ASSESSMENT OF SALIVARY NEUTROPHIL ELASTASE LEVELS IN WOMEN WITH PCOS AND PERIODONTITIS

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ABSTRACT

Background: One well-known hormonal disease, polycystic ovarian syndrome (PCOS), is linked to an elevated risk of periodontal inflammation. Whether or not (PCOS) is present, periodontitis, a chronic inflammatory illness of the gums.

Aim of study: Comparing salivary levels of neutrophil elastatse (NE) in healthy controls and patients with polycystic ovarian syndrome who have periodontitis is the primary goal of this investigation.

Methodology: From January to July, 2022, the study was conducted. There were 85 participants in the current study, ranging in age from (20-40) years. The participants in this study were divided into four groups: those without periodontitis (n=10), those with periodontitis and (PCOS) (n=25), and those with both conditions (n=25).

Results: Periodontitis group had the highest median plaque index (PI), followed by mixed group, PCOS group, and finally groupings ($p < 0.001$). Compared to the periodontitis and combination groups, the control and PCOS groups exhibited considerably decreased bleeding on probing median level ($p<0.001$). However, compared to the periodontitis and mixed groups, the PCOS group had a much lower level ($p<0.001$). In contrast to combined group, the periodontitis group had a considerably lower median level of PPD ($p<0.001$). The median levels of NE were found to be dropping in the following order: periodontitis, control, mixed, and PCOS groups, according to Intra-group comparison.

Conclusion: Those with polycystic ovary syndrome (PCOS) are more likely to experience periodontal inflammation and tissue damage than those without PCOS. Reducing neutrophil elastase production is one mechanism by which anti-inflammatory drugs may alleviate periodontal inflammation.

Keywords: *Neutrophil elasatase, polycystic ovarian syndrome, periodontit*

INTRODUCTION

According to Nazir (2017) and Gasner and Schure (2022), Periodontal disease is the most prevalent dental issue impacting individuals as much as 90% of the global population [1],[2]. Periodontal diseases encompass conditions affecting the gums, alveolar bone, cementum, and periodontal ligament, are known as periodontal diseases[3]. Inflammatory biomarkers such as C-reactive protein (CRP) and other neutrophil and macrophage molecules, including tumor necrosis factor-alpha (TNF-α), matrix metalloproteinases (MMPs), and interleukins (IL-1 and IL-8), constitute this response[4],[5].

Hyperandrogenism, inability to ovulate, and polycystic ovarian morphologic characteristics define polycystic ovary syndrome. Between 6 and 10% of reproductive-age women have "classic" polycystic ovary syndrome according to NIH diagnostic criteria (i.e., hyperandrogenism in conjunction with ovulatory failure)[6],[7],[8],[9],[10], however. The number of cases may be twice as elevated when employing the more broad Maastricht criterion.

Periodontal diseases, including gingivitis and CP, are positively associated with polycystic ovary syndrome (PCOS), according to one meta-analysis. When comparing PCOS women to controls, periodontal exams showed much greater levels of PD, BOP, and PI. Finding abnormal CAL also helped in diagnosing CP. Patients with polycystic ovary syndrome may have been more vulnerable to periodontal disease due to their abnormal periodontal response and associated proinflammatory condition[11],[12]. Periodontitis can be confirmed by ruling out gingivitis with pseudopockets (false pockets), which can affect the

measurement of PD, PI, and BOP. All three of these variables —PD, BOP, and PI—are indicators of peridontal membrane destruction. Since CAL measures insertion loss, which stands for the irreversibility of the procedure, it has been included in periodontal evaluations by a number of writers. These findings suggest that periodontitis and other periodontal illnesses, gingivitis, are more common in PCOS patients compared to those without the condition[13].

An rise in range of serum hsCRP values noted in women with PCOS and periodontal conditions, suggesting a possible periodontally mediated synergistic etiologic component[14]. According to MPO and neutrophil elastase, two additional mediators that are linked to these reactions[15]. Neutrophils are crucial in the first host inflammatory response that targets periodontal bacteria. These mediators amplify and promote inflammation both locally and systemically, as well as the activation of matrix metalloproteinases (MMPs), bond loss, and bone resorption[16]. In addition, oxidative stress is worse in PCOS patients who have chronic periodontitis because their serum and saliva oxidative stress indicators are higher and their total serum antioxidant status levels are lower[17].

METHODOLOGY

Sample collection

The study was carried out during the period from January 2022 to July 2022. The study participants' salivary samples were taken between 9:00 am and 1:00 pm. The present study included a total of 85 subjects with an age range of (20-40) years. The current study included a control group (n = 10), patients suffering from periodontitis (n = 25), patients suffering from polycystic ovary syndrome (PCOS) (n=25) and patients suffering from combined pathologies periodontitis and PCOS (n=25). Those patients were visiting the dental center at Adiwniyah teaching hospital during the p^2eriod of the study. Before any oral examination took place, they were gathered. The entire saliva was collected using the passive saliva drooling method. Each participant was given a plastic cup, and instructed to let saliva flow into the cup for five minutes in a quiet, isolated setting without stimulation or spitting. From the examination were the wisdom teeth.

Clinical biomarker assessment
Full mouth PI, full mouth BOP, PPD, and CAL were the clinical parameters that were measured for the entire dentition that was already present. The ELISA technique was employed to quantify neutrophil elastase. All samples will be allocated in saliva sample collection tubes containing lyophilized protease inhibitor solution.

Assessment is summarized in following steps:

1. Human sample: Total number 85 subjects with an age range of 20 to 40 years selected according to the inclusion criteria of the study 2. Data were obtained from each patient according to specialized case sheet3. Salivary sample was obtained from each participant 4. Periodontal examination was performed to report PI, BOP, CAL, and PPD 5. Subjects were accordingly divided into 4 groups:
• Control group (n = 10) •
• PCOS group (n = 25) •
Periodontitis group (n = 25)
• PCOS and Periodontitis group (n = 25)
6. Biochemical test was performed to estimate salivary NE.

Statistical

With the help of SPSS version 23 and Microsoft Office Excel 2010, we were able to gather, summarize, analyze, and display the data. For quantitative (numerical) variables, the first step was to check their normality distribution using the Kolmogorov-Smirnov test. If they weren't normally distributed, the data were presented as midpoint and range between the two extremes, which represent the distribution and mean, correspondingly. Statistical tests that were utilized were: Mann When the numeric variables were not normally distributed, we used the Whitney U test to find the difference in median between any two groups. When the variables were normally distributed, we used the Kruskal Wallis test to compare the groups. After that, we used pos hoc Dunns' test to find the difference in mean values between the groups that were mainly tested with the Kruskal Wallis test. Finally, we used Spearman correlation to find the correlation between any two numeric variables, and we expressed the results as the correlation coefficient (r) and the level of significance (P). A p-value of 0.05 or below was determined to have a substantial impact. At a p-value of 0.01 or below, we regarded the level of significance to be strong.

RESULTS

Comparison of median age among patients and controls subjects is shown in (Table 1). The results showed that PCOS group had the lowest median age median which was not significantly different from that of control group (p=0.541), but it was markedly inferior to that of combined and periodontitis groups ($p \leq 0.001$). On the other hand, the median age in the control group was not significantly different in comparison with combined and periodontitis groups (p>0.05). In addition, median age in the combined

group was not significantly different from that of periodontitis group (*p*=1.000).

Table 1. Description and statistical test of age among patients and control subjects

Characteristic	Control (n = 10)	PCOS (n = 25)	Periodontitis (n = 25)	Combined (n = 25)	Kruskal Wallis		
		Age (years)			Test statistic	df	*p*-value
Median	28.50	23.00	35.00	31.00	31.529	3	< 0.001***
IQR	7.50	3.50	7.00	7.50			
Range	21 -36	20 -35	20 -40	24 -39			

n: amount of incidents; **IQR**: inter-quartile range; ***: significant at $p \leq 0.001$

(Table 2) displays the outcomes of comparing the patients' and control participants' median plaque index and bleeding on probing. Compared to the periodontitis and mixed groups, the control group had a considerably reduced median plaque index level (*p*<0.001). This level was not statistically different from the PCOS groups, though. Alternatively, compared to the periodontitis and mixed groups, the PCOS group had a noticeably lower level (*p*<0.05). Furthermore, there was a significant difference in levels between the combined group and the periodontitis group (*p*< 0.01). While the control group did not vary substantially from the PCOS groups in terms of bleeding on probing median level, it was markedly inferior to the periodontitis and combination groups (*p*<0.001). However, compared to the periodontitis and mixed groups, the PCOS group had a much lower level (*p*<0.001). The combined group also demonstrated a statistically significant level, but the periodontitis group did not (*p*=1.000). Shown a statistically significant degree

Table 2. Descriptive statistic tests of plaque index among patients and control subjects

Characteristic	Control (n = 10)	PCOS (n = 25)	Periodontitis (n = 25)	Combined (n = 25)	Kruskal Wallis		
		Plaque index			Test statistic	df	*p*-value
Median	11	32	100	63	61.152	3	< 0.001***
IQR	18.75	30	0	47.5			
Range	2 -40	12 -80	62 -100	24 -100			
		BOP index					
Median	5.5	6	100	100	65.368	3	< 0.001***
IQR	5.25	3.50	7.50	8.50			
Range	1 -9	2 -9	67 -100	73 -100			

n: number of cases; **IQR**: inter-quartile range; ***: significant at $p \leq 0.001$

Medians Probing pocket depth (PPD) of periodontitis group and combined group were shown in (Table 3). The median level of PPD of periodontitis group was markedly diminished in periodontitis group than that of combined group (*p*<0.001). A small variance in the median level of CAL was seen between the periodontitis and mixed groups (*p*=0.116).

Table 3. Descriptive and statistical analysis of median Probing pocket depth (PPD) between periodontitis group and combined group

Characteristic	Periodontitis (n=25)	Combined (n=25)	Mann Whitney U test	
		PPD	Test statistic	*p*-value
Median	5	6	72	< 0.001 ***
IQR	1	1.75		
Range	4 -6	5 -7		
		PPD		
Median	3.4	3	234	0.116 NS
IQR	1	1.25		
Range	2.3 -5	2 -5		

n: number of cases; **IQR**: inter-quartile range; ***: significant at $p \leq 0.001$;NS:non significant

Descriptive statistics of neutrophil elastatse (NE) among patients and healthy controls is displayed in (Table 4 and Figure 1). Intra-group comparison revealed that the median levels of NE in descending order was as following:

periodontitis then control group then combined group and lastly PCOS group. Kruskal Wallis test demonstrated high significance difference ($p<0.001$). The results showed that PCOS group had the lowest NE median level which was not significantly different from that of control and combined groups ($p>0.05$), but it was significantly lower than that of periodontitis group ($p<0.001$). The combined group, in contrast, had a considerably lower level compared to the control and periodontitis groups ($p<0.05$). Compared to the periodontitis group, the control group had a considerably greater level ($p<0.001$).

Table 4.Descriptive and statistical test of median neutrophil elastatse (NE) among patients and control subjects

Characteristic	Control (n=10)	PCOS (n=25)	Periodontitis (n=25)	Combined (n=25)	Kruskal Wallis		
					Test statistic	df	*p*-value
NE							
Median	24.32	13.72	27.57	21.30	21.143	3	< 0.001 K ***
IQR	15.24	13.92	9.58	9.83			
Range	10.36 -42.69	5.50 -38.21	16.71 -46.27	8.18 -52.95			

n: number of cases; **IQR**: inter-quartile range; ***: significant at $p\leq0.001$;K:Kruskal-Wallis test statistic
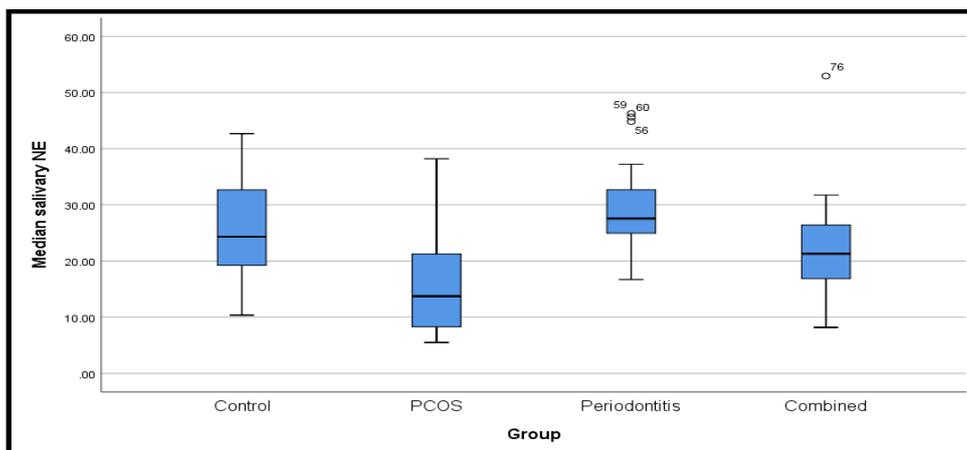

Figure 1. Box plot showing comparison of median neutrophil elastatse (NE) among patients and control subjects

The results showed that salivary neutrophil elastase (NE) did not correlate with clinical parameters in control and PCOS groups ($p>0.05$), (Table 5). In addition, there was no significant correlation between salivary neutrophil elastase (NE) and clinical parameters in periodontitis and combined groups ($p>0.05$) (Table 6).

Table 5. Correlation between salivary neutrophil elastase (NE) and clinical parameters in control and PCOS groups

Characteristic	Correlation index	Control	PCOS
Plaque index	*r*	-0.321	-0.322
	p	0.365	0.117
Bleeding on probing	*r*	-0.453	-0.234
	p	0.189	0.260

Table 6. Correlation between salivary neutrophil elastase (NE) and clinical parameters in periodontitis and combined groups

Characteristic	Correlation index	Periodontitis	Combined
Plaque index	*r*	0.126	0.117
	p	0.548	0.577
Bleeding on probing	*r*	0.000	0.234
	p	0.998	0.260
PPD	*r*	0.081	0.187
	p	0.701	0.371
CAL	*r*	0.087	-0.078
	p	0.680	0.711

DISCUSSION

The median plaque index varied significantly throughout the research groups, with individuals suffering from periodontitis, PCOS, combined periodontitis and PCOS, PCOS alone, and finally the control group showing the lowest values. Moreover, the POB index was determined to be greatest in periodontitis patients and those with PCOS and periodontitis combined, with a somewhat higher level in the PCOS group in contrast to control group with generally good health. We observed a statistically significant disparity in median PPD between the combined and periodontitis groups, but no such difference in CAL.

According to several earlier studies, oral examinations showed that PCOS individuals exhibited significantly elevated levels of PD and BOP compared to control subjects[18,19,20,21,15,22]. Simultaneously, the PI values were significantly different between the two groups, with PCOS women showing greater determinations. The CAL variable was assessed in five research, with three of them finding a higher index among PCOS subjects[21,23,17,24].

The Patients with polycystic ovary syndrome had a higher PI index compared to systemically healthy patients. Past studies have focused on the periodontal parameters in females with polycystic ovarian syndrome[21,14]. Periodontal disease indicators were more common among PCOS women as comparison to healthy controls, according to our research. Periodontal disease indices were significantly higher in female with polycystic ovary syndrome, which is in line with our findings[21].

Consistent with the known effect of Hyperandrogenism in vascular flora, patients with PCOS exhibited greater BOP rates compared to the control group[23]. An increased likelihood of inflammatory process activation is observed in PCOS patients. Periodontal disease, involvement of gingival supporting tissues, depth of the gingival sulcus, and bone loss are all affected by these inflammatory processes[23].

PCOS is positively related with periodontal disorders, specifically gingivitis and periodontitis. Periodontal exams revealed that PD, BOP, and PI were much superior in PCOS female compared to controls[13]. The presence of periodontitis was also confirmed by pathological CAL. The abnormal periodontal response in PCOS participants was linked to a proinflammatory condition, that could have rendered these patients more susceptible to periodontal disease[13].

A highest concentration of NE in saliva was detected in the periodontitis group., followed by the control group, combined group, and lowest in the PCOS group. This suggests that PCOS is linked to a lower level of NE, while periodontitis is associated with a higher level.

Research conducted in 2017 by Akcal et al. examined the levels of several enzymes in saliva and blood, including matrix metalloproteinase (MMP)-9, myeloperoxidase (MPO), neutrophil elastase (NE), and the ratio of MMP-9 to tissue inhibitor of MMP-1 (TIMP)-1. The subjects included both healthy controls and patients with polycystic ovary syndrome (PCOS), and gingivitis was either present or absent. Researchers found that female with polycystic ovary syndrome (PCOS) and gingivitis had higher levels of salivary MMP-9 and NE than PCOS in women who are periodontally healthy, and that women with PCOS and gingivitis had higher levels of serum MMP-9 and MPO than periodontally healthy PCOS women. Thus, when it comes to salivary NE, Ackal et al. (2017) found results that are in agreement with our own. Consistent with our result, a study by Nizam et al. (2014) found that periodontitis is linked with higher levels of salivary NE. This suggests that NE can activate MMP-9 at the site of inflammation.

Since Dursun et al. (2011) found a correlation between periodontal disease and polycystic ovary syndrome, only few studies have looked into the matter further[21]. The infectious nature of periodontal infections causes periodontitis, a localized inflammatory response that lasts just a short while. Inflammation of the gums can be influenced by polycystic ovary syndrome (PCOS). When a person has polycystic ovary syndrome (PCOS), systemic inflammation exacerbates this local inflammation. PCOS patients often have altered lipid profiles, which aid in developing etiology of periodontal disease by, among other things, releasing hormones that promote inflammation, including tumor necrosis factor (TNF)[25,26].

Here, Zçaka et al. (2012) discovered that healthy individuals alongside gingivitis had greater quantities of salivary TNF with reduced IL-6 levels in GCF, serum, and saliva, but PCOS patients with gingivitis had a more pronounced inflammatory response[12]. Women who have polycystic ovary syndrome are more likely to have periodontitis and have greater levels of inflammation and periodontal breakdown compared to those who do not have PCOS[12].

An elevated hsCRP level in the blood is associated with a higher probability of a heart attack[26]. Patients with polycystic ovary syndrome who also have periodontal disease tend to have higher levels of systemic hsCRP., suggesting a possible periodontally mediated synergistic etiologic factor[14,24]. Both neutrophil elastase and monocyte chemoattractant protein (MPO) are important components of the first host inflammatory response to periodontal infections and are associated with these processes[15]. These mediators promote and intensify systemic and local inflammation, matrix metalloproteinase (MMP) activation, attachment loss, and bone resorption[22,15]. Furthermore, oxidative stress is exacerbated in PCOS patients with chronic periodontitis because of the correlation between elevated levels of oxidative stress indicators in blood and saliva and decreased levels of overall condition of antioxidants[17]. Original publication year: 2014, the study investigated the correlation between PCOS, salivary microbiome, antibodies to serum levels, and inflamed gum tissue[15]. When comparing PCOS patients to controls with periodontal disease, they found significant differences in the microbiological components. Salivary P. gingivalis and F. nucleatum concentrations were higher in PCOS patients with gingivitis, and serum antibodies to P. intermedia, P. gingivalis, and S. oralis were also higher in these patients. PCOS is a systemic endocrine condition that may have a significant influence considering the make-up of the microbiota in the mouth. As a result, some members of this community may trigger an increased systemic response in people with PCOS[13,27].

CONCLUSION

Those with polycystic ovary syndrome (PCOS) are more likely to experience periodontal inflammation and tissue damage than those without PCOS. Whether a woman has polycystic ovary syndrome or not, salivary matrix neutrophil elastase is not a good indicator of periodontitis. Reducing neutrophil elastase production is one mechanism by which anti-inflammatory drugs may alleviate periodontal inflammation.

DECLARATIONS

Funding

Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent Not applicable.

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