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Salivary levels of suPAR, HIF-1α and TNF-α in different grades of stage III periodontitis

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Abstract

Background The aim of the study is to evaluate saliva levels of Soluble urokinase plasminogen activator receptor (suPAR), Hypoxia-inducible factor-1 alpha (HIF-1α) and tumor necrosis factor-alpha (TNF-α) in stage III grade A, grade B, grade C periodontitis and periodontal health and to understand the roles of these molecules in periodontal inflammation process and also to compare the three biomarkers' discriminative efficacy in periodontal disease.

Methods A total of 80 individuals, 20 with stage III grade A periodontitis (group A), 20 with stage III grade B periodontitis (group B), 20 with stage III grade C periodontitis (group C) and 20 with healthy periodontium (group H) were recruited for this study. Full-mouth clinical periodontal measurements were recorded in periodontal charts. Whole saliva samples were collected to determine the levels of suPAR, HIF-1α and TNF-α in study groups using enzyme-linked immunosorbent assay (ELISA) method.

Results The saliva concentration of suPAR, HIF-1α, and TNF α was significantly higher in group A, group B, and group C compared with group H ($p < .05$). Additionally, salivary suPAR concentration was significantly higher in group C than in groups A and B ($p < .05$). Positive statistically significant correlations were observed between three biomarkers and all clinical parameters ($p < .05$).

Conclusions Increased levels of saliva suPAR, HIF-1α, and TNF α suggest that these molecules may play a role in periodontitis. In addition, the higher salivary suPAR levels in grade C periodontitis compared to other grades suggest that suPAR may be one of the potential molecules that can be used to predict disease progression and periodontal disease classification.

Trial registration Before starting the study, the study plan was uploaded to clinicaltrials.gov.tr and an identification number was obtained (Date: 21.05.2024, İdentification number: NCT06430450).

Keywords Hypoxia-inducible factor-1α, Periodontitis, Saliva, SuPAR, Tumor necrosis factor alpha

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Introduction

As a result of the host response to periodontopathogens found in microbial dental plaque, a large number of inflammatory mediators are produced. These biological agents play a crucial role in both the onset and progression of periodontal disease [1]. Various studies have demonstrated that detecting cytokines and biomarkers involved in inflammation in serum, saliva, and gingival crevicular fluid (GCF) samples from individuals with periodontal disease can provide significant insights into the development and pathogenesis of the disease [2–6].

The soluble urokinase plasminogen activator receptor (suPAR), an indicator of systemic immunological activation, inflammation, and thrombogenesis, has recently been recognized as a mediator in several inflammatory disorders [7]. suPAR is produced upon the cleavage of the urokinase plasminogen activator receptor (uPAR) from the cell membrane. It has been identified as a biological mediator of the body's immunological response and inflammation [8]. suPAR is present in plasma, cerebrospinal fluid, urine, and other regions of the body characterized by active inflammation and immunological responses [9, 10] Elevated plasma suPAR levels have been documented in various illnesses including cancer, diabetes, cardiovascular disease, and rheumatoid arthritis [8, 11, 12].

Studies examining the relationship between salivary suPAR levels and periodontal diseases found positive correlations between salivary suPAR levels and clinical parameters [13, 14]. In addition, a significant positive relationship was shown between salivary suPAR concentration and putative periodontopathogens (*Fusobacterium nucleatum, Tannerella forsythia, Parvimonas micra, Prevotella nigrescens, Campylobacter rectus, Prevotella intermedia, and Porphyromonas gingivalis*) [15] Skottrup et al. stated that the determination of salivary suPAR levels can aid in the early diagnosis and intervention of periodontitis. Furthermore, some studies also suggested that suPAR could be a potential biomarker for future disease detection and treatment using saliva samples [15, 16].

In periodontitis, elevated levels of Gram-negative anaerobic bacteria cause edema and increased inflammatory cell infiltration in the diseased periodontal tissues, which impairs capillary perfusion and disrupts the balance between oxygen consumption and supply [17, 18]. Hypoxia-inducible factor-1 alpha (HIF-1α), a crucial transcriptional regulator of oxygen balance, is the first mediator of adaptive cellular responses to hypoxia. HIF-1α stimulates the expression of numerous genes that play a crucial role in wound healing and tissue repair [19]. In clinical studies, higher levels of HIF-1α molecule were detected in saliva and GCF samples of periodontitis patients compared to healthy patients [20–22]. Additionally, when gingival samples from patients with periodontitis were examined, higher levels of HIF-1α protein expression were found compared to healthy patients [23, 24]. However, some studies have reported that the TNF-α molecule, which is found at elevated levels in periodontitis patients, increases HIF-1α protein expression and leads to an increase in the total amount of HIF-1α in GCF [20, 23, 24]. These data indicate a potential correlation between HIF-1α and the pathophysiology of periodontal inflammation [20, 21, 23, 24].

TNF-α is a cytokine synthesized by T cells and macrophages and exhibits immunomodulatory and proinflammatory effects on various cell types [25]. It has been suggested that TNF-α plays a key role in the progression of periodontitis and facilitates the degradation of periodontal tissues by inducing the synthesis of prostaglandin E2 (PGE2), interleukin-1 (IL-1), and matrix metalloproteinases [26, 27]. In this study, we chose to investigate the relationship between suPAR, HIF-1α and TNF-α a proinflammatory cytokine known to be associated with periodontal diseases, to better understand their roles in the periodontal inflammation process.

To the best of our knowledge, no study has comparatively analyzed the levels of suPAR, HIF-1α and TNF-α 1 in saliva samples of patients with stage III grade A, stage III grade B and stage III grade C periodontitis and healthy periodontium. Considering the data mentioned above, we hypothesized that suPAR and HIF-1α may play a role in periodontal disease and its varying degrees and that these molecules may help distinguish periodontal disease from a healthy periodontium. The present study had three aims: First, to comparatively investigate the salivary levels of suPAR and HIF-1α in groups with stage III grade A, stage III grade B, and stage III grade C periodontitis, as well as in individuals with a healthy periodontium. Second, to evaluate the correlations between both molecules and full-mouth clinical parameters, as well as TNF-α. Third, to compare these three biomarkers'discriminative efficacy in periodontal disease.

Materials and methods

This cross-sectional study included 80 systemically healthy, non-smoking individuals (47 females and 33 males; age range 18–70); 20 of these individuals had stage III grade A periodontitis, 20 had stage III grade B periodontitis, 20 had stage III grade C periodontitis and 20 were periodontally healthy. The study was conducted in accordance with the Helsinki Declaration and has been approved by the Karamanoglu Mehmetbey University Faculty of Medicine Local Scientific Medical Research Ethics Committee (Date: 13.09.2023, No:08–2023/04). The trial was registered on "clinicaltrials.gov" (United

States National Library of Medicine), with the identification number NCT06430450.

Study participants were selected from patients referred to the Karamanoglu Mehmetbey University, the Faculty of Dentistry, Department of Periodontology, Karaman, Turkey, between July 2024 and September 2024. Approximately 350 individuals who applied to the periodontology clinic were examined for eligibility for the study. To participate in the study, participants were informed about the study, invited to participate and examined for exclusion and inclusion criteria. The study's objectives and protocol were thoroughly explained to all participants, and written informed consent was obtained from those willing to participate.

The following criteria were applied for inclusion: individuals with different periodontal health conditions (Stage III Grade A periodontitis, Stage III Grade B periodontitis, Stage III Grade C periodontitis, and periodontally healthy individuals), individuals without any systemic diseases, individuals who do not use any tobacco products, including those who use smokeless forms of tobacco, and individuals with at least 20 teeth present in the mouth, excluding third molars.

The exclusion criteria were as follows: individuals with any systemic disease, smokers and consumers of tobacco products, individuals who have undergone periodontal therapy within the past six months, individuals who have been administered any anti-inflammatory medications in the past three months or antibiotics in the past six months, pregnant or breastfeeding individuals, and individuals with fewer than 20 permanent teeth.

Participants who met the inclusion criteria and consented to join the study were gradually enrolled in the study groups throughout the data collection period until the required sample size was achieved.

A comprehensive periodontal examination, including full-mouth charting and radiographic assessments, was performed on each participant to evaluate their periodontal health.

Participants were categorized into 4 groups according to their periodontal health status [28, 29]. While including the patients in stage III periodontitis, the following criteria were taken into consideration after clinical and radiological examination. BOP $\geq$30% in the whole mouth, interdental CAL $\geq$5 mm (at the site of the greatest loss), radiographic bone loss extending to the mid-third or apical third of the root. Additionally, patients classified as Stage II periodontitis based on CAL and radiographic bone loss assessments, but who possessed at least one complexity factor (Probing depths $\geq$6 mm, vertical bone loss $\geq$3 mm, furcation involvement class II or III, moderate ridge defects), were also included in the study as stage III periodontitis, as their stage shifts to stage III due to these factors. 30% or more of the teeth had CAL (generalized) in terms of disease extent [28, 29].

- Healthy periodontium (group H) (n = 20, 9 males/11 females, mean age: 29.85): Intact periodontium or reduced periodontium in a non-periodontitis patient. Bleeding on probing (BOP) <10% in the whole mouth, probing depth $\leq$3 mm.
- Stage III grade A periodontitis (group A) (n = 20, 9 males/11 females, mean age: 49.65): Having stage III periodontitis and % of bone loss/age <0.25 in the site of the tooth with the greatest loss.
- Stage III grade B periodontitis (group B) (n = 20, 7 males/13 females, mean age: 41.15): Having stage III periodontitis and % of bone loss/age 0.25–1.0 in the site of the tooth with the greatest loss.
- Stage III grade C periodontitis (group C) (n = 20, 8 males/12 females, mean age: 43.25): Having stage III periodontitis and % of bone loss/age >1.0 in the site of the tooth with the greatest loss.

Clinical measurements

An experienced clinician (İ.T.) conducted a clinical periodontal examination for all participants, assessing gingival index (GI) [30], plaque index (PI) [31], bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) to determine periodontal status. CAL and PD were measured at six different sites per tooth: distobuccal, mesiobuccal, mid-buccal, mesiolingual/mesiopalatal, disto-lingual/disto-palatal, and mid-lingual/mid-palatal. Meanwhile, GI, PI, and BOP were measured at four sites per tooth: mesial, distal, buccal, and lingual/palatal aspects.

Saliva sampling

Saliva samples were collected 1–2 days after clinical measurements were made. Prior to the collection of the saliva sample, all participants refrained from eating and drinking for one hour, which took place in the morning. Unstimulated whole saliva was collected by having participants spit into a plastic cup. Then, the saliva collected in the plastic cup was taken with a sterile syringe and transferred to the centrifuge tube. To eliminate cells and food debris and reduce saliva turbidity, which could affect analysis accuracy, the samples were centrifuged at room temperature for 10 min at 45 ×g – 600 RPM. Subsequently, 0.5 mL portions of saliva were transferred into sterile polypropylene tubes and preserved at − 80 °C until biochemical analysis.

Measurement of suPAR, HIF-1α, and TNF-α in saliva samples

ELISA kits (Elabscience, Houston, TX, USA) were employed to assess the levels of salivary suPAR, HIF-1α, and TNF-α. Saliva samples and calibration standards were precisely dispensed into wells pre-coated with specific antibodies. The plates were incubated for ninety minutes at 37 °C. Afterward, each well received a 1-h incubation at 37 °C with a biotin-conjugated antibody. The wells were then washed three times using 350 µl of washing buffer. Following the addition of streptavidin HRP enzyme, the wells were incubated for 30 min at 37 °C, and then subjected to five automatic washes with 350 µl of washing buffer. A substrate for HRP enzyme was added, and the plate was incubated at 37 °C in the dark. After that, H2SO4 was added to stop the reaction. Using an ELISA plate reader, absorbance was measured at 450 nm. (BioTek, ELX 800, BioTek Instruments, Winooski, VT, USA) and a standard curve was used to compare the absorbance values in order to determine the concentration.

Sample size calculation and statistical analyses

The sample size was determined using a software program (G*Power version 3.0.8, Heinrich Heine University, Düsseldorf, Germany) following a prior study that investigated saliva TNF-α levels in healthy individuals and periodontitis [32]. For this study, 20 volunteers were needed for each group, with a 0.59 effect size, a 99% power, and $\alpha = 0.05$. A software application (SPSS v.29.0; IBM Corporation, Armonk, New York, USA) was used to perform statistical analysis. The Shapiro–Wilk test was employed to assess the data for normal distribution. Demographic, clinical and biochemical data were normally distributed. ANOVA and Tukey tests were used to compare clinical and biochemical parameters, age and tooth number. The distribution of genders among the groups was examined using the Chi-square test. The Pearson correlation test was used to examine the correlations for biochemical and clinical data. The capacity of three biomarkers to distinguish between periodontal health and periodontitis, as well as between grades A, B, and C of stage III periodontitis, was evaluated using receiver-operating characteristic (ROC) curves and area under the curve (AUC) analysis. The $p < 0.05$. threshold was established for statistical significance.

Results

The demographic data and clinical results for the study groups is presented in Table 1. The mean age was higher in periodontitis groups compared to group H and the mean age was higher in group A compared to group B ($p < 0.001$). The number of teeth was lower in group C compared to group H ($p < 0.05$) and the other groups were similar ($p > 0.05$).

Clinical results

PD and CAL values were significantly higher in periodontitis groups than in group H ($p < 0.001$) and these values were significantly higher in group B and group C than in group A ($p < 0.001$). GI, PI and BOP (%) values were higher in periodontitis groups than in group H ($p < 0.001$), and these values were similar in the periodontitis groups ($p > 0.05$).

Biochemical results

The biochemical results data for the study groups and saliva concentrations of suPAR, TNF-α and HIF-1α are presented in Table 2. Saliva concentration of suPAR was significantly higher in periodontitis groups compared to group H ($p < 0.05$), and it was significantly higher in group C compared to group A ($p < 0.001$) and group B

Table 1 Patient characteristics and clinical periodontal parameters of study groups (Mean ± SD)

Patient characteristic	Group H ($n = 20$)	Group A ($n = 20$)	Group B ($n = 20$)	Group C ($n = 20$)	*p* value
Age (y)	29.85 ± 6.81	49.65 ± 9.22[a]	41.15 ± 8.97[a b]	43.25 ± 8.89 [a]	<.001
Sex (males/females)	9/11	9/11	7/13	8/12	>.05
Number of teeth	27.30 ± 1.34	25.35 ± 2.68	25.25 ± 3.02	24.80 ± 2.64 [a]	<.05
Periodontal parameters					
PD (mm)	1.45 ± 0.18	2.97 ± 0.42[a]	3.62 ± 0.52[ab]	3.66 ± 0.63[ab]	<.001
CAL (mm)	0.00 ± 0.00	3.12 ± 0.46[a]	3.88 ± 0.58[ab]	3.95 ± 0.79[ab]	<.001
GI	0.25 ± 0.17	1.50 ± 0.24[a]	1.48 ± 0.40[a]	1.61 ± 0.32[a]	<.001
PI	0.44 ± 0.25	1.77 ± 0.33[a]	1.70 ± 0.31[a]	1.77 ± 0.44[a]	<.001
BOP (%)	0.85 ± 2.23	56.04 ± 20.68 [a]	64.10 ± 25.12[a]	68.07 ± 18.83[a]	<.001

[a] Significant difference from group H

[b] Significant difference from Grade III periodontitis Grade A group

Table 2 Saliva levels of biochemical parameters in study groups

Saliva concentration (Mean ± SD)	Group H ($n = 20$)	Group A ($n = 20$)	Group B ($n = 20$)	Group C ($n = 20$)	*p* value
suPAR (ng/mL)	9.06 ± 5.62	22.07 ± 11.82 [a]	29.38 ± 12.62[a]	42.43 ± 16.91[abc]	<.001
HIF-1α (pg/mL)	77.12 ± 37.04	125.74 ± 54.29[a]	127.53 ± 35.76[a]	122.89 ± 60.49[a]	<.05
TNF-α (pg/mL)	4.69 ± 3.01	10.29 ± 5.08[a]	14.13 ± 12.24[a]	15.99 ± 7.73[a]	<.05

[a] Significant difference from group H

[b] Significant difference from Grade III periodontitis Grade A group

[c] Significant difference from Grade III periodontitis Grade B group

($p < 0.05$). Saliva concentration of TNF-α and HIF-1α was significantly higher in periodontitis groups compared to group H ($p < 0.05$), and saliva concentrations of TNF-α and HIF-1α were similar in the periodontitis groups ($p > 0.05$).

Correlations

Correlations for clinical and biochemical parameters for the study are presented in Table 3. There were strong positive correlations between saliva suPAR levels and all clinical parameters ($p < 0.01$). Saliva HIF-1α levels had strong positive correlations with GI, PI and BOP (%) ($p < 0.01$), and positive correlations with PD and CAL ($p < 0.05$). Saliva TNF-α levels had strong positive correlations with CAL and PI ($p < 0.01$), and positive correlations with PD, GI and BOP (%) ($p < 0.05$). There were strong positive correlations between saliva suPAR levels and saliva TNF-α levels ($p < 0.01$), and there were positive correlations between saliva suPAR levels and saliva Hif-1α levels ($p < 0.05$). No correlation between saliva levels of TNF-α and HIF-1α was observed ($p > 0.05$).

ROC analyses

ROC analysis was performed to assess discriminative efficiencies of salivary suPAR, HIF-1α, and TNF-α between periodontal health and periodontitis and between different grades of stage III periodontitis (Table 4). Receiver operating characteristic curves of salivary suPAR, HIF-1α and TNF α are shown in Fig. 1. Salivary HIF-1α has acceptable discriminatory power to distinguish between healthy periodontium and periodontitis (AUC = 0.781), salivary TNF α has very good discriminatory power to distinguish between the healthy periodontium and periodontitis (AUC = 0.835), and salivary suPAR has excellent discriminatory power to distinguish between the healthy periodontium and periodontitis (AUC = 0.955). Salivary suPAR also has acceptable discriminatory power to distinguish grade A from grade C (AUC = 0.793) and grade B from grade C (AUC = 0.761).

Discussion

The current study compared the salivary levels of suPAR, HIF-1α, and TNF-α in those with healthy periodontal tissue and those with stage III, grade A, grade B, and grade C periodontitis. According to the literature, this is the first study on the levels of suPAR, HIF-1α, and TNF-α in saliva in relation to stage III periodontitis grades and periodontal health. When the periodontally healthy group and periodontitis groups were compared, suPAR, HIF-1α and TNF-α were found to be significantly higher in all periodontitis groups compared to the healthy group. In the evaluation of stage III periodontitis groups according to grades, no difference was found in TNF-α and HIF-1α levels, while a significant difference was found in grade C suPAR levels compared to grade A and grade B periodontitis groups.

It has been demonstrated that infectious and inflammatory diseases such rheumatoid arthritis, bacteremia with endotoxemia, HIV infection, viral infections, and malaria raise serum suPAR levels. It is well recognized that leukocyte activation and inflammatory stimulation raise SuPAR levels in many body fluids [8]. It has been reported that suPAR is a stable biomarker for detecting disease activity in rheumatoid arthritis and high serum

Table 3 Correlations of clinical and biochemical parameters

	PD (mm)	CAL (mm)	GI	PI	BOP (%)	suPAR (ng/mL)	HIF-1α (pg/mL)	TNF-α (pg/mL)
suPAR (ng/mL)	.502[b]	.520[b]	.495[b]	.514[b]	.504[b]	1	.322[a]	.481[b]
Hif-1α (pg/mL)	.271 [a]	.307[a]	.394[b]	.491[b]	.327[b]	.322[a]	1	.228
TNF-α (pg/mL)	.274[a]	.321[b]	.241[a]	.346[b]	.274[a]	.481[b]	.228	1

[a] Correlation significant at the.05 level (2-tailed)

[b] Correlation significant at the.01 level (2-tailed

Table 4 Receiver operating characteristic analysis of salivary suPAR, Hif-1α and TNF-α in periodontal health and periodontitis

Group	AUC	Cutoff value (ng/mL)	Sensitivity	Specificity	95% CI	*p* value*
Periodontal health vs periodontitis (suPAR)	0.955	> 16.015	84.00	93.33	0,873–0,991	<.001
Stage III grade A vs. B periodontitis (suPAR)	0.639	> 24.45	52.94	93.33	0,451–0,801	.195
Stage III grade A vs. C periodontitis (suPAR)	0.793	> 24.45	72.22	93.33	0,616–0,913	.001
Stage III grade B vs. C periodontitis (suPAR)	0.761	> 33.565	72.22	88.24	0,588–0,889	.002
Periodontal health vs periodontitis (Hif-1α)	0.781	> 107.072	66.67	81.25	0,663–0,873	<.001
Stage III grade A vs. B periodontitis (Hif-1α)	0.542	> 67.768	100.00	22.22	0,366–0,711	.683
Stage III grade A vs. C periodontitis (Hif-1α)	0.535	> 226.432	18.75	100.00	0,356–0,707	.738
Stage III grade B vs. C periodontitis (Hif-1α)	0.568	≤ 107.828	62.50	64.71	0,385–0,739	.523
Periodontal health vs periodontitis (TNF-α)	0.835	> 8.16	73.08	93.75	0,726–0,914	<.001
Stage III grade A vs. B periodontitis (TNF-α)	0.524	≤ 9.49	56.25	58.82	0,343–0,700	.821
Stage III grade A vs. C periodontitis (TNF-α)	0.593	> 11.9	68.42	70.59	0,417–0,753	.357
Stage III grade B vs. C periodontitis (TNF-α)	0.599	> 12.57	63.16	68.75	0,420–0,760	.337

Abbreviations: *AUC* area under the curve, *CI* confidence interval

* Significance level at $p < .05$

suPAR levels are associated with tissue destruction and erosion in rheumatoid arthritis [33, 34].

In studies examining salivary suPAR levels in periodontal diseases, salivary suPAR levels were found to be significantly higher in both gingivitis and periodontitis compared to the healthy group and salivary suPAR levels have been shown to positively correlate with clinical periodontal parameters [13–15, 35]. In addition, a study reported that salivary suPAR levels were significantly higher in severe and moderate gingivitis than in mild gingivitis and that salivary suPAR levels may be related to the severity of the disease [35]. In our study, similar to previous studies, salivary suPAR levels were found to be significantly higher in all periodontitis groups compared to the healthy group and were significantly higher in group C compared to groups A and B. Additionally, in this study, salivary suPAR levels showed a strong positive correlation with all clinical parameters, similar to previous studies [13, 14]. It was an important finding that it was higher in periodontitis with an aggressive destruction pattern than in slow and moderate destruction patterns. This suggests that suPAR may be one of the potential biomarkers that can be used to determine disease severity and progression rate. In a scoping review examining potential salivary biomarkers associated with periodontitis, suPAR was reported as one of the potential biomarkers that provide valuable information from early diagnosis of periodontal disease to severity assessment and treatment planning [36]. The results of our study have once again proven this situation stated in this scoping review. As is known, TNF-α is one of the molecules known to play a key role in the pathogenesis of periodontal disease [37, 38]. Taşdemir et al. found a very strong positive correlation between GCF TNF-α levels and GCF suPAR and saliva suPAR levels in their study. Similar to the previous study, a very strong positive correlation was found between saliva TNF-α levels and saliva suPAR levels in this study. This raises the question of whether suPAR, like TNF-α may be one of the cytokines that play a key role in the pathogenesis of periodontal disease.

According to previous studies, anaerobic bacterial biofilm, enhanced inflammatory cell infiltration, and microthrombosis cause a hypoxic gradient to form in deep periodontal pockets [20, 39]. In comparison to healthy tissues, the density of HIF-1α-positive cells and the quantities of HIF-1α protein are noticeably higher in inflammatory gingival tissues next to periodontal pockets [24, 40].

In their study evaluating salivary and GCF HIF-1α levels in chronic and aggressive periodontitis, Afacan et al. found a significant difference in the chronic and aggressive periodontitis groups compared to the healthy group, but they could not find a significant difference between chronic and aggressive periodontitis [20]. They attributed this situation to the fact that the aggressive and chronic periodontitis patients included in the study had similar GI and BOP values. Similar to previous studies showing that HIF-1α levels in saliva samples were significantly higher in periodontitis groups than in healthy groups, in this study, salivary HIF-1α levels were found to be significantly higher in periodontitis groups than in the healthy group [20]. However, no significant difference was found

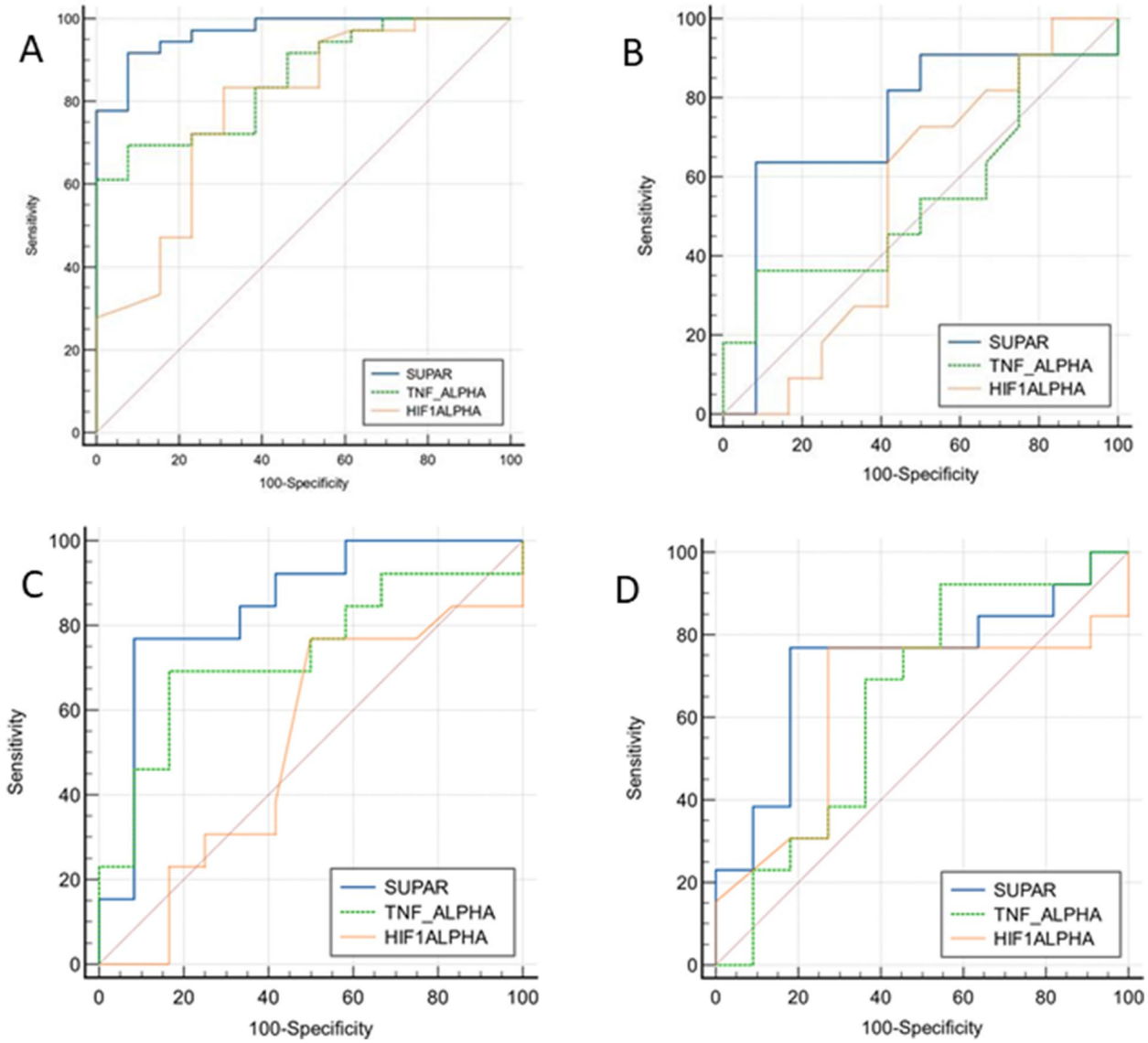

Fig. 1 Receiver operating characteristic curves of salivary suPAR, HIF-1α, and TNF α. **A** Periodontal health vs. stage III periodontitis. **B** Stage III grade A vs. grade B periodontitis. **C** Stage III grade A vs. grade C periodontitis. **D** Stage III grade B vs. grade C periodontitis

between the periodontitis groups. Positive correlations were found between salivary HIF-1α concentrations and PD and CAL levels, while strong positive correlations were found between PI, GI and BOP. Considering the results of previous studies and the results of this study, it is conceivable that HIF-1α may play a role in inflammatory responses and bone resorption and attachment loss during the progression of periodontal disease [20, 41, 42]. However saliva HIF-1α level was thought to be insufficient to determine the progression rate and progression pattern of periodontitis.

TNF-α is a pro-inflammatory cytokine that has a significant role in the etiology of periodontal disease. Salivary TNF-α levels were considerably greater in periodontitis groups than in the healthy group in our study, which is consistent with previous studies [43–45]. But no significant difference was found between stage III grade A, grade B and grade C. While there is no study in the literature examining salivary TNF-α in different grades of periodontitis, only one study investigated whether there was a difference between the grades in the gingival crevicular fluid of patients with stage IV periodontitis, and in this study, similar to our study, no significant difference was found between the grades of periodontitis in terms of TNF-α levels [46]. In studies conducted before the current classification in 2017, no difference was found

between salivary TNF-α levels in patients with aggressive periodontitis and chronic periodontitis, and while it was stated that TNF-α correlated with clinical periodontal parameters indicating the current clinical status of the disease, but no significant relationship was found with the rate of progression and destruction pattern of the disease [47–49].

The present study has some limitations. First, the cross-sectional design of the study limits the ability to clearly and definitively establish the relationship between the different grades of stage III periodontitis and the salivary levels of these three cytokines and more comprehensive studies are needed in the future. Secondly, serum and DOS samples could be collected from the patients to investigate the relationship between the different grades of stage III periodontitis and serum and DOS levels of suPAR, HIF-1α, and TNF-α. Finally, changes in the levels of these three cytokines could have been examined in individuals with the disease following treatment. Another limitation of the study is that the mean age of the periodontitis groups was higher than the healthy group. This may be considered as a confounding factor in terms of interpreting the data. There is only one study in the literature examining the relationship between salivary suPAR levels and age, and in this study, salivary suPAR levels were found to be 3.79 ng/mL in individuals aged 74–89 years, while they were found to be 3.16 ng/mL in individuals aged 24–66 years, and it was stated that saliva suPAR levels were statistically significantly higher in the group aged 74 and over [50]. In our study, there were only three patients aged 60 and over, and the patient with the highest age was 68 years. We could not find a study in the literature examining the relationship between salivary HIF-1α levels and age. Previous studies investigating the relationship between salivary TNF-α levels and age have reported a significant decline in TNF-α concentrations with advancing age [51, 52]. In light of these findings, lower TNF-α levels might have been expected in our study as well, considering that the mean age of the periodontitis groups was higher than the healthy group. However, our results demonstrated that salivary TNF-α levels were significantly elevated in the periodontitis groups compared to the healthy controls. This finding may indicate that the increase in salivary TNF-α levels associated with periodontitis is particularly pronounced, independent of age-related variations.

Conclusion

Within the limits of this study, salivary levels of suPAR, HIF-1α and TNF-α were found to be increased in periodontal disease groups. Both molecules may have an important role in the pathogenesis of periodontal disease. Salivary concentrations of both molecules can potentially be used as diagnostic markers for periodontal disease. In particular, salivary concentrations of suPAR were higher in group C compared to groups A, B, and healthy periodontium groups. This result suggests that salivary suPAR concentration may be a potential inflammatory risk indicator/biomarker in the classification of periodontitis and the prediction of the progression rate of periodontitis. Our findings supported previous articles evaluating the relationship between these molecules and inflammatory diseases. In addition, studies examining the salivary concentrations of these molecules in different stages and grades of periodontitis may further clarify the role of these molecules in the pathogenesis, prediction of progression rate, and classification of periodontal diseases.

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Authors' contributions

İT performed data collection, formal analysis, funding acquisition, development of research methodology, project management, writing, review, and editing of the article. ÖÖ gave ideas in terms of methodology. HEY performed and interpreted the biochemical analyses of the study. EK performed collection and analysis of literature information required for the study ŞA performed collection and analysis of literature information required for the study. MS performed formal analysis, development of research methodology, project management, writing, review and editing of the article.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Helsinki Declaration and has been approved by the Karamanoglu Mehmetbey University Faculty of Medicine Local Scientific Medical Research Ethics Committee (Date: 13.09.2023, No:08–2023/04).

The study's objectives and protocol were thoroughly explained to all participants, and written informed consent was obtained from those willing to participate.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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