Research Article

Association between salivary and serum Interleukin-39 and osteopontin in periodontitis patients: case-control study

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Abstract: Background: Cytokines have a crucial role in developing chronic inflammation and the inflammatory response. Some interleukins, such as interleukin-39 (IL-39), have unknown specific roles in the pathogenesis of periodontitis. Osteopontin has been shown to be a multifunctional cytokine and a unique modulator of innate and adaptive immune responses. Therefore, the study's objective was to measure IL-39 and osteopontin level and their ratio in the saliva and serum of periodontitis patients in Iraq. Materials and methods: This case-control study include 78 individuals with an age range of 25-60 years (40 periodontitis patients without clinical intervention and 38 healthy volunteers). Levels of salivary, serum IL-39 and osteopontin were determined for both groups, utilizing the Enzyme-Linked Immunosorbent Assay technique. The Social Science Statistical Package (Version 26; SPSS, IBM) was utilized for statistical analysis. Results: Serum levels of IL-39 and osteopontin were significantly elevated in periodontitis patients compared to healthy participants ($p < 0.001$ and $p = 0.001$, respectively). A similar pattern was observed in saliva, with significantly higher levels of IL-39 and OPN in periodontitis patients. Positive correlations were detected between serum and salivary levels of IL-39 ($p = 0.037$) and OPN ($p = 0.003$). Diagnostic validity tests demonstrated that IL-39 and OPN assays exhibited high sensitivity and negative predictive value (100%), and high accuracy (94.87% to 100%). The area under the curve values approached 1.0. Conclusion: Elevated saliva interleukin-39 and Osteopontin levels and elevated serum parameters are strongly associated with periodontitis.

Keywords: IL-39, Osteopontin, Saliva, Periodontitis

Introduction

Periodontitis is an inflammatory condition affecting the supportive tissues of the teeth. It is characterized by alveolar bone loss and pocket development. In Iraq, a recent study utilizing the 2017 classification system revealed that the prevalence of periodontitis in an Iraqi population was 36.5%, with severe forms of the disease (Stages 3 and 4) being the most dominant, accounting for 77.3% of cases. Understanding the local prevalence and severity of periodontitis is crucial for developing targeted prevention and treatment strategies [1]. The disease results from a combination of genetic factors and dysbiotic microbial communities from dental plaque. The immune system has a significant impact in the progression and development of periodontitis [2]. The host's response to microbial dysbiosis involves the production of inflammatory substances, including matrix metalloproteinases (MMPs), cytokines, and prostaglandins, which trigger an immune-inflammatory reaction [3, 4]. Among the many inflammatory cytokines involved, Interleukin-12 (IL-12) is a significant player, contributing to the breakdown of periodontal tissues and acting as a crucial regulatory cytokine in periodontitis [5]. In recent years, the role of cytokines such as IL-6, IL-30, IL-35 and IL-39 has garnered increasing attention for their involvement in both pro-inflammatory and anti-inflammatory pathways, contributing to the pathogenesis of various immune-related diseases,

including periodontitis[6-8]. Periodontitis have common risk factors including but not limited to obesity [9], smoking [10], diabetes [11], genetic factors [12] .

IL-39 is a glycoprotein composed of Ebi3 and IL-23p19 subunits and a recently discovered member of the interleukin-12 family. IL-39 is critical in managing inflammatory diseases and regulating both innate and adaptive immunity. It influences various immune pathways by modulating the activity of immune cells, including macrophages and T lymphocytes, and by interacting with other cytokines involved in inflammatory processes [13, 14]. In autoimmune diseases and other inflammatory conditions, IL-39 has emerged as a potential therapeutic target due to its role in driving chronic inflammation [15]. Recent research indicates that IL-39 contributes to the pro-inflammatory environment in periodontitis by activating STAT1/STAT3 signaling pathways, which are key in the progression of the disease [16]. Its involvement in periodontitis, a condition marked by persistent inflammation and tissue destruction, suggests that IL-39 might contribute to the disease's progression and could offer novel insights into potential therapeutic approaches [16-18].

Osteopontin (OPN), a multifunctional glycoprotein, plays a significant role in the immune system, functioning in adaptive and innate reactions of immunity as a cytokine and an adhesion molecule [19, 20]. OPN is secreted by immune cells like dendritic cells, T lymphocytes, and macrophages. It is highly concentrated at sites of tissue injury, particularly where osteoclasts attach to bone surfaces, indicating its role in bone metabolism and immune responses. It is also considered a soluble tumor necrosis factor receptor-like factor that inhibits osteoclast differentiation [21,23]. It is involved in regulating phagocyte retention at sites of injury, facilitating tissue repair, and modulating the inflammatory response. OPN also acts as a potent chemoattractant, directing the migration of macrophages to inflammatory sites, and enhances the production of other inflammatory cytokines like IL-12, which further stimulates the immune response [11]. Research has shown that OPN levels in gingival crevicular fluid increase in correlation with the severity of periodontal disease, and these levels decrease significantly following non-surgical periodontal treatment [24]. In the context of periodontitis, elevated levels of OPN could reflect ongoing tissue destruction and inflammatory activity, making it a valuable biomarker for assessing disease severity and progression [25].

Saliva provides a unique vantage point for observing systemic inflammation due to its dynamic composition, which reflects variations in the body's inflammatory state [26, 27]. The non-invasive, painless, and cost-effective nature of saliva collection makes it an attractive alternative to other bodily fluids, especially during health crises like a pandemic [28]. Despite the known roles of IL-39 and OPN in inflammation, their correlation with periodontitis has not been thoroughly investigated. Specifically, there is a scarcity of studies examining these biomarkers in periodontitis patients within the Iraqi population. This study aims to address this gap by exploring the association between salivary and serum IL-39 and OPN levels in periodontitis patients in Iraq. Understanding their role could provide new insights into the pathogenesis of periodontitis and enhance our ability to monitor and manage this chronic inflammatory disease effectively.

Materials and Methods

Study design and sample size

The study employed an observational case-control design to investigate the association between salivary and serum IL-39 and OPN levels in periodontitis patients. A total of seventy-eight individuals were included, comprising 40 periodontitis patients who visited the Department of Periodontics at the College of

Dentistry at the University of Baghdad, for the first time without prior clinical intervention, and 38 healthy control subjects. This sample size was chosen to provide adequate power for detecting significant differences in biomarker levels between the groups while balancing practical constraints. The sample size aligns with similar studies in periodontology and allows for preliminary insights into the role of IL-39 and OPN in periodontitis [29]. The sample size of 78 participants (40 cases, 38 controls) corresponds to detecting a moderate effect size (~0.6–0.65) with $\alpha = 0.05$ and 80% power. Whether it is based on proportions (e.g., 60% vs. 30%) or means (moderate standardized difference), the math justifies ~39 per group. The study was conducted between December 2023 and March 2024. Approval was granted by the ethical committee of the College of Dentistry, Baghdad University (Approval No. 868723, Date: 3-12-2023). Every participant in the study was completely educated about the aim of our research, and a consent form was obtained from them.

Inclusion Criteria

Healthy Periodontium: Participants with BOP <10%, probing pocket depth (PPD) $\leq$ 3 mm, and an intact periodontium (no probing attachment loss) were included as controls. These criteria ensured the selection of individuals with a healthy periodontium to serve as a baseline comparison for patients with periodontitis.

Periodontitis Cases: Participants with detectable clinical attachment loss (CAL) of $\geq$2 non-adjacent teeth or CAL $\geq$3 mm on buccal or lingual/palatal surfaces with pocketing >3 mm at $\geq$2 teeth were included. These criteria were chosen based on established diagnostic guidelines for periodontitis to ensure that the study population had a clear and clinically relevant diagnosis of periodontitis.

Exclusion criteria for periodontitis patients

Recent Periodontal Treatment or Antibiotics, autoimmune Disorders or Systemic Inflammation, Chronic Systemic Diseases, smoking, pregnancy, xerostomia, presence of Malignancy, oral Lesions Unrelated to Periodontitis.

Diagnosis of periodontitis

Periodontitis was clinically diagnosed and evaluated by a periodontal specialist following the World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions (2017) criteria. This approach was chosen to standardize the classification and ensure that the study's periodontitis cases met internationally accepted diagnostic criteria. periodontitis was assessed using two main clinical parameters: probing pocket depth (PPD) and clinical attachment level (CAL). Both were measured at six sites per tooth with a periodontal probe. CAL was measured from the cementoenamel junction (CEJ) to the base of the pocket, and PPD from the gum margin to the pocket base. Periodontitis was diagnosed when CAL $\geq$3 mm was present at two or more non-adjacent teeth, following the 2017 World Workshop criteria.

Laboratory measurements

Saliva sample collection: 3 ml of whole saliva samples in unstimulated condition were collected from participants between 9-11 am, using the spitting method. Participants were instructed to avoid eating and

drinking for one hour before saliva collection. Saliva was collected into a labeled, graduated plastic tube for 10 minutes. The saliva samples were processed immediately using a centrifuge at 3000 rpm for 10 minutes. The clear supernatant was separated using a micropipette and transferred into Eppendorf tubes. Samples were then stored at -20 °C to preserve the integrity of the biomarkers until biochemical analysis. This method ensures that saliva samples are collected and processed in a controlled environment, minimizing potential contamination or degradation of biomarkers.

Blood sample collection: Approximately 3ml of venous blood was drawn from the antecubital vein using disposable syringes and 21-gauge stainless steel needles. The blood was collected into sterile gel separation tubes and allowed to clot briefly before centrifugation at 3000 rpm for 10 minutes. The serum was then transferred to Eppendorf tubes and stored at -20 °C.

Measurement of Il-39 and OPN levels in Saliva and Serum

IL-39 and OPN levels in both saliva and serum were measured using sandwich ELISA (Enzyme-Linked Immunosorbent Assay) kits. The Human IL-39 ELISA kit (catalog NO YLA3996HU) and Human OPN ELISA kit (catalog NO YLA0342HU) from Shanghai, China, were employed.

Standard solutions were prepared from the original concentration provided in the kit, creating a series of dilutions (48 pg/mL, 24 pg/mL, 12 pg/mL, 6 pg/mL, and 3 pg/mL) to generate a calibration curve. The assay involved multiple steps to ensure precision: the necessary number of microplate strips was determined based on the number of samples and standards, with each tested in triplicate. Samples were then added to designated wells, along with IL-39 antibodies and streptavidin-HRP. After gently shaking to mix, the plate was sealed and incubated at 37°C for 60 minutes.

Post-incubation, the plate was washed thoroughly with a prepared washing solution, repeating the process five times to eliminate any unbound substances. Following this, chromogen solutions A and B were added, and the plate was incubated at 37°C for 10 minutes to develop the color. The enzymatic reaction was stopped by adding a stop solution, which caused an immediate color change from blue to yellow. The optical density (OD) of each well was measured at 450 nm using a microplate reader within 10 minutes of adding the stop solution to ensure timely and accurate results.

Data analysis involved plotting the OD values against the known concentrations of the standards to create a standard curve. This curve, along with linear regression analysis, allowed for precise calculation of the biomarker concentrations in the samples. The use of these analytical techniques further enhanced the accuracy and reproducibility of the results.

The choice of ELISA for this study was due to its high specificity and sensitivity, making it a widely accepted method for detecting low levels of biomarkers such as IL-39 and OPN in biological samples. The technique's reliability and ability to provide quantitative results make it a gold standard in biomarker research.

Statistical analysis

We conducted the statistical analysis using Version 26 of the SPSS statistical program (SPSS, IBM), and Microsoft Office Excel 2010 to create the figures, except for the receiver operating characteristic (ROC)

curve. The normality of continuous data was estimated using Shapiro–Wilk, and Kolmogorov–Smirnov. Normally distributed data was presented as mean ± standard deviation (SD). For categorical data, we used frequency and percentage for descriptive analysis. We conducted a student's t-test to compare quantitative variables between the studied groups (Age [years], IL – 39 saliva, IL – 39 serum, OPN saliva and OPN serum). We used the Pearson chi-square test ($\chi 2$) to compare qualitative variables between the studied groups, such as Age groups (years) and Sex. The Pearson correlation test was used to identify the correlation between immunological assays. The ELISA test's diagnostic accuracy was assessed using an ROC curve, a cut-off value, and various metrics, including the area under the curve (AUC), sensitivity, positive predictive value (PPV), specificity, and negative predictive value (NPV). The statistical significance threshold (P-value) was set at $P<0.05$ (statistically significant) and $P<0.01$ (highly statistically significant).

Results

In this study, we reviewed the results for patients afflicted with periodontitis and healthy controls as follows: The periodontitis group included 40 patients aged between 24 and 60 years. Additionally, healthy individuals (n=38) served as control subjects, with ages ranging from 21 to 56 years. Specifically, 55% of the periodontitis patients were aged between 41 and 60 years, while 78.9% of the control group were younger, within the 20 to 40 years age range. This difference was statistically significant ($p = 0.001$). Additionally, the mean age of the periodontitis group was significantly higher than that of the control group (42.81 vs. 32.89 years, $p = 0.009$).

Gender differences were also notable, with the control group having a higher percentage of males (73.7%) compared to the periodontitis group (65%). Conversely, the periodontitis group had a higher percentage of females (35% vs. 26.3% in the control group), and this gender distribution difference was statistically significant ($p = 0.003$) (Table 1).

Table 1: Association between demographic variables and studied groups

Demographics			Studied groups		P - Value
			Healthy Control (N = 38)	Periodontitis (N = 40)	
Age Groups / Year	20 - 40	N	30	18	0.001
		%	78.9%	45%	
	41 - 60	N	8	22	
		%	21.1%	55%	
Gender	Male	N	28	26	0.003
		%	73.7%	65%	
	Female	N	10	14	
		%	26.3%	35%	
Mean			32.89	42.81	0.009
Std. Deviation			10.708	12.271	
Std. Error			2.457	2.744	

P<0.01 is a highly significant difference.

The mean ± SD of IL-39 levels in saliva was statistically significantly higher in periodontitis patients (11.054 ± 1.313) compared to healthy controls (4.209 ± 1.009), with a p-value of 0.007. Similarly, the mean IL-39

serum level was statistically significantly higher in periodontitis patients (29.847 ± 7.711) compared to controls (7.582 ± 1.301), with a p-value of 0.003.

Additionally, the mean OPN saliva level was statistically significantly higher in periodontitis patients (16.851 ± 1.708) compared to the control group (6.713 ± 1.387), with a highly significant difference (p = 0.002). Finally, the mean OPN serum level was statistically significantly higher in periodontitis patients (17.785 ± 1.398) than in controls (9.697 ± 2.137), with a p-value of 0.005 (Table 2).

Table 2: Comparison of Mean distributions of Assays between studied groups

Assays	Groups (N)	Mean	Std. Deviation	Std. Error	p – Value
IL39 saliva	**Healthy control (38)**	4.209±	1.009	0.231	0.007
	Periodontitis (40)	11.054±	1.313	0.294	
IL39 serum	**Healthy control (38)**	7.582±	1.301	0.298	0.003
	Periodontitis (40)	29.847±	7.711	1.724	
OPN saliva	**Healthy control (38)**	6.713±	1.387	0.318	0.002
	Periodontitis (40)	16.851±	1.708	0.382	
OPN serum	**Healthy control (38)**	9.697±	2.137	0.491	0.005
	Periodontitis (40)	17.785±	1.398	0.313	

P<0.01 highly significant difference by using (t-test)

The correlations between age and immunological assays in periodontitis patients revealed significant findings. There was a significant inverse (negative) correlation between age and IL-39 serum levels (r = -0.47, P = 0.038), OPN saliva levels (r = -0.53, P = 0.016), and OPN serum levels (r = -0.55, P = 0.012). However, no significant correlation was observed between age and IL-39 saliva levels.

In terms of the relationships between the immunological assays, there was a statistically significant positive correlation between IL-39 saliva and IL-39 serum levels (r = 0.469, P = 0.037). No significant correlation was found between IL-39 saliva and OPN saliva or serum levels. Additionally, a statistically significant positive correlation was noted between IL-39 serum levels and both OPN saliva levels (r = 0.663, P = 0.001) and OPN serum levels (r = 0.559, P = 0.011). Finally, there was a statistically significant positive correlation between OPN saliva and OPN serum levels (r = 0.627, P = 0.003) (Table 3).

Table 3: Correlation between the immunological assays and the studied groups- Pearson correlation analysis.

		Pathological Periodontitis			
Pearson Correlation		**Age Year**	**IL39 Saliva**	**IL39 serum**	**OPN saliva**
IL39 saliva	**r**	-0.121			
	p-value	0.612			
IL39 serum	**r**	-0.47	0.469		
	p-value	0.038	0.037		
OPN saliva	**r**	-0.53	0.216	0.663	
	p-value	0.016	0.361	0.001	
OPN serum	**r**	-0.55	-0.03	0.559	0.627
	p-value	0.012	0.891	0.011	0.003

p>0.05 = non-significant difference

The diagnostic test accuracy analysis revealed that the immunological assays, including IL-39 saliva, IL-39 serum, OPN saliva, and OPN serum, are highly effective in diagnosing and monitoring periodontitis. These assays demonstrated exceptional sensitivity and NPV, both reaching 100%, indicating a strong ability to correctly identify true positives and true negatives. The specificity and PPV values, although slightly lower, were still notably high, particularly with OPN serum, which achieved 100% across all metrics. The accuracy of these assays ranged from 94.87% to 100%, further supporting their reliability. The area under the curve (AUC) values, close to or at 1.0, underscore the assays' excellent diagnostic performance. These results suggest that IL-39 and OPN levels in saliva and serum can serve as robust biomarkers for the diagnosis and follow-up of periodontitis, with statistically significant differences observed ($p < 0.01$) (Table 4, and figure 1)

Table 4: Validity tests of IL 39 saliva, IL-39 serum, OPN saliva and OPN serum were performed using the ROC test in the sera of periodontitis and control.

Validity tests	IL-39 saliva	IL-39 serum	OPN saliva	OPN serum
Sensitivity	100%	100%	100%	100%
Specificity	94.7%	89.5%	89.5%	100%
Positive predictive value (PPV)	95.2%	90.9%	90.9%	100%
Negative predictive value (NPV)	100%	100%	100%	100%
Accuracy	97.43%	94.87%	94.87%	100%
Area under curve	0.974	0.947	0.947	1
Cut off value	Up to 6.1$_{pg/ml}$	Up to 9.95$_{pg/ml}$	Up to 9.23$_{pg/ml}$	Up to 13.1$_{pg/ml}$
p- value	p = 0.008	p = 0.002	p = 0.002	p = 0.001

p<0.01 = Highly significant difference.

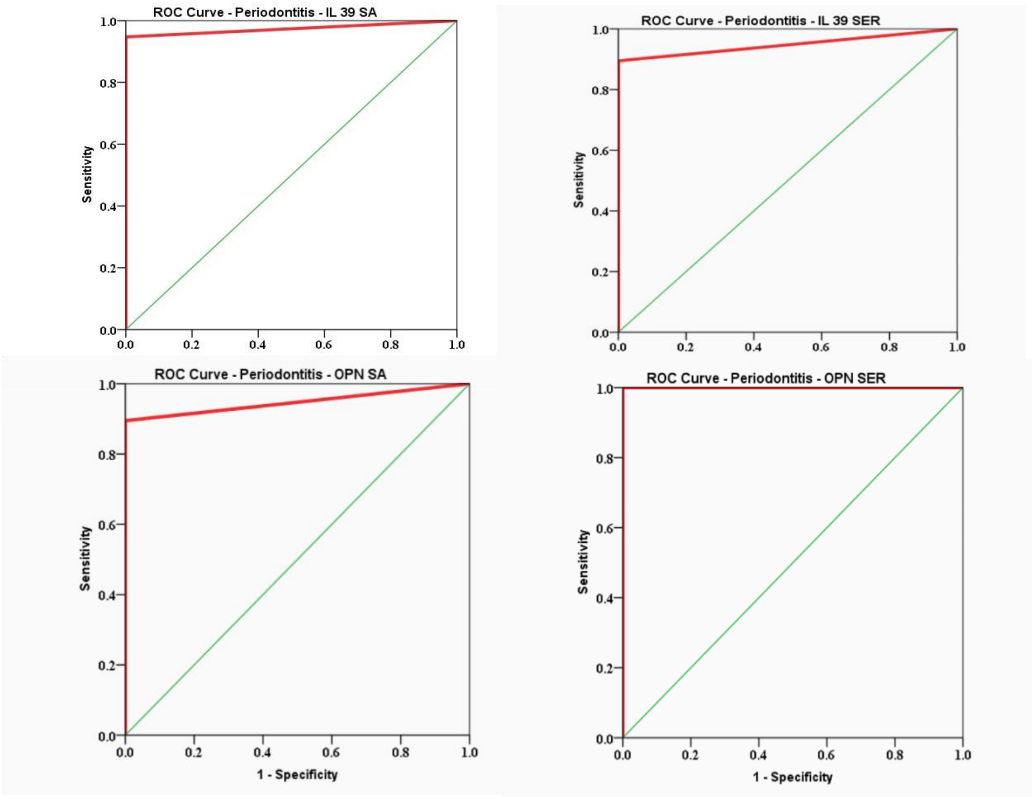

Figure 1: Validity tests of IL 39 SA, IL39 SER OPN SA & OPN SER by using ROC test in sera of Periodontitis and control.

Discussion

In this study, we compared patients with periodontitis to healthy controls, revealing significant differences in age, gender distribution, and biomarker levels. Results highlight periodontitis patients were older on average (42.81 years) compared to controls (32.89 years) and had higher levels of IL-39 and OPN in both saliva and serum. Specifically, IL-39 and OPN levels were markedly elevated in periodontitis patients, with significant differences between the two groups ($p < 0.01$). Age was negatively correlated with certain biomarker levels, indicating that older patients might show different biomarker profiles. The study also found strong positive correlations between various biomarkers, suggesting a linked response to periodontitis.

Similar to our findings another case-control study by Sari et al. reported levels of IL-1β, periostin, and IL-39 in gingival crevicular fluid were significantly elevated in individuals with periodontitis and gingivitis compared to those with healthy periodontal conditions ($p < 0.001$). Positive correlations were observed between these biochemical markers in gingival crevicular fluid and clinical periodontal parameters ($p < 0.05$). Multivariate generalized linear regression analysis indicated that IL-39 levels in gingival crevicular fluid were significantly associated with periodontitis ($\beta = 37.6$, 95% CI = 22.9–52.4) and gingivitis ($\beta = 28.4$, 95% CI = 15.8–41) ($p < 0.001$). Elevated IL-39 levels corresponded with increases in IL-1β and periostin levels in the presence of periodontal disease, suggesting that IL-39 may play a role in periodontal inflammation. As a novel cytokine in the IL-12 family, IL-39 could serve as a potential biomarker for predicting periodontal diseases [29]. Another Egyptian case-control study revealed that IL-39 levels were highest in patients with diabetic periodontitis but decreased significantly after surgery. IL-39 is a proinflammatory marker that plays a role in the development and advancement of periodontitis [30]. Additionally findings from another pilot study from Iraq demonstrated that in both periodontitis groups, clinical parameters showed comparable deterioration, accompanied by a significant increase in OPN expression compared to controls. Additionally, OPN levels in gingival crevicular fluid were sufficiently sensitive and specific to differentiate between healthy individuals and those with periodontitis, even in the presence of type 2 diabetes mellitus. This suggests that osteopontin could serve as a valuable diagnostic biomarker for periodontal disease [31-33]. Although both these studies reported elevated IL-39 and osteopontin levels in patients with periodontitis, it should be noted that their cohort consisted of individuals with diabetes, which differs from our study's population. Moreover, findings from periodontal studies suggest that OPN concentrations in gingival crevicular fluid increase with disease progression. Furthermore, nonsurgical periodontal treatment significantly decreases OPN levels in gingival crevicular fluid. While further long-term prospective studies are required, current evidence indicates that OPN may be a promising salivary biomarker for monitoring periodontal disease progression [34]. Another comparative study highlighted that in individuals with generalized chronic periodontitis, the average plasma OPN level was significantly elevated at 153.08 ng/ml compared to 55.09 ng/ml in those with healthy periodontium. Following treatment for generalized chronic periodontitis, the plasma OPN level reduced to 91.53 ng/ml [35]. Another case-control study by Dong et al. indicated that OPN is crucial in the progression of periapical periodontitis and exhibits a dual role in bone metabolism during this condition [36]. In a study conducted by Sharma and Pradeep involving 30 subjects divided into healthy, gingivitis, and chronic periodontitis groups, the researchers found that OPN levels were highest in periodontitis patients, with mean levels of 1575.01 ng/mL in gingival crevicular fluid and 1273.21 ng/mL in plasma. This study used enzyme immunoassays to quantify osteopontin, similar to our methodology but focused on a smaller sample size [37]. Our study's findings

further validate these results, demonstrating comparable osteopontin elevations in periodontitis, highlighting its potential as a biomarker for periodontal destruction. Furthermore, our study demonstrated a significant increase in IL-39 and osteopontin levels in periodontitis patients, which parallels the findings of elevated antimicrobial peptides such as cathelicidins and beta defensins-1 in periodontitis cases which was studied by Al-Daragi et al. in an iraqi case control study which was done in 2024 on 50 patients with periodontitis [4].

It is important to highlight that, in reviewing existing literature, we found that most studies evaluating IL-39 and OPN focused on their levels in gingival crevicular fluid, with only one study assessing these biomarkers in plasma. In contrast, our study uniquely measured these biomarkers in both saliva and serum. Our analysis demonstrates that these biomarkers exhibit high diagnostic accuracy, with exceptional sensitivity, specificity, and overall accuracy, underscoring their potential as reliable tools for diagnosing and monitoring periodontitis. This approach is novel, as it encompasses the assessment of all three biomarkers IL-39, OPN, and their interactions in our study population. Most existing research examines these markers individually or in limited combinations, making our study's comprehensive evaluation a significant contribution to the field.

While emphasizing more in this context, a key advantage of using oral fluids for periodontal diagnostics is the ability to identify individuals at high risk of developing disease before it becomes clinically apparent. Early detection and diagnosis can greatly enhance the management of periodontal conditions by enabling earlier intervention with less invasive and more cost-effective treatment options [38]. Salivary biomarkers hold significant potential for differentiating between healthy individuals and those with periodontal disease due to notable differences in biomarker levels observed in saliva samples from these groups. Evaluating disease severity is crucial for clinical decision-making and treatment planning, as these biomarkers reflect ongoing inflammation and tissue damage in the oral cavity. Increased biomarker levels correlate with greater disease severity, highlighting their role in complementing traditional clinical evaluations. By integrating salivary biomarkers with conventional clinical assessments, clinicians gain a more objective and quantifiable perspective on periodontal health, which enhances diagnostic accuracy and allows for more personalized and effective treatment strategies [39].

Protein-based salivary profiles have been extensively utilized to detect physical conditions and identify factors contributing to oral disorders, though their application in evaluating the prognosis or treatment of oral diseases remains limited. Recent research indicates that measuring levels of IL-1β, TNF-α, and other inflammatory cytokines can effectively track the progression, treatment efficacy, and prognosis of periodontal disease. Clinical findings also suggest that inflammatory biomarkers can provide insights into therapeutic outcomes and reflect the current state of periodontal disease. Despite this, research specifically investigating the potential of inflammatory biomarkers to predict treatment success for periodontal disease is still scarce [40].

This study boasts several strengths. It uniquely assesses both salivary and serum levels of IL-39 and OPN, offering a comprehensive analysis of these biomarkers in different biological fluids. By comparing periodontitis patients with healthy controls, the study effectively identifies biomarkers associated with periodontal disease, demonstrating high sensitivity, specificity, and overall diagnostic accuracy. This rigorous approach not only enhances the reliability of the findings but also fills a significant research gap in the

region, making it the first of its kind in the region to the best of our knowledge. The study's novel insights into these biomarkers' roles in periodontitis have the potential to impact clinical practices by providing new tools for accurate diagnosis and monitoring, thereby improving patient management and treatment outcomes. Additionally, the determination of IL-39 represents a novel advancement in the field of dentistry and periodontics, as it has emerged relatively recently and is not yet widely studied. IL-39, a proinflammatory cytokine, offers a new perspective on the inflammatory processes underlying periodontitis, providing potential insights into disease mechanisms and progression. The scarcity of research on IL-39 in this context highlights the importance of our study, which is among the few to evaluate OPN and IL-39. By investigating these biomarkers, our study contributes to a deeper understanding of their roles in periodontitis and underscores the potential of IL-39 as a valuable diagnostic and prognostic tool in periodontal disease management.

Limitations and future research directions

This study has several limitations to consider also. Firstly, the relatively small sample size may limit the generalizability of the results and reduce the statistical power to identify significant associations. Additionally, the study's comparisons with existing literature are limited, which hinders the ability to contextualize the findings within the broader research landscape and assess how they align or contrast with previous studies. These limitations could impact the robustness and applicability of the study's conclusions. Despite its limitations, this study is important to publish due to its contribution to understanding the roles of IL-39 and OPN in periodontitis. By exploring these biomarkers, the study provides initial insights that could guide future research and clinical practice. The clinical implications are significant as identifying reliable biomarkers for periodontitis could enhance early diagnosis, monitor disease progression, and evaluate treatment efficacy. Additionally, these findings may pave the way for more extensive studies with larger sample sizes and detailed comparisons, ultimately improving patient management and therapeutic strategies in periodontology.

Future research should focus on expanding the study's scope to address several key areas. Increasing the sample size and diversity will help validate the associations between IL-39, OPN, and periodontitis, making the findings more generalizable. Longitudinal studies are needed to observe and analyze changes in these biomarkers over time, which will improve understanding of disease progression and treatment effectiveness. Additionally, comparing these results with existing literature can provide deeper insights and help identify patterns or inconsistencies. Investigating the biological mechanisms behind the roles of IL-39 and OPN in periodontitis could reveal new therapeutic targets. Furthermore, studies should explore how these biomarkers can be used in clinical settings for early diagnosis and monitoring, ultimately enhancing patient management and treatment strategies for periodontitis.

Conclusion

This study significantly advances the understanding of periodontitis by evaluating the roles of salivary and serum IL-39 and OPN as biomarkers. The comprehensive analysis demonstrated that both biomarkers are elevated in periodontitis patients compared to healthy controls, with high sensitivity and specificity in distinguishing between the two groups. The novel approach of measuring these biomarkers in both saliva and serum enhances diagnostic accuracy and provides valuable insights into their potential as tools for diagnosing and monitoring periodontal disease. As the first study of its kind in the region to

the best of our knowledge, it fills an important research gap and sets the platform for future investigations into the application of IL-39 and OPN in periodontal diagnostics and treatment. The findings underscore the potential of these biomarkers to improve clinical management and patient outcomes in periodontitis.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

S. J. Faisal: Contributed to the conception of the study, the design of the study, the analysis of the data, interpretation, drafting of the manuscript, and critical revision. B. AL-Drobie and Anas Salami: Supervisor and contributed to the study's conception and design, data analysis, as well as interpretation, drafting, and critical revision of the manuscript.

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Informed consent

Informed consent was obtained from all individuals, or their guardians included in this study.

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رابط جديد بين إنترلوكين-39 وأوستيوبونتين في اللعاب والمصل ومرض اللثة

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المستخلص:

خلفية: تلعب السيتوكينات دورًا حاسمًا في المساهمة في تطوير الالتهاب المزمن والاستجابة الالتهابية. لذلك، تلعب الإنترلوكينات دورًا مهمًا في التسبب في التهاب اللثة. بعض الإنترلوكينات، مثل (IL-39)، لها أدوار غير معروفة في التسبب في التهاب اللثة، حيث تعزز تنشيط الخلايا المناعية والاستجابات الالتهابية. في السنوات الأخيرة، تم إثبات أن أوستيوبونتين هو سيتوكين متعدد الوظائف وجزيء لاصق للخلايا، وهو منظم فريد للاستجابات المناعية الفطرية والتكيفية. لذلك، كان الهدف من الدراسة هو قياس مستويات (IL-39) وأوستيوبونتين ونسبتهما في اللعاب والمصل لدى مرضى التهاب اللثة في العراق.

المواد والطرق: شملت هذه الدراسة ثمانية وسبعين فردًا من كلا الجنسين تتراوح أعمارهم بين (25-60) سنة (بما في ذلك 40 مريضًا بالتهاب اللثة لم يخضعوا لأي تدخل سريري و38 متطوعًا صحيًا) في بحثنا. تم تحديد مستويات الإنترلوكين-39 وأوستيوبونتين في اللعاب والمصل لكل مشارك، بما في ذلك مرضى التهاب اللثة ومجموعة التحكم، باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA). تم استخدام حزمة الإحصاءات للعلوم الاجتماعية الإصدار (IBM SPSS 26) للتحليل الإحصائي.

النتائج: أظهرت مستويات المصل من IL-39 وأوستيوبونتين ارتفاعًا كبيرًا لدى مرضى التهاب اللثة مقارنةً بالمشاركين الأصحاء $p < 0.001$ و $p = 0.001$ على التوالي. وتم ملاحظة نمط مماثل في اللعاب، حيث كانت مستويات IL-39 و OPNأعلى بشكل كبير لدى مرضى التهاب اللثة. بالإضافة إلى ذلك، تم الكشف عن ارتباطات إيجابية بين مستويات المصل واللعاب لـ-IL 39 ($r = 0.469$، $p = 0.037$) و OPN($r = 0.627$ ، $P=0.003$).كما أظهرت اختبارات الصلاحية التشخيصية أن فحوصات IL-39 و OPNأظهرت حساسية عالية وقيمة تنبؤية سلبية (100%)، ودقة عالية (94.87% إلى 100%). كانت قيم منطقة تحت المنحنى قريبة من 1.0، مما يشير إلى أداء تشخيصي ممتاز. تشير هذه النتائج إلى أن المستويات المرتفعة من-IL 39و OPN في المصل واللعاب هي علامات حيوية موثوقة لتشخيص ومراقبة التهاب اللثة، مع دقة تشخيصية قوية وفروقات إحصائية هامة.

الخلاصة: ترتبط المستويات المرتفعة من IL-39 وأوستيوبونتين في اللعاب والمصل بشكل قوي مع التهاب اللثة.