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Does smoking influence tryptophan metabolism in periodontal inflammation? A cross-sectional study

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Abstract

Objectives: The aim of this study was to identify the effects of smoking and periodontal inflammation on tryptophan-kynurenine metabolism as well as the correlation between these findings and clinical periodontal parameters.

Background: It has been shown that the tryptophan amino acid's primary catabolic pathway, the kynurenine pathway (KP), may serve as a key biomarker for periodontal disease. Although there are studies investigating the effect of smoking on KYN-TRP metabolism, the effect of smoking on periodontal disease through KP has not been revealed so far.

Methods: The salivary and serum samples were gathered from 24 nonsmoker (NS-P) stage III, grade B generalized periodontitis and 22 smoker (S-P) stage III, grade C generalized periodontitis patients, in addition to 24 nonsmoker (NS-C) and 24 smoker (S-C) periodontally healthy control individuals. Saliva and serum IL-6, kynurenine (KYN), and tryptophan (TRP) values, and KYN/TRP ratio were analyzed by liquid chromatography-mass spectrometry. Clinical periodontal measurements were recorded.

Results: Salivary TRP values were significantly higher in both periodontitis groups than control groups ($p < .05$). Salivary KYN values were highest in NS-P group ($p < .05$). Salivary KYN values did not differ significantly between periodontitis groups ($p = .84$). Salivary KYN/TRP ratio was significantly lower in NS-P group compared to other groups ($p < .001$). Serum TRP value is higher in S-P group than other groups; however, significant difference was found in S-C group ($p < .05$). Serum KYN values were significantly lower in smokers than nonsmokers. Serum KYN/TRP ratio is higher in NS-P group. NS-P group has the highest salivary IL-6 levels, NS-C group has the lowest values ($p < .05$).

Conclusions: Our results point out that smoking exacerbates inflammation in the periodontium and increases TRP destruction and decreases IDO activity by suppressing KP in serum. As a result, kynurenine and its metabolites may be significant biomarkers in the link between smoking and periodontal disease.

KEYWORDS
kynurenine pathway, periodontitis, smoking

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1 | INTRODUCTION

Periodontal disease is a chronic inflammatory condition that affects tooth-supportive tissues. It can cause tooth loss and systemic inflammation.[1] The severity of periodontal disease depends on various risk factors, including age, genetic predisposition, poor oral hygiene, and systemic diseases such as diabetes that affect the health of the periodontium; however, the most recognized risk factor today is smoking.[2,3]

Smoking is associated with serious pathologies such as cancer, cardiovascular diseases, chronic bronchitis, emphysema, and periodontitis.[4–6] It has been confirmed that there is a strong association between worse periodontal status due to an increased amount of plaque and calculus in smokers.[4,7,8] Clinical studies have shown clinical, biochemical, and microbiological aspects of tobacco use that correlate with the prevalence and severity of periodontal diseases.[5] Smoking, alters the host response to bacterial biofilm specifically by triggering the release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1), IL-8, IL-6, and transforming growth factor-β (TGF-β).[9] Smoking decreases gingival vascularization, suppression of the immune response, disruption of the morphological and functional healing of the periodontium, and alter the biofilms complexity.[10]

Tryptophan is an essential amino acid that cannot be synthesized in the human body and has the lowest concentration compared with the other amino acids. It is used for protein synthesis with a rate-limiting role in human body.[11] Tryptophan is also the pioneer of two metabolic pathways; the kynurenine pathway (KP) and the serotonin pathway.[12] The primary mechanism of tryptophan catabolism is KP, and the metabolites of KP are linked to inflammatory reactions, neurological disorders, and immune regulation.[13] Furthermore, in our previous study, we demonstrated that KP may be crucial to the pathophysiology and development of periodontal disease.[14] We evaluated the TRP, KYN, and KYN metabolites between nonsmoker stage III periodontitis patients and periodontally healthy individuals. The results of this study revealed that salivary TRP, KYN, KYN metabolites, and serum KYN, KYN/TRP ratio were significantly higher in periodontitis patients than in healthy controls.[14] So it is possible to serve as a predictive biomarker of KP for these conditions.

Indoleamine 2,3-dioxygenase (IDO) is the first rate-limiting enzyme in KP, which catalyzes tryptophan. Studies have shown that IDO plays a vital role in the immune tolerance.[15] When an infection, inflammation, or immunological response is triggered, the IDO enzyme is expressed more frequently then KP is activated, and its products begin to accumulate.[16] The Kynurenine/Tryptophan (KYN/TRP) ratio is an appropriate indicator of TRP degradation and IDO activity.[17] It has been shown that serum IDO activity decreases in smokers inversely with increasing serum cotinine concentration in smokers as a result of tobacco use.[18]

Although there are studies investigating the effect of smoking on KYN-TRP metabolism, the effect of smoking on periodontal disease through KP has not been explored/scrutinized/revealed on salivary and serum samples so far.[19,20] Salivary and serum analyses have been reported as laboratory tests to identify periodontitis.[21–24] Saliva is the biofluid most studied and analyzed in the literature.[25] It is a noninvasive and safe diagnostic specimen that is especially valuable in large-scale studies.[26] Saliva from individuals who have periodontitis includes many kinds of enzymes, cytokines, and indicators of connective tissue degradation and bone turnover.[27,28] Several earlier investigations examined the serum levels of different cytokines in patients with periodontitis.[23,29,30] Although the outcomes are inconclusive and disputed, it is possible to conclude that chronic periodontal inflammation leads to a persistent systemic inflammatory state.[24]

Considering that KP is an important biomarker in periodontitis, we hypothesized that the change in this pathway due to smoking may also directly affect the course of periodontitis. In this study, it is aimed to determine the effects of smoking and periodontal inflammation on saliva and serum KYN-TRP metabolism and to reveal the relationship of these data with clinical periodontal parameters.

2 | MATERIALS AND METHODS

106 patients who were received to Istanbul Medipol University, Faculty of Dentistry, Department of Periodontology were appropriate for this cross-sectional study. 4 of these 106 individuals refused to participate, 5 patients were excluded because of drug usage during the last 6 months, and 3 patients were excluded because they had received periodontal therapy in last 3 months (Figure 1). As a result, the final study population was 94.

The protocol for this study was approved by the Ethics Committee on Human Research of İstanbul Medipol University (No:63 Date:06.01.2022). The Declaration of Helsinki was followed when conducting this cross-sectional study. Each participant received comprehensive information regarding the procedures, and they all signed written consent forms. To report the data, the STROBE checklist was utilized.

2.1 | Patient selection

Systemically healthy, 24 nonsmokers with stage III grade B generalized periodontitis (NS-P), 22 smokers patients (≥10 per day) with stage III grade C generalized periodontitis (S-P), 24 periodontally healthy nonsmokers (NS-C) and 24 periodontally healthy smoker individuals (S-C) were included in this present study. Individuals using drugs that may affect periodontium, pregnant or lactating women, and individuals that received periodontal treatment in the last 3 months were not included in this study.

2.2 | Periodontal examination and clinical measurements

Clinical and radiological examinations of all participants were performed for diagnosis of periodontal health and periodontitis.[31] The

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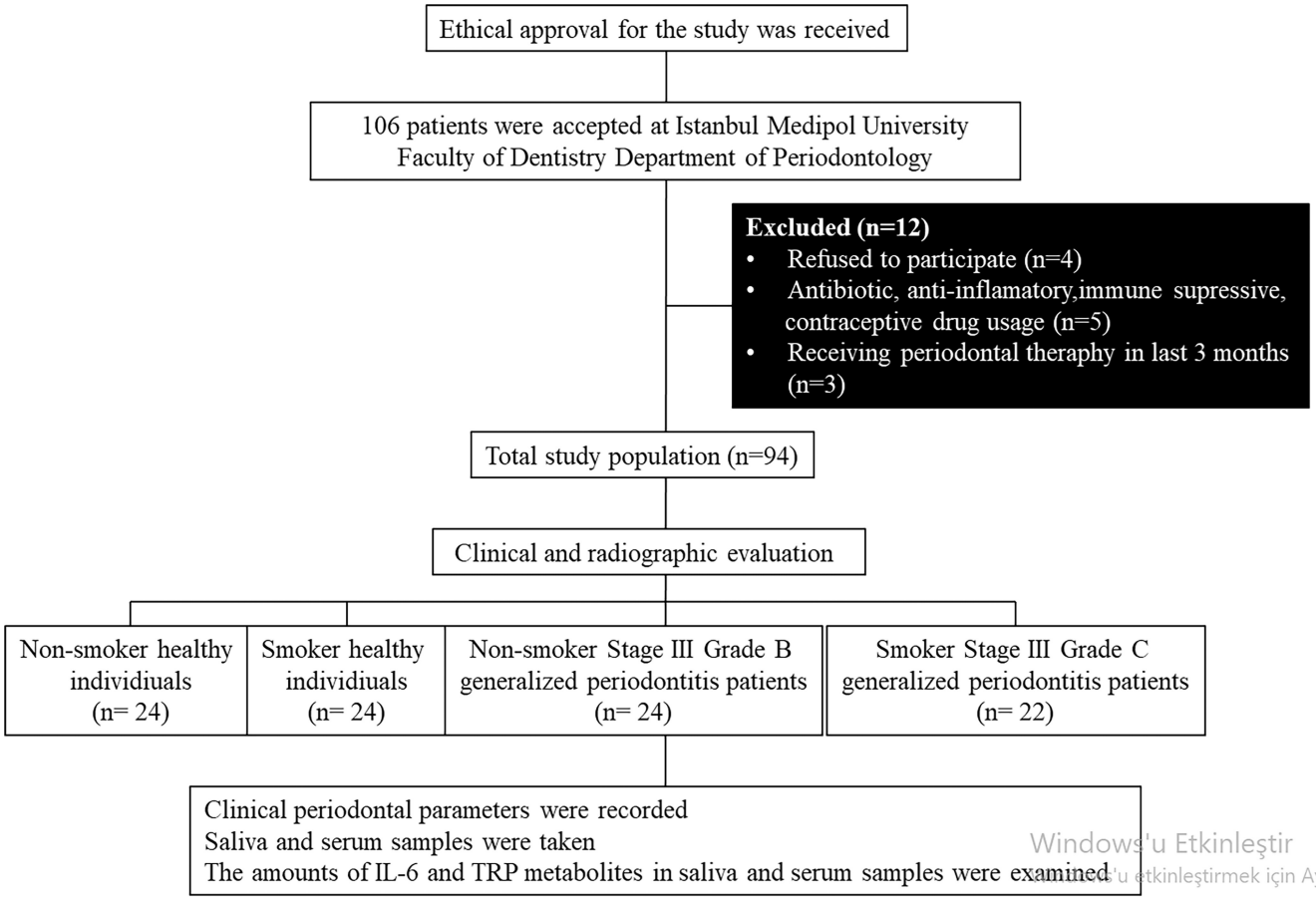

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FIGURE 1 Flowchart of the study.

same investigator (NB) measured six regions of all teeth to record all clinical periodontal measurements, including plaque index (PI), probing pocket depth (PPD), attachment loss (AL), and bleeding on probing (BOP). Individuals in nonsmoker groups (NS-P, NS-C) were required to have never smoked for at least the last 2 years, while individuals in smoker groups (S-P, S-C) were required to have smoked ≥10 cigarettes per day, for at least 5 years.[32]

Periodontal health was stated as having a probing pocket depth (PPD) ≤ 3 mm and no indication of inflammation (NS-C, S-C). Patients with ≥2 mm detectable interdental AL in nonadjacent teeth were included in periodontitis groups (NS-P, S-P). According to the previously mentioned clinical and radiographic standards, periodontal health and periodontitis were clinically diagnosed.[31] The greatest interdental attachment lost side was enrolled for each tooth and individuals with ≥5 mm AL were diagnosed with stage III periodontitis. Patients diagnosed with periodontitis were classified in accordance with bone loss/age index. NS-P group was graded as grade B, and S-P group as grade C.

2.3 | Salivary and serum specimens

Unstimulated salivary specimens were collected between 8 am-11 am. In order to avoid contamination (bleeding etc.) clinical periodontal measurements were performed subsequent to saliva collection. The participants had refrained not to perform any oral hygiene practices in the morning before sample collection, and not to eat and drinking anything at least 2 h before sampling. All participators were warned to rinse their mouths with distilled water just prior to sample collection. Then, saliva was sampled by spitting directly into a sterile tube. The sample collection procedure was continued for 5 min. Samples were then centrifuged at 10000 rpm for 10 min.[33]

Participants had conventional venous punctures to obtain 10 cc of venous blood samples, which were then kept at room temperature for 30 min to allow for coagulation and then centrifuged at 4000 rpm for 10 min.[34]

All samples were preserved at −80°C until the day of the analysis.

2.4 | IL-6 analysis

A commercial ELISA kit was used to measure the IL-6 levels in serum and saliva samples. The manufacturer's instructions were followed for each test in detail. Analyte concentrations were calculated according to the test standard curve. Intra- and interassays precision values were 10% and 12%, respectively. 7.8–500 pg/

*Human IL-6 ELISA Kit, Uscn, Cloud-Clone Corp.

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mL was the assay's detection range. After averaging the results, all samples were run twice and the average values of the results were calculated.

2.5 | KP analysis

TRP, KYN, and KYN metabolites were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) in accordance with our previous paper, which is a modified version of the method defined by Tömösi et al.[35]

2.6 | Examiner calibration

The examiner (NB) was calibrated before the study. Five subjects in all were evaluated twice, with a one-hour gap between each assessment. The recordings were performed in two sets, with the second set blinded to the first, and the reproducibility assessment revealed that 85% of the sites had repeat probing mean values that were within ±1 mm. An interexaminer evaluation of the scores of probing depths indicated good repeatability ($\kappa = .826$).

2.7 | Statistical analyses

Sample size analysis was based on the study of Kurgan et al.[14] Alpha error is selected as .05. For a two-tailed hypothesis of power 95%, the sample size was planned as at least 20 for each group.

Commercially accessible statistical software was used to perform all analyses. To determine normally distributed data, the Shapiro–Wilk test was used. Categorical variables were subjected to the chi-square test.

The Bonferroni test was done as a "one-way ANOVA" multiple comparison test to evaluate variables among the four groups. For data that were not normally distributed, the Kruskal–Wallis test was used. The Spearman correlation analysis was used to find correlations between biochemical and clinical markers. Significant data was determined as $p < .05$.

3 | RESULTS

3.1 | Study populations and clinical periodontal measurements

Table 1 displays the demographical and clinical data of the periodontitis and control groups. A total of 94 systemically healthy participants were included in this study, comprising 24 nonsmokers with stage III grade B generalized periodontitis (NS-P: 10 F/14 M mean age 41.00 [32–56]), 22 smokers with stage III grade C generalized periodontitis (S-P: 9 F/13 M mean age 42.00 [33–53]), 24 periodontally healthy nonsmokers (NS-C: 15 F/9 M mean age 38.50 [28–55]) and 24 periodontally healthy smokers (NS-C: 12 F/12 M mean age 39.00 [32–58]). There were no differences between the groups according to age and gender (respectively $p = .316$, $p = .415$). All clinical periodontal measurements were significantly higher in both periodontitis groups ($p < .001$).

3.2 | Biochemical parameters

Figure 2 displays the concentration of TRP, KYN, and KYN metabolites in saliva and serum. Salivary TRP levels were significantly higher in both periodontitis groups than control groups ($p < .05$). The salivary TRP level in the S-P group was significantly lower than in the NS-P group ($p = .003$). Although salivary KYN levels were highest in the NS-P group, there was no significantly difference between the periodontitis groups ($p = .84$). Although the periodontitis groups had higher salivary KYN levels than the controls, a significant difference was found only with the S-C group. Salivary KYN/TRP ratio of NS-P group was the significantly lowest ($p < .001$).

Serum TRP value is higher in S-P group than the other groups, and significant differences were found between S-P group and S-C group ($p < .05$). Serum KYN values were significantly lower in the S-P group than in the NS-P group ($p < .001$) and in the S-C group compared to the NS-C group ($p < .05$). Serum KYN/TRP ratio was significantly the lowest among all groups ($p < .001$).

In our study, the levels of salivary KYN metabolites (KYNA, 3OHKYN, PA, QA) were higher in the NS-P group than the S-P group, while the levels of serum KYN metabolites were lower in the NS-P group than the S-P group. Yet, significant difference between NS-P and S-P groups was obtained only for salivary QA levels among all KYN metabolites.

Figure 3 displays the salivary and serum amounts of IL-6. While salivary IL-6 levels were found higher in both periodontitis groups than controls, serum IL-6 levels were just the opposite.

3.3 | Correlation analyses

Table 2 displays correlation analyses between biochemical metabolites and periodontal parameters. There is a significant positive ($p < .05$) correlation between clinical parameters (PI, PPD, BOP, AL) and salivary TRP and KYN levels, and a significant negative ($p < .001$) correlation between salivary KYN/TRP ratio. It has been shown that there is a significant positive correlation among clinical parameters (PI, PPD, BOP, AL) and salivary IL-6 level ($p < .05$), and that there is a significant positive correlation among clinical parameters (PI, PPD, BOP) and serum KYNA and QA values ($p < .05$). Salivary TRP levels significantly positive correlated with salivary IL-6 levels ($p < .05$). Salivary KYN/TRP ratio had significantly

[†]TSQ Quantum Access MAX Triple Stage Quadrupole Mass, Thermo Scientific.

[‡]SPSS for Windows v.26, IBM SPSS Inc.

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TABLE 1 Demographic, and clinical parameters of periodontitis and control groups in smokers and nonsmokers.

Parameters	Control (NS-C) $n=24$	Smoker-Control (S-C) $n=24$	Periodontitis (NS-P) $n=24$	Smoker-Periodontitis (S-P) $n=22$	p
Age (year)[a]	38.50 (28–55)	39.00 (32–58)	41.00 (32–56)	42.00 (33–53)	.316
Gender F/M	15/9	12/12	10/14	9/13	.415
PI	0.58±0.31	0.84±0.36	1.50±0.53	1.72±0.52	**<.001**
PPD (mm)	1.59±0.22	1.56±0.53	2.79±0.75	2.91±0.53	**<.001**
BOP (%)	6.96±4.25	7.48±4.52	41.76±20.98	26.55±17.29	**<.001**
AL (mm)	0.35±0.70	0.47±0.75	3.56±0.68	3.92±0.87	**<.001**

Note: Data shown as mean±standard deviation. Statistical difference with the control group $p<.05$. Significantly different values are shown in boldface type.

Abbreviations: AL, attachment loss; BOP, bleeding on probing; PI, plaque index; PPD, probing pocket depth.

[a]Data shown as median (min–max).

negative correlation with serum KYN level and serum KYN/TRP ratio ($p<.05$).

4 | DISCUSSION

Numerous studies have shown that KP, which is the primary catabolic pathway of the TRP amino acid, is effective in the immune response in various systemic inflammatory diseases such as diabetes, cardiovascular diseases, multiple sclerosis, and schizophrenia.[36–38] Our previous study demonstrated that KP is crucial for the etiopathogenesis of periodontal diseases.[14] In the literature, it was shown that smoking has impacts on KP.[18,19,39] Based on this information, in this study, the influence of smoking and periodontal inflammation on salivary and serum TRP-KYN metabolism was investigated and it was revealed that the KYN/TRP ratio was significantly decreased in saliva in association with periodontitis. Strikingly, while the salivary KYN/TRP ratio was lowest in the NS-P group, the serum KYN/TRP ratio was the lowest in the S-P group.

Proinflammatory cytokines that increase as a result of periodontal inflammation initiate the catabolism of TRP amino acid. The enzyme responsible for metabolizing the TRP amino acid in case of inflammation is IDO. Due to inflammation, the immune system is activated, which in turn increased IDO expression leading to induction of KP.[16] Studies have demonstrated that IDO is expressed in human gingival epithelium and human PDL cells during the pathogenesis of periodontitis.[40,41] Additionally, it has been reported that the number of Langerhans cells, one of the responsible cells that express IDO in epithelium, were increased in periodontal inflammation.[42] Therefore, it can be inferred that the increase has an effect on IDO activity and salivary TRP level in advanced periodontitis cases.[43,44] The KYN/TRP ratio, which is the product of this pathway, also indicates the activation of IDO. In our previous study, we have demonstrated that serum KYN/TRP ratio was higher in periodontitis patients.[14]

In the current study, we investigated the alterations in TRP and its metabolites on both salivary and serum samples in smokers with periodontitis. Saliva is useful because it is plentiful and easily accessible via noninvasive techniques.[45] It can be sampled in much higher quantities without the requirement for clinical facilities, the collection is affordable, and no trained specialists are required.[22] It has been proposed that saliva content reflects the inflammatory condition of the entire mouth. Some high-abundance proteins in saliva, on the other hand, can cause issues with the detection of low-abundance proteins.[46]

We determined that both periodontitis groups had significantly higher salivary TRP level than control groups. This result is in line with the results of previous studies.[14,47–50] Gingival crevicular fluid (GCF) is a serum-derived fluid and is also included in the content of saliva. TRP is transported by blood to target organs. The amount of TRP delivered into the GCF is influenced by the rise of TRP concentration in the blood vessels of the gingival connective tissue as a result of periodontal inflammation, which also influences the amount of TRP transferred from the GCF to the saliva. Although salivary TRP level was significantly lower in smokers compared to nonsmokers among periodontitis patients, in the control groups this was the opposite. There are no other studies in the literature examining the effect of smoking on salivary TRP levels. Numerous studies in the literature have shown that smoking increases the periodontal destruction and affects the course of the disease by exacerbating inflammation in periodontal disease.[7,51–53] It is thought that smoking affects TRP metabolism in two ways. First, smoking suppresses the IDO enzyme, leading to a decrease in TRP degradation.[18,54,55] This condition is actually characterized by an increase in salivary TRP level. It is possible to see the suppressive effect of smoking on salivary IDO activity when the control groups were compared. However, smoking also causes an increase in proinflammatory cytokines in periodontal disease.[5,7] The increase in proinflammatory cytokines related to both periodontal inflammation and smoking increases IDO activity.[5,40,41] This is characterized by a reduction in salivary TRP level. As a result, the reduced salivary TRP level in the periodontitis group can be attributed to the severity of the periodontal inflammation.

In both periodontitis groups, salivary KYN levels were higher than the control groups in this study. In addition, the levels of salivary KYN metabolites were lower in smokers compared to nonsmokers among periodontitis patients. As we mentioned above, it is an expected result that TRP in saliva will be broken down into KYN more effectively due to exacerbation of periodontal inflammation,

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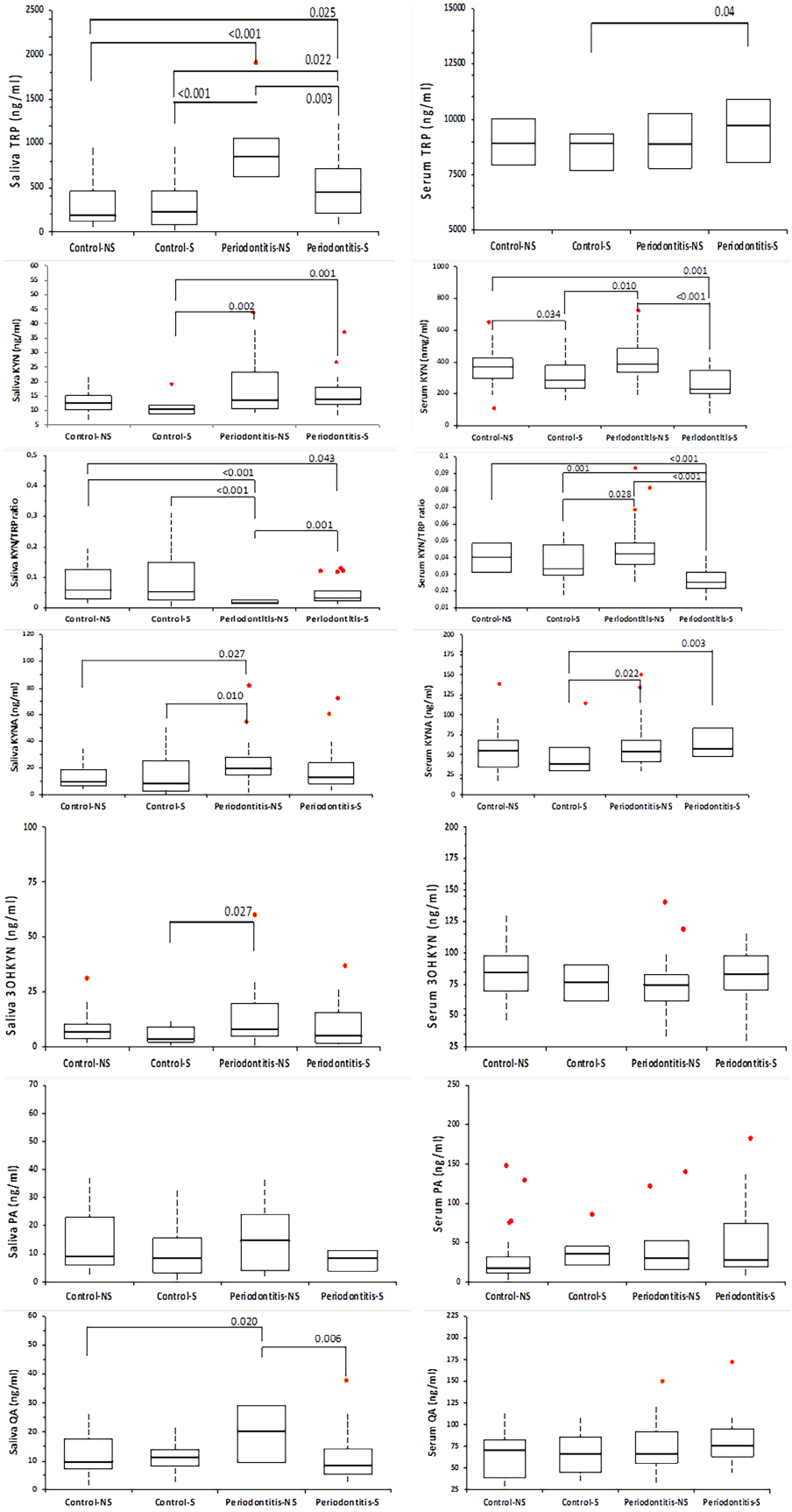

FIGURE 2 Salivary and serum levels of TRP, KYN, and KYN metabolites (KYNA, 3OHKYN, PA, QA) with NS-C, S-C, NS-P, and S-P groups. Box-and-whisker plots with the median (horizontal line), interquartile range (box), and outlier (circles) values are shown. Significantly different ($p < .05$).

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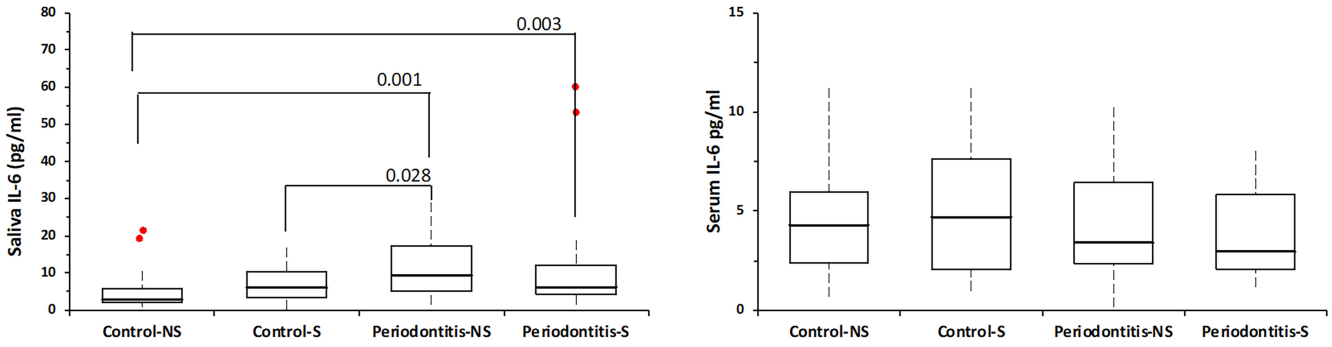

FIGURE 3 Saliva and serum levels of IL-6 with NS-C, S-C, NS-P, and S-P groups. Box-and-whisker plots with the median (horizontal line), interquartile range (box), and outlier (circles) values are shown. Significantly different ($p < .05$).

and accordingly, the level of salivary KYN and its metabolites will be high in these groups. The results regarding the levels of salivary KYN metabolites in the NS-P group were similar to the findings of our previous study,[14] and at the same time, there was a positive correlation between salivary KYNA, 3OHKYN, QA levels, and salivary TRP levels. In our study, according to the KYN/TRP ratio results, salivary KYN/TRP ratio observed in the NS-P group was the lowest of all groups. The increase in TRP amount in saliva as a result of increased vascularization and destruction of protein structures in periodontal inflammation may account for the result. On the other hand, the significantly higher KYN/TRP ratio in the S-P group in comparison to its NS-P counterpart can be explained by the vascularization-reducing effect of smoking in the periodontium, which, in turn, decreases the amount of TRP transferred from the GCF to the saliva.

Serum levels of tryptophan and its metabolites were also evaluated in this study. The serum TRP level observed for the S-P group was the highest among all groups, while its serum KYN level and accordingly KYN/TRP ratio were lowest among all groups. The levels of serum KYN metabolites observed for the NS-P group were lower than that of the S-P group. The suppressive effect of smoking on KP has previously been shown in other studies.[18,54,55] IDO, the first limiting enzyme of KP, plays an active role in immune response and is mostly induced by interferon-γ (IFN- γ).[16] Carbon monoxide (CO) released in the cigarette smoke potently suppresses IFN-γ production. This information supports the hypothesis that exposure to CO in smokers is associated with downregulation of all biochemical pathways induced by IFN-γ.[56–58] As a result, serum TRP levels increase because of decreased serum IDO activity in smokers, and the level of KYN in the serum decreases.[59] Serum KYN and TRP levels in this study were compatible with the literature. In our study, salivary and serum TRP levels showed opposite results. It has been shown that the KYN/TRP ratio, which is an indicator of IDO activity, is significantly different between NS-P and S-P groups, and this difference is opposite in direction between saliva and serum samples. This result is consistent with studies that showed the suppressive effect of smoking on serum IDO activity. In addition to the effect of smoking on this pathway, KP is more active in saliva in the SP group; this can be explained by the more dominant effect of exacerbating periodontal inflammation. These results agree with the literature stating that TRP catabolism in saliva increases due to inflammation. In light of this information, we can conclude that KYN metabolites may serve as significant indicators of periodontal inflammation.

Salivary IL-6 levels were higher in both periodontitis groups than in the control groups in this study. This result is consistent with studies showing that IL-6 plays a critical role in the etiology of periodontal disease.[14,60–62] It has been demonstrated that there was a relationship between TRP catabolism and IL-6.[63] IL-6 is an inflammatory marker and the primary stimulant of IDO expression. Thus, IL-6 activation of IDO results in high KYN levels and an increases in the KYN/TRP ratio. A positive correlation between kynurenine and the KYN/TRP ratio with IL-6 was reported in patients with SARS-CoV-2.[64]

Kim et al. reported that IL-6 expression in rodents plays a role in pain and depression by increasing IDO activity both in vitro and in vivo via the JAK/STAT pathway.[65] Another study showed that STAT3 activation mediated by IL-6 expressed in association with hepatic carcinoma stimulates IDO production in dendritic cells.[66] In periodontal inflammation, bacterial stimulus especially *Porphyromonas gingivalis* increases IL-6 expression in gingival fibroblasts. On the other hand, when fibroblasts are stimulated with IL-1 and IL-6, they play a role in tissue destruction by producing MMP.[67] As a result, the amount of TRP amino acid released indirectly increases in saliva.[14] The data of our study is consistent with this information. We also demonstrated that there is a positive correlation between saliva IL-6 and saliva TRP levels.

The major strength of this study is that for the first time kynurenine and tryptophan levels were measured in salivary and serum samples among smokers with periodontitis in a standardized study population. The primary limitation of this study is its cross-sectional design. The GCF samples were not examined, which is a further limitation. GCF will definitely disclose more specifically the effect of periodontal disease on biomarkers in the dentogingival area. However, saliva has recently gained attention as an alternate source of biomarkers.[68,69] Collecting saliva samples is a noninvasive and simple procedure. Saliva content is a substance that reflects the clinical periodontal status and indicates

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TABLE 2 Correlations between biomarkers and periodontal clinical parameters (Spearman correlation coefficients, *r values*) ($n = 94$).

Variables	Serum KYN	Serum TRP	Serum KYN/TRP	Serum KYNA	Serum 3OHKYN	Serum PA	Serum QA	Saliva KYN	Saliva TRP	Saliva KYN/TRP	Saliva KYNA	Saliva 3OHKYN	Saliva PA	Saliva QA	Saliva IL-6
Serum TRP	**0.342***														
Serum KYN/TRP	**0.861****	−0.079													
Serum KYNA	0.153	0.028	0.168												
Serum 3OHKYN	0.194	0.002	**0.237***	0.167											
Serum PA	**−0.237***	0.052	**−0.232***	**0.212***	0.002										
Serum QA	0.203	0.088	0.194	**0.366****	0.201	0.087									
Saliva KYN	−0.012	−0.169	0.093	**0.314***	0.181	0.156	**0.296***								
Saliva TRP	0.167	0.059	0.185	**0.265***	−0.017	0.128	**0.224***	**0.436****							
Saliva KYN/TRP	**−0.237***	−0.134	**−0.222***	**−0.254***	0.025	−0.074	−0.164	−0.072	**−0.888****						
Saliva KYNA	0.067	0.002	0.093	0.143	−0.052	0.150	0.100	**0.369****	**0.558****	**−0.457****					
Saliva 3OHKYN	0.087	−0.048	0.157	0.113	−0.011	−0.005	−0.023	0.081	**0.306***	**−0.320***	0.191				
Saliva PA	0.018	−0.040	0.052	0.052	0.015	0.000	0.005	−0.050	0.188	−0.191	0.169	**0.210***			
Saliva QA	**0.213***	−0.033	**0.285***	−0.123	0.005	0.083	0.167	0.196	**0.358***	**−0.301***	**0.305***	0.142	**0.204***		
Saliva IL-6	−0.071	0.027	−0.079	0.176	−0.096	**0.285***	0.052	0.188	**0.269***	−0.183	0.192	0.180	−0.051	0.017	
Serum IL-6	0.120	0.105	0.095	0.014	0.065	0.066	0.180	−0.077	−0.025	0.023	−0.064	−0.128	−0.004	−0.042	−0.066
Age	0.006	0.188	−0.105	0.006	−0.148	0.002	−0.005	0.110	0.052	−0.010	0.073	−0.033	0.032	0.082	0.126
PI	0.076	**0.286***	−0.051	**0.259***	0.089	0.116	**0.317***	**0.215***	**0.422****	**−0.377****	0.177	−0.012	−0.105	0.030	**0.330***
PPD	0.001	0.119	−0.085	**0.223***	0.138	0.086	**0.237***	**0.382****	**0.527****	**−0.389****	**0.227***	0.061	−0.063	0.089	**0.301***
BOP%	0.111	0.072	0.056	**0.267***	0.026	0.171	**0.226***	**0.347***	**0.584****	**−0.477****	**0.311***	**0.237***	0.084	0.144	**0.453****
AL	0.001	0.167	−0.120	0.191	0.022	0.108	0.191	**0.353***	**0.495****	**−0.389****	**0.249***	0.042	0.035	0.039	**0.260***

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

Significantly different values are shown in boldface type.

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the inflammatory state of the entire mouth. Furthermore, many of the promising GCF indicators have been identified as possible saliva biomarkers of periodontitis.[70]

Only periodontitis patients were included in this study to investigate the impact of smoking, which is a major risk factor for periodontal diseases, via tryptophan metabolites. However, gingivitis, the most prevalent form of periodontal disease, can proceed to periodontitis in susceptible individuals if left untreated, emphasizing the significance of early detection and treatment of gingivitis.[71] The transition from gingivitis to the early stages of periodontitis has been associated with an increase in proinflammatory cytokines.[72] The identification of critical salivary biomarkers in gingivitis serves to detect these instances and observe the progression of periodontal disease.[73] Patients with gingivitis and periodontitis at various stages should be included in future researches that investigate at the local and systemic alterations in KYN and its metabolites caused by periodontal inflammation, as well as how they interact with clinical periodontal parameters.

5 | CONCLUSION

In our study, it was revealed that smoking exacerbates inflammation in the periodontium and increases TRP catabolism, and suppresses KP in serum, by decreasing IDO activity. Although we consider that KP is an active pathway in the pathogenesis of periodontal disease, many complex factors can coexist in the oral environment that can simultaneously affect this biochemical pathway. The interaction of these factors with each other and their occasionally dominant features may cause the activity of this pathway in the mouth to occur in the opposite direction of its systemic activity. As a result, kynurenine and its metabolites may be significant biomarkers in the link between smoking and periodontal disease.

AUTHOR CONTRIBUTIONS

The hypothesis of the research was developed by Canan Önder and Şivge Kurgan. Nur Balcı submitted the application to the ethics committee and collected the data for the study. Canan Önder, Nihan Akdoğan, Şivge Kurgan, Nur Balcı, Ceyhan Ceran Serdar, and Meral Günhan all made contribution to the interpretation of the results and authoring of the article. Ceyhan Ceran Serdar and Muhittin A. Serdar performed biochemical analysis and testing. Muhittin A. Serdar also performed the statistical analysis. All authors reviewed and approved the submitted final manuscript.

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.

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