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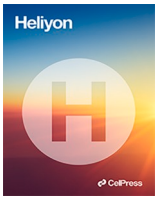

Research article

Salivary and serum nitric oxide synthase, macrophage inflammatory protein 1 alpha and macrophage migration inhibitory factor levels in periodontal disease

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ABSTRACT

Objective: Periodontal disease is multifactorial inflammatory disease involving both gingivitis and periodontitis. Inducible nitric oxide synthase (iNOS), macrophage inflammatory protein 1 alpha (MIP-1α) and macrophage migration inhibitory factor (MIF) are mediators contributing to the progression of periodontal diseases with distinct functions. The aim of this study is to evaluate the local and systemic iNOS, MIP-1α and MIF concentrations in patients having periodontal disease with different severities.

Design: The study was conducted on 88 individuals equally divided into four groups; 1) Periodontally Healthy 2) Gingivitis 3) Stage I–II Periodontitis 4) Stage III–IV Periodontitis. Saliva and serum samples were obtained from each individual and then periodontal examinations were performed. Plaque and bleeding on probing indexes, probing depths and clinical attachment levels were measured on each tooth to determine the periodontal status. Concentrations of iNOS, MIP-1α and MIF were measured with enzyme-linked immunosorbent assay.

Results: Patients with stage I–II and stage III–IV periodontitis had more iNOS levels than periodontally healthy people in serum and saliva ($p \leq 0{,}001$ for serum; $p < 0{,}05$ for saliva). Stage III–IV periodontitis group had significantly more serum-iNOS levels than that in gingivitis group ($p = 0{,}005$). When compared with periodontally healthy individuals, MIP-1α levels in stage III–IV periodontitis patients were measured significantly more in saliva; ($p = 0{,}016$) but less in serum ($p = 0{,}006$) samples. More serum-MIF concentrations were observed in stage I–II periodontitis groups than that in periodontally healthy individuals ($p < 0{,}05$).

Conclusion: Increased salivary and serum iNOS and serum-MIF levels in different stages of periodontitis suggest that these molecules might be involved in periodontal disease pathogenesis. Also, oral microenvironment may stimulate the enhanced MIP-1α concentration in advanced periodontitis cases.

1. Introduction

Periodontal disease is multifactorial disease primarily caused by the accumulation of microbial dental plaque on tooth surface. Periodontal disease, as a term, involves both gingivitis and periodontitis; gingivitis is the inflammation of soft tissues surrounding the

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teeth [1]; but in periodontitis, inflammation extends to tooth-supporting tissues causing permanent tissue loss [2]. Periodontitis formerly classified into chronic and aggressive types; chronic periodontitis was representing the slowly progression form of periodontitis, meanwhile the disease was progressing rapidly with high destructive functions in aggressive periodontitis. According to AAP/EFP 2017 Classification, which forms the basis of this study, chronic and aggressive periodontitis are classified under one group named as 'periodontitis'. Staging and grading system is used to categorize the severity of periodontitis [3].

Nitric oxide (NO) is a free radical produced from L-arginine through NO synthase (NOS) enzyme activity. Inducible nitric oxide synthase (iNOS), isoform of NOS, has the highest NO producing capacity [4]. iNOS mediated NO production is associated with killing bacteria in early response, but it stimulates bone destruction in progressive forms of inflammatory diseases [5,6]. Periodontopathogen bacteria *Aggregatibacter actinomyecetemcomitans (Aa)* and *Porphyromonas gingivalis (Pg)* were found to stimulate iNOS expression [7,8]; and elevated iNOS levels leading to enhanced NO production and progression of periodontitis have been shown with several studies [9–11].

Gram (−) bacterial endotoxins stimulate secretion of chemokine-like inflammatory cytokine named as macrophage migration-inihibitory factor (MIF) [12]. MIF has wide range of pro-inflammatory functions such as stimulating the production of tumor necrosis factor (TNF), interleukin-1β (IL-1β), NO and several matrix metalloproteinases [12,13]. MIF also has crucial role in the progression of periodontitis by being involved in the alveolar bone loss [14,15]. Neutralizing MIF has been suggested to be used as a therapeutic target to prevent pathogenic bone loss in periodontitis [14].

Macrophage inflammatory protein 1 alpha (MIP-1α) (also named as C–C motif ligand 3, CCL3) is pro-inflammatory chemokine inducing chemotaxis and transendothelial migration of immune response cells [16]. MIP-1α also stimulates osteoclastic bone resorption [17,18] and due to its strong relation with alveolar bone loss and decreasing levels after periodontal therapy, MIP-1α has been suggested to be used as salivary biomarker in periodontitis. Elevated levels of MIP-1α have been accepted as an indicator of presence of periodontitis [19–21].

According to the previous classification system, increased iNOS, MIF and MIP-1α levels in periodontitis have been shown previously with distinct studies [22–24]; however, to our knowledge little is known about the concentrations of these mediators based on the 2017 classification scheme. There are still discussions about the difficulties of adaption of new classification to clinical practice; biochemical analyses of specific mediators can be beneficial to supply supportive information in here [25]. iNOS, MIF and MIP-1α are three mediators that can easily be detected from body fluids. In the pathogenesis of periodontal diseases they have crucial functions in each step of the host response and they strongly contribute to the progression of the disease [10,14,19]. These features make these mediators important candidates to be used as adjunct markers in detecting the severity of periodontal disease in the new classification scheme. Therefore, in this study we hypothesized that secretion profiles of iNOS, MIF and MIP-1α increase in line with the severity of periodontal disease. We aimed to examine and compare iNOS, MIF and MIP-1α concentrations in serum and saliva samples of the patients with periodontally healthy, gingivitis, stage I–II and stage III–IV periodontitis.

2. Material-method

2.1. Study selection

A total of 88 participants (52 males/36 females) applied to the Department of Periodontology, School of Dentistry, Ataturk University, Erzurum, Turkey between December 2021 and 2022 were involved in the study. Participants were chosen among the patients applied to our clinic. Systematically healthy, non-smoker patients who had not received periodontal treatment or antibiotic therapy in the last six months were enrolled. Four groups (22 individuals in each group) were generated according to their periodontal status; 1) Periodontally Healthy 2) Gingivitis 3) Stage I–II Periodontitis 4) Stage III–IV Periodontitis. The study was conducted in full accordance with ethical principles including the World Medical Association's Declaration of Helsinki and approved by the Ethics Committee of the Ataturk University, School of Medicine (B.30.2.ATA.0.01.00/682). The study protocol was explained to each individual before periodontal examination and biosample collection; and written informed consent was received. Medical and dental histories were obtained initially. The participants; i) having systemic/immunsuppresive disease ii) have undergone periodontal therapy in the last six months iii) used antibiotics/anti-inflammatory drugs in the last three months iv) currently smoking or stopped smoking in the last six months were excluded from the study; also pregnant and breastfeeding women were not involved.

2.2. Saliva and serum sampling

Unstimulated whole saliva samples were collected from each patient early in the morning (between 9:00 a.m. and 10:00 a.m.) before periodontal measurements and interventions. Participants were requested an overnight fasting and not drinking (except water) or chewing gum. Patients were informed to sit comfortably and accumulate saliva samples in the floor of the mouth by keeping their mouths open for 5 min and then spit out into 5 ml polypropylene tubes (ISOLAB SantrifugeTube, 078.02.001, Eschau, Germany). Saliva samples were then centrifuged at 1000×*g* for 20 min; cell debris was removed and supernatants were transferred into Eppendorf tubes [26].

Five milliliters of venous blood were taken from the antecubital vein by standard venipuncture method into non-ethylene diamino tetra-acetic acid containing test tube (BD Medical 366,566 SST II Vacutainer, New Jersey, USA) and blood was let to clot at room temperature for 2 h. Then the blood samples were centrifuged at 1000×*g* for 20 min to obtain serum. All samples were stored at −80 °C until biochemical analyses done by enzyme-linked immunosorbent assay (ELISA).

2.3. Clinical periodontal measurements

After saliva and serum collection, periodontal examination was performed by one calibrated experienced examiner (FCO). Silness-Löe plaque index (PI), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) were measured gently on six sites (mesio-buccal, buccal, disto-buccal, lingual, disto-lingual) of each tooth, except third molars using periodontal probe (Williams, Hu-Friedy, Chicago, IL). BOP was recorded positive if bleeding occurred within 15 s after periodontal probing dichotomously by visual examination. CAL was measured between cemento-enamel junction and the base of the periodontal pocket. Individuals were diagnosed in accordance with the clinical criteria stated in the consensus reports of "2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions" [3,27] Table 1 shows the clinical diagnostic criteria used in the study.

2.4. Measurement of MIF, MIP-1α and iNOS in Saliva and serum samples

Commercial ELISA kits were purchased to determine MIF (human MIF ELISA kit, Wuhan USCN Business Corporation, Lot: L221207992, USA), MIP-1α (human MIP-1α ELISA kit, Wuhan USCN Business Corporation, Lot: L221207980, USA), and iNOS (human NOS_2 ELISA kit, Wuhan USCN Business Corporation, Lot: L221207994, USA) levels in serum and saliva. All measurements were done in accordance with the manufacturer's instructions. All the assays were duplicate and significant cross-reactivity or interference was not observed. The inter-assay co-efficients of variation (%CV) were 11%–9%–10% while intra-assay %CV were 9%. The measuring range of the MIP-1α, MIF and NOS_2 kit were between 15.6 and 1.000 pg/ml, 0,312-20 ng/ml and 0.156–10 ng/ml, respectively. The minimum detectable dose of MIP-1α, MIF and NOS_2 were typically less than 6.3 pg/ml, 0.128 ng/ml and 0.054 ng/ml, respectively.

2.2. Statistical analyses

The sample size was determined by one-way ANOVA test using the G* Power 3.1 Software Package based on the previous studies [28]. When effect size was taken as $f = 0.973$, it was calculated that at least 22 participants should be included in each group with a confidence interval of 95% at 80% power. IBM SPSS 20 program was used for statistical analysis. Data were presented as mean, standard deviation, median, minimum, maximum, percentage and number. The normal distribution of continuous variables was evaluated with the Shapiro Wilk-W test, Kolmogorov Smirnov test, Q-Q plot, skewness and kurtosis. In the comparison of continuous variables with more than two independent groups, the ANOVA test was used when the normal distribution condition was met, and the Kruskal Wallis test was used when it was not. Tukey test for homogenous variances and Tamhane's T2 test for non-homogenous variances were performed as post-hoc test after ANOVA analysis. After Kruskal Wallis test, Kruskal Wallis 1-way ANOVA (k samples) test was used for post-hoc tests. In 2×2 comparisons between categorical variables; Pearson Chi-square test was used if the expected value is > 5, chi-square Yates test was used if the expected value was between 3 and 5 and the Fisher's Exact test was used if the expected value was <3. In the comparison of two quantitative variables, Pearson correlation was used if there was normal distribution and Spearman correlation test was used if there was not. Covariance analysis was used to examine the periodontal parameters and biomarker levels after adjusting for age; data shown in figures and table are representing the adjusted results. Differences were counted as significant when p value was less than 0.05.

3. Results

3.1. Demographic and clinical periodontal parameters

Fifty-two male and thirty-six female participants were involved in the study and significant difference was not observed between the genders. Mean age of the patients with stage I–II and stage III–IV periodontitis were significantly more than the patients with gingivitis and periodontally healthy ($p < 0,05$) (Table 2). Periodontally healthy group had the least PI score with significant difference from stage I–II periodontitis ($p = 0,001$) and stage III–IV periodontitis ($p < 0,001$) groups. Stage III–IV periodontitis group had significantly more PI score than the gingivitis group; ($p < 0,001$) however, difference was not observed between stage I–II and stage III–IV periodontitis groups. Stage III–IV periodontitis group had significantly more BOP than periodontally healthy ($p < 0,001$), gingivitis ($p = 0,001$) and stage I–II periodontitis ($p < 0,005$) groups. There was significant difference in BOP between gingivitis and periodontally healthy groups ($p < 0,05$). The PD of stage I–II and stage III–IV periodontitis were significantly more than periodontally healthy and gingivitis groups, ($p < 0,001$) but there was no difference between periodontally healthy and gingivitis; and between stage I–II and stage III–IV periodontitis groups. However, CAL in stage III–IV periodontitis group was significantly more than that in stage I–II periodontitis group ($p < 0,05$) (Table 3).

Table 1
Diagnostic criteria for periodontal disease.

Periodontally Healthy	<10% BOP, ≤3 mm PD
Gingivitis	≥10% BOP, ≤3 mm PD
Stage I Periodontitis	1–2 mm greatest iCAL, Bone loss in the coronal third of the root
Stage II Periodontitis	3–4 mm greatest iCAL, Bone loss in the coronal third of the root
Stage III Periodontitis	≥5 mm iCAL, Bone loss extending to mid-third and beyond, Having 4 or less tooth loss due to periodontitis
Stage IV Periodontitis	≥5 mm iCAL, Bone loss extending to mid-third and beyond, Having 5 or more tooth loss due to periodontitis

Table 2
Demographic data.

	Periodontally Healthy	Gingivitis	Stage I–II Periodontitis	Stage III–IV Periodontitis
Gender (m/f)	14M/8F	12M/10F	13M/9F	13M/9F
Age (years)	32 ± 7	31 ± 10	42 ± 98[a]	41 ± 1[a]

Mean ± SD.
[a] Significantly more than gingivitis and periodontally healthy groups.

3.2. Concentration of iNOS was positively correlated with the severity of periodontal disease

The levels of iNOS in saliva and serum were increased in parallel with the severity of periodontal disease and the highest amount of iNOS was observed in patients with stage III–IV periodontitis. Patients with stage III–IV and stage I–II periodontitis had significantly more serum-iNOS levels than individuals with periodontally healthy ($p = 0{,}001$ and $p < 0{,}001$, respectively). Also, in serum samples stage III–IV periodontitis group had significantly more iNOS levels than that in gingivitis group ($p = 0{,}005$); however, difference between stage I–II periodontitis and gingivitis groups were not significant. In saliva, both stage I–II and stage III–IV periodontitis groups had significantly more iNOS amount than that in periodontally healthy group ($p < 0{,}05$) but not from gingivitis group. Although iNOS levels in saliva and serum samples were more in patients with stage III–IV periodontitis than that in patients with stage I–II periodontitis; the differences between these groups were not significant (Table 4, Fig. 1).

3.3. Highest MIP-1α concentration in saliva was observed in stage III–IV periodontitis group

A dramatic reduce was seen in serum MIP-1α levels of stage III–IV periodontitis group and it was significantly less than these in periodontally healthy ($p = 0{,}006$) and stage I–II periodontitis ($p < 0{,}05$) groups. No difference was observed among periodontally healthy, gingivitis and stage I–II periodontitis groups. In contrast, saliva-MIP-1α levels were significantly more in stage III–IV periodontitis group than that in periodontally healthy group ($p = 0{,}016$). Although slight increase was observed in these levels in parallel with the severity of disease, differences did not reach statistical significance (Table 5, Fig. 2).

3.4. Highest MIF concentration in serum was observed in stage I–II periodontitis group

Difference was not observed between gingivitis and periodontitis in terms of MIF concentrations in serum and saliva. In serum samples patients with stage I–II periodontitis had significantly more MIF levels than that in periodontally healthy individuals ($p < 0{,}05$). Although patients with stage I–II periodontitis had slightly more serum-iNOS concentrations than patients with gingivitis and stage III–IV periodontitis, the differences were not significant. Saliva-MIF concentration was reached the highest level in stage III–IV periodontitis group but differences with the other groups were not statistically significant. Patients with gingivitis had less amount of saliva-MIF than the other groups; but not with significant difference (Table 6, Fig. 3).

4. Discussion

In the present study iNOS, MIP-1α and MIF concentrations in saliva and serum were investigated in patients with periodontally healthy, gingivitis, stage I–II and stage III–IV periodontitis. As excepted, PD, CAL, PI and BOP were increased in line with the severity of periodontal disease. Although mean PD difference between stage I–II and stage III–IV periodontitis groups was not counted significant due to the gingival recessions, more CAL was observed in stage III–IV periodontitis. Biochemical analysis results showed that iNOS concentration increases in line with the severity of periodontal disease; and stage III–IV periodontitis patients had the highest amount

Table 3
Clinical periodontal parameters.

Periodontal Parameters	Periodontally Healthy	Gingivitis	Stage I–II Periodontitis	Stage III–IV Periodontitis
PI	1.4 ± 0.1	1.5 ± 0.2	1.8 ± 0.4[b]	2 ± 0.4[a]
BOP (%)	6.5	19[b]	58.7[b]	66.7[a,d]
PD (mm)	1.5 ± 0.3	1.7 ± 0.4	2.5 ± 0.6[a]	3 ± 0.7[a]
CAL (mm)	1.6 ± 0.2	1.8 ± 0.6	3.7 ± 1.1[a]	7.8 ± 1.5[a,d]

Mean ± SD.
[c]Significantly more than gingivitis groups.
[a] Significantly more than gingivitis and periodontally healthy groups.
[b] Significantly more than periodontally healthy groups.
[d] Significantly more than Stage I–II periodontitis groupsPI = Plaque index, BOP = Bleeding on probing, PD = Probing depth, CAL = Clinical attachment level.

Table 4
Mean iNOS Concentrations in Serum and Saliva.

iNOS levels (ng\mL)	Serum	Saliva
Periodontally Healthy	0.946 ± 0.971	1.112 ± 1.295
Gingivitis	1.446 ± 1.722	2.477 ± 1.324
Stage I–II Periodontitis	2.695 ± 1.429	2.578 ± 2.473
Stage III–IV Periodontitis	3.998 ± 2.04	2.76 ± 2.26

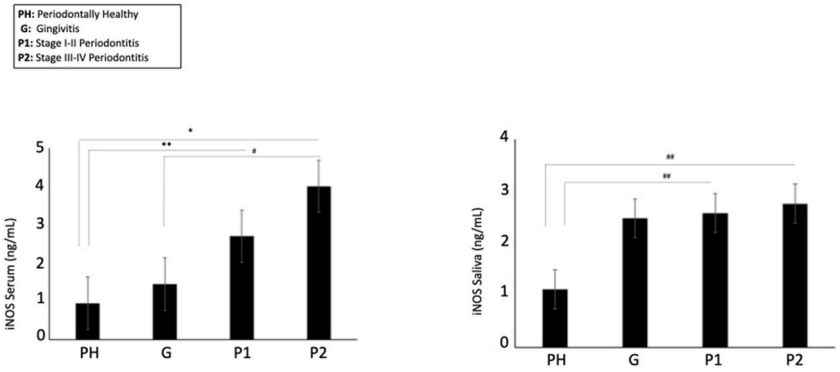

Fig. 1. Shows inducible nitric oxide synthase (iNOS) concentrations in serum and saliva of patients with periodontally health (PH), gingivitis (G), stage I–II periodontitis (P1) and stage III–IV periodontitis (P2). Data are expressed as mean ± standard error. Differences were considered significant when p value was less than 0.05. *p = 0,001; **p < 0,001; #p = 0,005; ##p < 0,05.

Table 5
Mean MIP-1α concentrations in serum and saliva.

MIP-1α levels (pg\mL)	Serum	Saliva
Periodontally Healthy	314.197 ± 223.357	164.548 ± 185.376
Gingivitis	245.695 ± 243.480	274.645 ± 217.443
Stage I–II Periodontitis	327.254 ± 226.562	283.66 ± 192.779
Stage III–IV Periodontitis	109.857 ± 110.049	348.255 ± 203.756

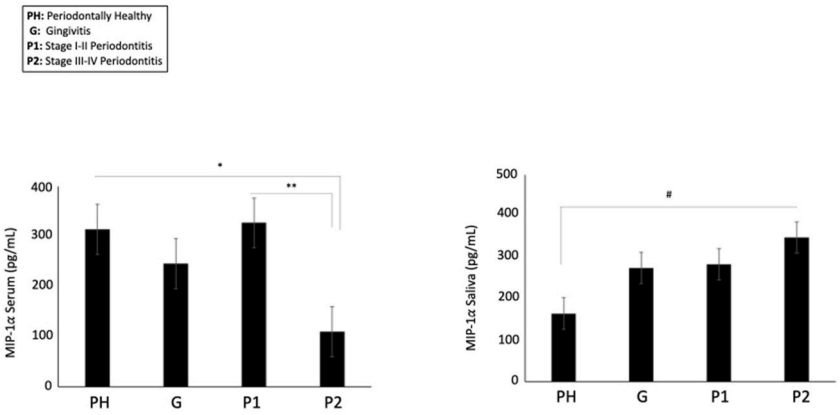

Fig. 2. Shows macrophage inflammatory protein 1 alpha (MIP-1α) concentrations in serum and saliva of patients with periodontally healthy (PH), gingivitis (G), stage I–II periodontitis (P1) and stage III–IV periodontitis (P2). Data are expressed as mean ± standard error. Differences were considered significant when p value was less than 0.05. *p = 0,006; **p < 0,05; #p = 0,016.

of iNOS levels in serum and saliva samples. Highest MIP-1α concentration was seen in stage III–IV periodontitis group in saliva, but not in serum. Amount of MIF reached the peak level in the circulation of stage I–II periodontitis patients.

iNOS production by gingival cells in periodontitis have been shown previously by preclinical and clinical studies [29,30]. In this study, it was suggested that enhanced expression of iNOS is not limited with tissue samples, but also can be detected in body fluids of

Table 6
Mean MIF concentrations in serum and saliva.

MIF levels (ng\mL)	Serum	Saliva
Periodontal Healthy	2.419 ± 5.187	7.361 ± 10.592
Gingivitis	3.833 ± 5.614	3.338 ± 1.103
Stage I–II Periodontitis	8.568 ± 7.901	6.49 ± 28.95
Stage III–IV Periodontitis	7.027 ± 8.105	9.42 ± 30.89

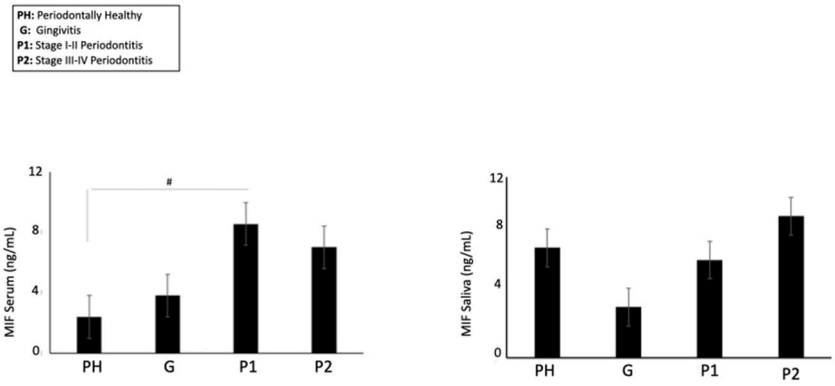

Fig. 3. Shows macrophage migration inhibitory factor (MIF) concentrations in serum and saliva of patients with periodontally healthy (PH), gingivitis (G), stage I–II periodontitis (P1) and stage III–IV periodontitis (P2). Data are expressed as mean ± standard error. Differences were considered significant when p value was less than 0.05. *p = 0,001; #p < 0,05.

patients. iNOS concentrations in blood have been used in determining the severity of certain systemic diseases previously [31]. Our results claim that iNOS levels in both saliva and serum samples are elevated in stage III–IV periodontitis. This supports the other studies showing significantly more NO/NOS levels in periodontitis [25] and advanced periodontitis cases [24] compared with periodontally healthy subjects. Keratinocytes, osteoclasts, osteoblasts and fibroblasts are some sources of iNOS in periodontal tissues but iNOS is mainly secreted by macrophages [32]. Therefore analyzing serum and saliva samples eliminate periodontal cells and may give a view while evaluating macrophage levels on the basis of iNOS generated NO production. Regarding the other inflammatory cell sources of iNOS such as dendritic cells and natural killer cells it is not possible to come to a definite conclusion about macrophage levels with results of this study. However, as macrophages are their main source [31]; our results speculate that on the basis of iNOS secretions, circulating macrophages may be increased in patients with stage III–IV periodontitis compared to the patients having gingivitis. This is not the case for salivary iNOS levels; as similar amount of concentration was observed in gingivitis and periodontitis.

In the present study, salivary MIP-1α concentration showed gradual increase in line with the severity of periodontal disease. These results support other studies showing elevated MIP-1α levels in saliva samples of patients with periodontitis [23,33]. MIP-1α was also accepted as one of the five candidate biomarkers for diagnosis of periodontitis from saliva [19] and regarding the results of this study, high MIP-1α levels in saliva may show the existence of stage III–IV periodontitis cases. However, involvement of MIP-1α in gingivitis is controversial; some studies showed more MIP-1α levels in patients with gingivitis than periodontally healthy individuals but there are other studies showing the reverse [28,34]. In our study, no difference was observed between gingivitis and periodontally healthy groups in terms of MIP-1α concentration.

Due to the current results, periodontally healthy subjects had similar amount of serum-MIP-1α levels with stage I–II periodontitis group, and surprisingly amount of MIP-1α in stage III–IV periodontitis is significantly less than both of these groups. In a previous study difference was not observed at serum MIP-1α levels between periodontitis and periodontally healthy groups. Although severity of periodontitis was not mentioned there, mean CAL was measured as 3.76 ± 1.11 [35], which is similar to CAL in stage I–II periodontitis groups in the present study (3.7 ± 1.1). Therefore it could not be wrong to tell that severity of periodontitis is similar to each other and MIP-1α levels in circulation does not show difference in stage I–II periodontitis. Our results moreover indicate that as the disease progresses to stage III–IV periodontitis, these levels tend to decrease. Serum MIP-1α concentration pattern conflicts with its salivary levels in this study in which oral microenvironment may be a contributing factor in here. Supporting this, it was previously shown that bacterial virulence factor lipopolysaccharide (LPS) and *Aa* stimulates MIP-1α secretion [36,37].

Increased serum-MIF concentrations in chronic periodontitis patients have been shown previously, but severity of periodontitis has not been mentioned there [38]. The present study shows more detailed information that MIF level was increased in stage I–II periodontitis compared with periodontally healthy group; however, no significant change was seen due to progression to stage III–IV periodontitis. There was no difference between periodontal health and diseases at salivary-MIF levels in this study. These results are contrary to other studies showing increased saliva-MIF levels in gingivitis [39] and periodontitis [38,39] when compared with periodontally healthy controls. However, in agreement with our results, in a previous study difference was not shown at MIF levels in saliva between periodontally healthy, gingivitis and periodontitis patients [40]. Mean age of periodontitis patients were significantly

more than the periodontally healthy and gingivitis patients in the present study that is not unexpected considering the immunopathogenesis of the periodontitis [41]. Due to declined immune response, less amount of MIF level was observed in spite of the higher plaque accumulation in elderly patients. The age was suggested to modify MIF secretions in here [42], which also could be the contributing factor in effecting MIF levels in this study. It was previously reported that *Pg*-LPS has inhibitor effect over MIF expression [43], that also could be a reason of relationship between locally suppressed MIF levels contrary to their increased amount in serum samples seen in this study. MIF deficiency in macrophages causes hyporesponsivity against Gram (−) microorganism stimulation through TLR4 down-regulation [44] and in the opposite side, decreased MIF levels in oral microenvironment may lead to progression of periodontal disease.

5. Conclusion

In conclusion, there is no difference between gingivitis and periodontitis in terms of MIP-1α and MIF concentrations; however, more serum-iNOS levels are observed in stage III–IV periodontitis compared with gingivitis. More salivary iNOS and MIP- 1α concentrations are seen in stage III–IV periodontitis group than periodontally healthy group. Local MIF level is suppressed with the progression of periodontal disease. Oral microenvironment stimulates MIP-1α secretion. Periodontal disease is not an inflammatory disease limited with oral cavity rather has systemic effects and it is under systemic influence [45]. Therefore using both serum and salivary levels enable us to have knowledge about local and systemic relation of these mediators with periodontal disease; and that is the strength of this study. Including different periodontal disease conditions according to new classification system is its another strength, as it can provide quantitative information to classify the periodontal disease according to their severities. Sufficient number of patients could not be reached in each stage of periodontitis therefore we had to divide periodontitis into two groups (stage I–II, stage III–IV); this is the limitation of this study.

Data availability statement

Data associated with this study has not been deposited into a publicly available repository. All data analyzed during this study are referenced in the article and also available from the corresponding author on reasonable request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Fatma Oner: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Faruk Cagri Onat:** Resources, Methodology. **Yerda Ozkan Karasu:** Supervision, Project administration, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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