoking.

The exclusion criteria for this study were as follows: the use of antibiotics, anti-inflammatory or immunosuppressive drugs, and

contraceptives within 3 months prior to the study; the use of medications known to cause gingival hyperplasia (such as nifedipine, cyclosporine A, and hydantoin); recent periodontal treatment within the last 3 months; presence of fewer than 16 natural teeth, excluding the third molars; being pregnant or lactating.

2.2 | GCF sampling

Due to their accessibility and comparatively minimal plaque and saliva contamination, single-rooted teeth were selected for collecting GCF. In the periodontitis groups, the mesiobuccal and distobuccal sampling sites were chosen based on the severity of the disease, specifically probing depths greater than 5 mm. For control groups, single-rooted teeth were randomly chosen. Standardized paper strips (Periopaper, ProFlow) were used to collect GCF samples. Three strips were sequentially placed at each sampling site for 30 s and then combined for analysis. Prior to sampling, the area was isolated, and supragingival plaque was gently removed using sterile curettes. The tooth surfaces were then gently dried with air. The extra sulcular method[23] was used to obtain GCF samples, and any samples contaminated with blood were excluded from the analysis. GCF amounts were measured using calibrated equipment (Periotron 8000, Oraflow Inc.). Instrument values were converted to volume (microliters) using a standard curve.[24] The samples were kept at −80°C until further examination.

2.3 | Biochemical analysis

Samples were eluted from the pooled paper strips. Eluted samples were analyzed for ZO-1, Occ, Tric, and TNF-α levels using a commercial ELISA kit (ELISA Cloud Immunoassay, Cloud-Clone Corp), according to the manufacturer's instructions. Concentrations of the proteins were determined using the assay standard curve. The intra- and inter-assay accuracy for ZO-1, Occ, Tric, and TNF-α was 10% and 12%, respectively. The minimum detection ranges for the assays were as follows: ZO-1 and Occ, 0.156-10 ng/mL; Tric, 0.312-20 ng/mL; TNF-α, 15.6-1000 pg/mL. All samples were analyzed in duplicate, and the average value was used for subsequent calculations.

2.4 | Statistical analyses

The sample size analysis was conducted a priori using specialized software (3.1.9.2 G*Power; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-undarbeitspsychologie/gpower.html). With an effect size of 0.40, 80% power, and a 5% alpha level, the calculated sample size was determined to be at least 18 participants for each group, resulting in a total of 72 individuals. To increase the study's power, we aimed to include 20 participants in each group. However, we were unable to reach this number in the PS group and completed the study with 18 participants in that group.

Commercially available statistical software (SPSS for Windows v.26, IBM SPSS Inc.) was used for all data analyses. The Shapiro–Wilk test was used to assess the normality of the data. Normally distributed data (clinical periodontal parameters, age, and gingival crevicular fluid volume) were analyzed by the ANOVA and the Tukey post hoc test. Non-normally distributed data were analyzed by the Kruskal–Wallis and the Bonferroni post hoc test. The chi-squared test was used to compare categorical variables between groups. The Spearman test was used to examine the correlations between biochemical and periodontal clinical data. A significance level of 0.05 was applied for all statistical tests.

3 | RESULTS

3.1 | Periodontal clinical parameters

Table 1 presents the data for periodontal clinical parameters. The gender and age distributions among the groups did not show any significant differences ($p > .05$). Clinical periodontal markers were

TABLE 1 Comparison of demographic and clinical periodontal parameters between the groups.

Clinical parameters	Control (C; $n = 31$)	Smoker–Control (CS; $n = 22$)	Periodontitis (P; $n = 28$)	Smoker–Periodontitis (PS; $n = 18$)	p
Age (year)	33 (29–38.8)	37 (33.7–40.7)	32 (37.5–47.2)	41.5 (32.7–43.1)	.137
Gender (F/M)	14/17	12/10	11/17	13/5	.184
PI	0.48 ± 0.28	0.84 ± 0.8	1.48 ± 0.58[a,b]	1.75 ± 0.5[a,b]	**<.001**
PD (mm)	1.40 ± 0.23	1.42 ± 0.39	3.59 ± 0.84[a,b]	2.86 ± 0.55[a,b]	**<.001**
BOP (%)	7.96 ± 4.76	5.81 ± 3.72	64.19 ± 23.34[a,b]	25.45 ± 16.98[a,b,c]	**<.001**
CAL (mm)	1.48 ± 0.45	2.56 ± 1.47	4.91 ± 1.29[a,b]	4.84 ± 0.65[a,b]	**<.001**
GCFV (μL)	1.18 ± 0.43	1.09 ± 0.73	3.70 ± 1.60[a,b]	1.36 ± 0.61[c]	**<.001**

Note: Significant values are shown as boldface type.

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment lost; GCVF, gingival crevicular fluid volume; PD, probing depth; PI, plaque index.

[a]Statistically significantly different from C group.

[b]Statistically significantly different from CS group.

[c]Statistically significantly different from P group.

significantly higher in the periodontitis groups compared with the control groups ($p < .001$).

3.2 | Biochemical parameters

Figure 1 and Table 2 present the GCF concentrations of ZO-1, Occ, Tric, and TNF-α, both in total amount and site-specific concentrations. Site-specific levels of TNF-α, ZO-1, Tric, and Occ were significantly higher in the periodontitis groups compared with the other groups ($p < .001$). The total amount of ZO-1, Tric, and Occ was also significantly higher in the periodontitis groups ($p < .001$, $p < .001$, $p = .00$, respectively). Although the difference was not statistically significant, the total amount of TNF-α was higher in the periodontitis groups compared to the control groups ($p = .125$).

In the PS group, TNF-α, Occ, Tric, and ZO-1 levels were significantly lower compared with the P group ($p < .05$). The levels of all biomarkers were higher in the PS group compared with the CS group, but the difference in Tric levels was not statistically significant ($p < .05$). When comparing the control groups, smoking was found to decrease TNF-α and Tric levels ($p < .0001$). When comparing the C and PS groups, it was observed that TNF-α levels increased in the PS group, while Tric levels decreased ($p < .0001$).

3.3 | Correlations between clinical periodontal and biochemical parameters

Data from all 99 patients were included in these analyses (Table 3). ZO-1 and Occ demonstrated positive correlation with TNF-α, PPD, and BOP ($p < .001$). Tric showed positive correlation with BOP, PD, and TNF-α ($p < .001$). All TJ proteins exhibited positive correlation with each other ($p < .001$).

4 | DISCUSSION

The objective of our study was to assess the GCF levels of TJ proteins in patients with periodontitis and examine the impact of smoking on these levels. The total amount and site-specific concentrations of TNF-α, ZO-1, Occ, and Tric in GCF were significantly elevated in individuals with periodontitis, and smoking was found to decrease their levels. However, this difference was not statistically significant in relation to the total amount of TNF-α.

It has been reported that the total amount is a more reliable indicator than the concentration of biomarkers in DOS.[24,25] Although repeated sampling did not result in any change in DOS volume, it exhibited significant differences between the healthy and patient groups. Therefore, emphasizing the total amount of biomarkers rather than their concentration may offer a more accurate representation of health or disease status.[26] Consequently, the results in the current study are discussed based on the total amount, while both concentration and total amount outcomes are presented in Table 2. As a result of our study, similar findings were observed concerning both total amount and concentration, indicating that the levels of biomarkers increased with periodontal inflammation and decreased with smoking.

Epithelial cells play a crucial role in interacting with the external environment and serving as a barrier against various factors in different areas of the body, including the airways, oral cavity, and gastrointestinal tract.[27] Preserving the integrity of this barrier is vital for overall health, as disruptions can contribute to the development of several diseases.[28] Periodontopathogens and inflammatory mediators, such as TNF-α, IL-1β, and reactive oxygen species, can affect TJ proteins, leading to their damage or redistribution and an increase in TJ-mediated permeability.[10]

TNF-α, a key mediator of inflammation,[29] is known to be significantly elevated in patients with inflammatory conditions like periodontitis.[30] It has been well established that TNF-α can increase intestinal TJ permeability both in vivo and in vitro,[10] although limited studies have explored this interaction specifically in the gingival epithelium.[31–33] TNF-α-induced inflammation has been shown to reduce the expression of intestinal ZO-1 through the NF-kB signaling pathway.[34] Interestingly, in our study, we observed higher levels of ZO-1 in the periodontitis groups compared with the control groups. Given the heightened activation of the NF-kB signaling pathway in periodontal inflammation, one would expect a decrease in ZO-1 mRNA expression.[35] However, it is important to note that the GCF, which was the focus of our study, contains various destructive materials released from deteriorated periodontal tissues, including TJ proteins. The deterioration of these junctions in periodontitis could result in higher amounts of TJ proteins being present in the GCF. Additionally, the observation of focal loss of TJ proteins, particularly on the endothelial surface, with inflammatory stimuli further supports this finding.[3] In our study, we found a positive correlation between ZO-1 and TNF-α, as well as with clinical parameters such as PD and BOP, indicating tissue breakdown at the base of the periodontal pocket.[36] Taking all these findings into consideration, it can be concluded that TJs are disrupted, and the presence of free ZO-1 in the environment increases with periodontal inflammation.

Occ is an integral membrane protein that plays a crucial role in the structure of TJs along with ZO-1.[37] While the silencing of the Occ gene is not life-threatening, it has been associated with diseases such as chronic gastritis and hearing loss.[38] Under normal conditions, ZO-1 and Occ are organized in a constant and regular manner to maintain the integrity of the epithelial barrier. However, in the presence of inflammatory stimuli like TNF-α, their localization can change, leading to barrier dysfunction.[39] TNF-α has been shown to down-regulate the expression of Occ, similar to its effects on ZO-1.[10] Some studies in the context of periodontal disease have demonstrated that lipopolysaccharide (LPS) from *P. gingivalis*, specifically, can significantly suppress Occ expression.[40,41] However, in our study, we observed significantly higher levels of Occ in the periodontitis groups compared with the control groups. Despite the expected decrease in Occ

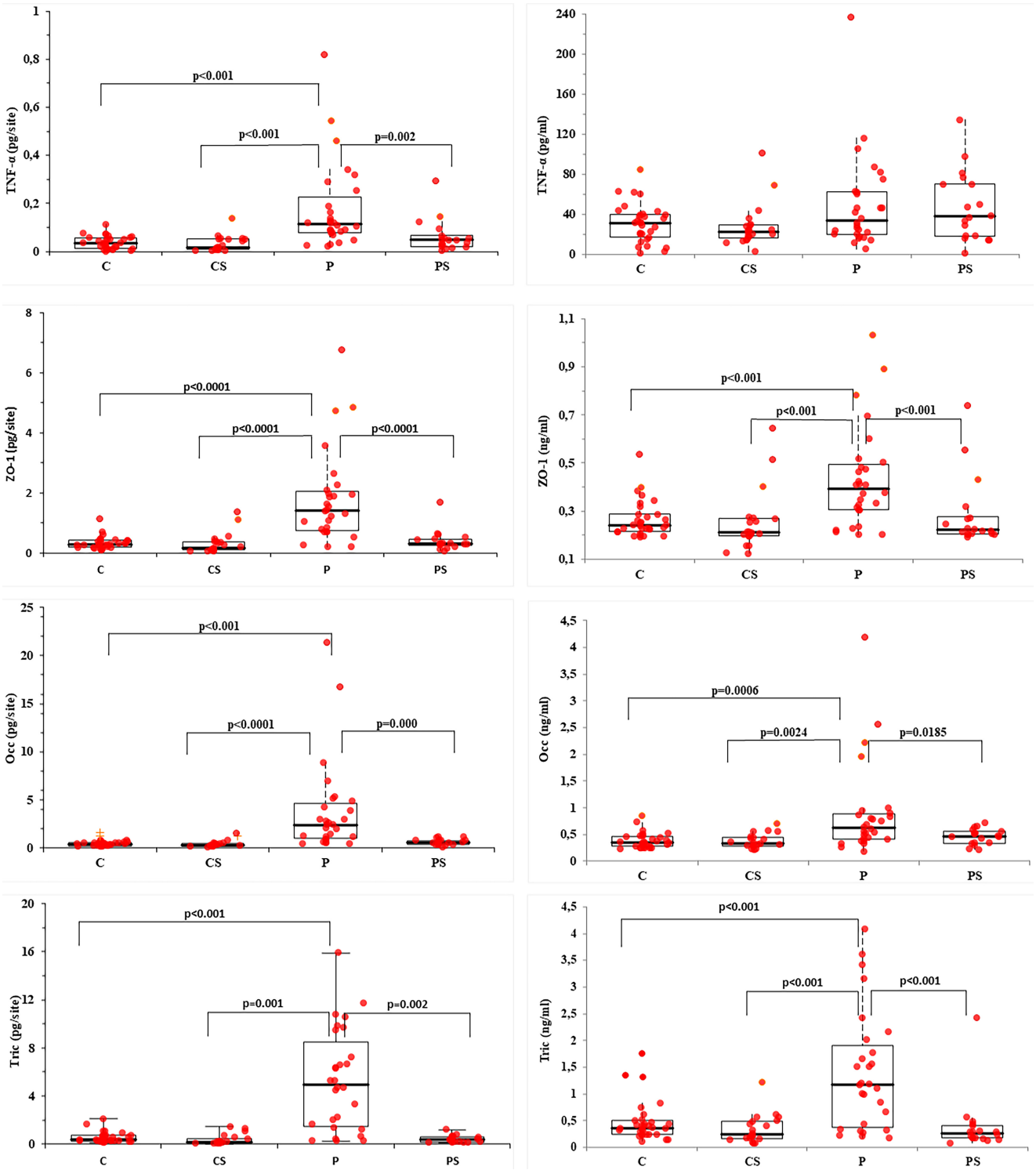

FIGURE 1 GCF levels of TNF-α, ZO-1, Occ, and Tric in healthy non-smokers (C), healthy smokers (CS), non-smoker periodontitis (P), and smoker periodontitis (PS) volunteers. Occ, occludin; TNF-α, tumor necrosis factor-alpha; Tric, tricellulin; ZO-1, zonula occludens-1. Box and whisker plots with the median (horizontal line), interquartile range (box), and outlier (circles) values are shown. *Statistically significant difference between the groups ($p < .05$).

protein levels due to decreased mRNA expression, it is possible that the dissolved and free Occ from disrupted TJs in response to periodontal inflammation led to an increase in Occ levels in the GCF. A more comprehensive understanding of this phenomenon can be achieved through histological examinations. Interestingly, a cell culture study using a blood-brain barrier model also demonstrated an increase in soluble Occ levels despite decreased mRNA expression.[42] Further research and investigations are needed to elucidate the underlying mechanisms and dynamics of Occ in the context of periodontal inflammation. Nonetheless, our findings

TABLE 2 GCF levels of TNF-α, ZO-1, OCC, and Tric.

Biomarkers	Control (C; $n=31$)	Smoker–Control (CS; $n=22$)	Periodontitis (P; $n=28$)	Smoker–Periodontitis (PS; $n=18$)	p
TNF-α (pg/site)	0.03 (0.01–0.06)	0.01 (0.01–0.5)	0.11 (0.08–0.22)[a,b]	0.05 (0.02–0.07)[c]	**<.001**
TNF-α (pg/mL)	31.35 (17.25–40.34)	22.93 (16.20–29.57)	33.90 (20.37–62.86)	37.95 (18.10–70.75)	.125
ZO-1 (pg/site)	0.28 (0.19–0.41)	0.17 (0.09–0.38)	1.42 (0.74–2.03)[a,b]	0.31 (0.26–0.47)[c]	**<.001**
ZO-1 (ng/mL)	0.24 (0.22–0.29)	0.21 (0.20–0.27)	0.39 (0.30–0.49)[a,b]	0.22 (0.21–0.28)[c]	**<.001**
Occ (pg/site)	0.41 (0.22–0.57)	0.27 (0.19–0.47)	2.41 (0.98–4.60)[a,b]	0.59 (0.34–0.79)[c]	**<.001**
Occ (ng/mL)	0.34 (0.28–0.46)	0.34 (0.28–0.44)	0.62 (0.40–0.88)[a,b]	0.46 (0.33–0.56)[c]	**.000**
Tric (pg/site)	0.35 (0.27–0.72)	0.20 (0.12–0.48)	4.98 (1.47–8.52)[a,b]	0.35 (0.17–0.63)[c]	**<.001**
Tric (ng/mL)	0.37 (0.24–0.50)	0.24 (0.16–0.48)	1.18 (0.37–1.91)[a,b]	0.25 (0.17–0.41)[c]	**<.001**

Note: Data are shown as median-interquartile range. Normally distributed data were evaluated with the ANOVA test, and non-normally distributed data were evaluated with the Kruskal–Wallis test. Significant values are shown as boldface type.

Abbreviations: Occ, occludin; TNF-α, tumor necrosis factor-alpha; Tric, tricellulin; ZO-1, zonula occludens-1.

[a]Statistically significantly different from C group.

[b]Statistically significantly different from CS group.

[c]Statistically significantly different from P group.

TABLE 3 Correlation of biomarkers between clinical periodontal parameters.

Variables	ZO-1 pg/site	Occ pg/site	TNF-α pg/site	Tric pg/site
Occ pg/site	**0.904****	–	–	–
TNF-α pg/site	**0.748****	**0.750****	–	–
Tric pg/site	**0.780****	**0.716****	**0.640****	–
PI	**0.275***	**0.301***	**0.271***	0.181
PD	**0.618****	**0.693****	**0.520****	**0.533****
BOP	**0.615****	**0.650****	**0.552****	**0.623****
CAL	**0.331***	**0.416****	**0.315***	**0.230***
GCVF	**0.945****	**0.923****	**0.756****	**0.790****

Note: Values in bold are different from 0 with a significance level alpha <0.05 (Spearmen correlation test). $r=0.20–0.40$ positive and low correlation. $r=0.40–0.60$ positive and mild correlation. $r=0.60–0.80$ positive and high correlation.

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment lost; GCVF, gingival crevicular fluid volume; Occ, occludin; PD, probing depth; PI, plaque index; TNF-α, tumor necrosis factor-alpha; Tric, tricellulin; ZO-1, zonula occludens-1.

*$p<.05$; **$p<.001$.

suggest that periodontal inflammation may contribute to the release of Occ from TJs, leading to increased Occ levels in the GCF.

Tric is a protein that is closely associated with Occ and is specifically localized to the tricellular junction region where three cells converge.[13] While Tric is predominantly found at the tricellular TJ, its degradation has been observed to cause structural abnormalities in both bicellular and tricellular TJs. This degradation leads to morphological changes, decreased transepithelial resistance, and increased paracellular permeability to molecules larger than 4 kDa.[13] Phylogenetically and structurally, Occ and Tric share similarities,[13] and it has been suggested that Tric may play a compensatory role in maintaining normal intestinal barrier function in Occ knockout mice.[14] Based on our findings, the higher levels of Tric in the periodontitis group may be attributed to a compensatory mechanism due to the loss of Occ from the cell membrane. As Occ levels decrease or undergo redistribution, Tric may be upregulated to help maintain the integrity and function of the epithelial barrier. This could explain the observed elevation of Tric levels in the context of periodontal inflammation. However, further research is needed to fully understand the intricate relationship between Occ and Tric and their specific roles in the pathogenesis of periodontal disease.

Smoking is widely recognized as one of the main environmental risk factors for periodontal diseases.[43] However, there is still no consensus on the precise mechanisms by which smoking contributes to these diseases. In our study, we observed that smoking was associated with decreased levels of all biomarkers in GCF. This finding contradicts some existing literature which suggests that smoking actually increases TNF-α expression.[44–46] However, Fredrikson et al. conducted a study in which they did not find a statistically significant difference in TNF-α levels released in response to bacterial stimulation between smoking and non-smoking groups.[47] They attributed this to the low sensitivity of peripheral lymphocytes to stimuli. Additionally, nicotine, a component of cigarettes, has been shown to inhibit the NF-κB signaling pathway in the airway epithelium, leading to a decrease in TNF-α expression. Nicotine is also known to possess anti-inflammatory properties.[48] It is important to note that studies involving cigarette smoking can be challenging to evaluate, and various factors such as cigarette content distribution, pack/year, number of cigarettes smoked, or the time interval between the last cigarette and sampling are crucial considerations. The observed decrease in TNF-α levels could be attributed to the anti-inflammatory activities of nicotine, its effects on endothelial function, reduced blood supply,[49] decreased immune cell infiltration,[50] suppression of intracellular signaling pathways,[49] and impaired receptor activation.[51] However, further research is needed to fully understand the complex interactions between smoking, inflammatory mediators such as TNF-α, and the pathogenesis of periodontal diseases.

Although the exact mechanism is not yet fully understood, smoking has been shown to increase epithelial permeability within minutes of smoking.[52] Some studies have suggested that cigarette smoke transiently increases the macromolecular permeability of the epithelium through protein tyrosine kinase (PTK)-dependent mechanisms.[20,49] Phosphorylation, which is influenced by PTK activity, can have both positive and negative effects on the amount and function of epithelial tight junctions.[53] For optimal tight junction formation and function, tyrosine phosphorylation of Occ and ZO-1 is necessary.[54] However, excessive tyrosine phosphorylation has been associated with loss of barrier function[55] and redistribution of tight junction proteins.[56] In our study, we found that the levels of ZO-1, Occ, and Tric in the PS group were significantly lower than those in the P group. Considering the inhibitory effect of TNF-α on the mRNA expression of ZO-1 and Occ, one would expect an increase in the levels of ZO-1 and Occ due to the decreased levels of TNF-α in smokers. However, the lower levels observed in the PS group may be associated with abnormal tyrosine phosphorylation. Tyrosine phosphorylation is a posttranslational modification that indicates insoluble TJ proteins readily available in the cytoplasm but not present at the junctions. This leads to a deterioration in barrier function despite the increased cytoplasmic abundance of these proteins. Studies have shown that tyrosine phosphorylation of Occ can cause its redistribution and increased macromolecular permeability at the blood–brain barrier.[57] Similarly, oxidative stress in an intestinal model has been found to induce tight junction loss through increased tyrosine phosphorylation of Occ and ZO-1.[4] These findings suggest that aberrant tyrosine phosphorylation of TJ proteins, such as Occ, may contribute to the disruption of epithelial barrier function observed in smokers and could be a potential mechanism underlying the increased epithelial permeability associated with smoking. However, further research is needed to fully elucidate the complex interactions and mechanisms involved.

The level of Tric was significantly lower in the PS group, while no statistically significant difference was observed between the CS group and the P group. The decreased Tric level in the PS group may be associated with the lower TNF-α levels observed in smokers in our study. Although there are no studies directly evaluating the effect of smoking on Tric, it has been observed that increased tyrosine phosphorylation resulting from smoking can lead to the redistribution of Tric from tricellular junctions to bicellular junctions.[58] This redistribution of Tric may occur as a compensatory mechanism to offset the impaired interaction between ZO-1 and Occ. The presence of Tric in bicellular junctions could potentially decrease the amount of Tric released into GCF. However, it should be noted that the expression of Tric can vary in different cell lines. Further studies are necessary to better understand the behavior of Tric, a protein whose functions have not yet been fully elucidated, in gingival epithelial cells.

Our study has some limitations. One of these limitations is that the selected proteins were evaluated only in GCF samples, and their intracellular distribution was not evaluated. Another limitation is the cross-sectional design of the study and the inability to evaluate the causality of the results. In addition, the inability to examine the change of TJ proteins in gingivitis is another limitation.

5 | CONCLUSION

This study is significant as it is the first to evaluate the barrier function of TJ proteins in relation to periodontal disease. Our findings indicate that periodontal inflammation increases permeability by altering the intracellular levels of TJ proteins. On the contrary, smoking may induce abnormal tyrosine phosphorylation of these proteins, leading to changes in their distribution within the cell membrane and subsequently affecting junction formation, rather than altering the total amount of insoluble forms within the cell. Particularly, the disruption of tricellular TJ by separating tricellular proteins from their junctions may weaken cell connections, allowing microorganisms and/or their products to pass into the subepithelial or intraepithelial space. These results highlight the importance of apical cell–cell junctions as vulnerable points in maintaining barrier function.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design of the study. ZG, ŞK, and CÖ have been involved in data collection, and ZG, ŞK, CÖ, and MAS have been involved in data analysis. ZG, ŞK, CÖ, MAS, ÖG, and MG have been involved in data interpretation, drafting the manuscript and revising it critically, and have given final approval of the version to be published.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PATIENT CONSENT STATEMENT

At the start of the trial, all participants provided written permission.

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