Evaluation of salivary matrix metalloproteinase -9 in patients with polycystic ovarian syndrome and periodontitis

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Abstract

The objective of study is to evaluate the salivary levels of (MMP-9) in polycystic ovarian syndrome women with inflammation of periodontium in comparison to healthy control. The present study included a total of 85 subjects with an age range of 20 to 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). The median value of plaque index (PI) was highest in periodontitis group followed by combined group then by PCOS group and lastly by groups ($p< 0.001$). The results showed that control and PCOS groups had the lowest bleeding on probing median level, but it was significantly lower than that of periodontitis and combined groups ($p< 0.001$). On the other hand, the level in the PCOS group was significantly lower than that of periodontitis and combined groups ($p< 0.001$). The median level of PPD of periodontitis group was significantly lower in periodontitis group than that of combined group ($p< 0.001$). Intra-group comparison revealed that the median levels of MMP-9 in descending order was as following: combined group then PCOS then periodontitis and lastly control group. Kruskal Wallis test demonstrated high significance difference ($p < 0.001$). PCOS is associated with more severe periodontal inflammation and tissue destruction in comparison with non-PCOS women. Salivary matrix metalloproteinase-9 is useful in predicting periodontitis in women with or without PCOS.

Key words: matrix metalloproteinase -9, polycystic ovarian syndrome, periodontitis

Introduction

Periodontal disease affects approximately 9 tenths of people globally, rendering it the highest prevalent illness of the oral cavity (1). Periodontal disorders are conditions that affect the periodontium, which is the supportive apparatus that surrounds the tooth and comprises periodontal ligament, cementum, alveolar bone and the gingival (2). One major part of this inflammatory response is the production and secretion of a number of biomarkers and molecules such as interleukins, matrix metalloproteinases (MMPs) and acute phase reactants (3, 4, 5). The syndrome of polycystic ovary is an illness that is characterized by morphologic features of ovarian numerous cysts, dysfunction of ovaries and raised levels of androgen (6). Based on the National Institutes of Health diagnostic criteria the disease affects approximately 6 to 10 % of females within their reproductive age; however, this prevalence rate may become double if Rotterdam criteria are taken into consideration (7).

In one systematic review, the association between periodontitis and polycystic ovaries has been confirmed. In this systemic review the authors stated that periodontal examinations shared the significantly higher levels of plaque index, bleeding on probing index and pocket depth index in females having PCOS when contrasted to women who were free of the disease. Moreover, the study has shown that pathological clinical attachment loss was a prominent feature in patients with chronic periodontitis. In PCOS women, the

periodontal changes were correlated with a state of proinflammatory inflammatory response and this may suggest the existence of increased susceptibility to periodontal tissue inflammation in those women. The use of pocket depth, bleeding on probing and plaque index is an essential step in establishing diagnosis of inflammation of periodontal tissues; however, it should be kept in mind that the presence of pseudopockets and inflammation of gingival may influence the determinations of these clinical parameters. For that reason, clinical attachment loss has been introduced by a number of authors to solve such a problem, since it stands for the irreversibility of the process as it estimates loss of insertion. According to above information, it appears that females having PCOS are more liable to develop inflammation of periodontal tissues when compared to women free of PCOS (8). The rise in highly specific C-reactive protein level in a systemic manner in women with PCOS and inflammation of periodontal tissues is an evidence of a synergistic causative process that can be periodontally mediated (9).

These inflammatory reactions are accompanied by the enhancement of other mediators such as neutrophil elastase and myeloperoxidase (10); both produced by neutrophil cells, which offer an essential contribution in the beginning of host inflammatory reaction against pathogens colonising periodontal tissues. These inflammatory mediators intensify and promote resorption of bone, attachment loss, matrix metalloproteinase stimulation and systemic and local inflammatory reaction (10). In addition, chronic periodontal inflammation in women with PCOS has been correlated to a rise in level of markers of oxidative stress in saliva and serum, and a reduction in serum levels of total antioxidant status, which play a role in potentiating oxidative stress (11).

Results and Discussion

Comparison of median age among control subjects and patients is demonstrated in table 1. The results showed that PCOS group had the lowest median age median which has no significant difference from that of control group ($p = 0.541$); however, it was lower significantly than that of combined and periodontitis groups ($p \leq 0.001$). Furthermore, the median age in the control group has no significant difference in comparison with combined and periodontitis groups ($p > 0.05$). In addition, median age in the combined category has no significant difference 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*
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			

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

Comparison of median bleeding on probing and plaque index among control subjects and patients is demonstrated in table 2. The results showed that control category had the lowest plaque index median level which has no significant difference from that of PCOS groups, but it was lower significantly than that of periodontitis and combined category ($p \leq 0.001$). On the other hand, the concentration in the PCOS category was lower significantly than that of periodontitis and combined categories ($p < 0.05$). In addition, the concentration in the periodontitis cattery was higher significantly

than that of combined category ($p< 0.01$). The results showed that control group had the lowest bleeding on probing median level which has no significance difference from that of PCOS groups, but it was lower significantly than that of periodontitis and combined categories ($p< 0.001$). On the other hand, the level in the PCOS group was lower significantly than that of periodontitis and combined categories ($p< 0.001$). In addition, the level in the periodontitis gro up showed no significant difference in comparison with combined group (p=1.000).

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*
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			

IQR: inter-quartile range; ***n***: number of cases; ***: 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 lower significantly in periodontitis group than that of combined category ($p< 0.001$). There was no significant variation in medina level of CAL between periodontitis and combined 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*
Median	5	6	72	< 0.001 ***
IQR	1	1.75		
Range	4 -6	5 -7		
PPD				
Median	3.4	3	234	0.116NS
IQR	1	1.25		
Range	2.3 -5	2 -5		

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

Descriptive statistics of Matrix metalloproteinase -9 (MMP-9) among control subjects and patients and are outlined in table 4 and figure 1. Intra-group comparison revealed that the median levels of MMP-9 in

descending order was as following: combined group then PCOS then periodontitis and lastly control group. Kruskal Wallis test demonstrated high significance difference (p < 0.001). The results demonstrated that control group had the lowest MMP-9 median level which was not different significantly from that of periodontitis

group, but it was lower significantly than that of PCOS and combined groups (p< 0.05). On the other hand, the level in the periodontitis category was not different significantly from that of PCOS (p = 0.113), but it was lower significantly than that of combined category

(p< 0.001). Moreover, the level in the PCOS category showed no significant difference in comparison with combined group (p = 0.190).

Table 4: Descriptive and statistical test of median Matrix metalloproteinase -9 (MMP-9) among control subjects and patients

Characteristic	Control n = 10	PCOS n = 25	Periodontitis n = 25	Combined n = 25	Kruskal Wallis		
MMP-9					Test statistic	df	p
Median	89.55	143.68	126.26	174.40	30.784	3	<0.001 ***
IQR	84.65	47.85	57.62	70.82			
Range	11.85 -199.71	87.27 -281.90	70.98 -223.54	117.94 -316.19			

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

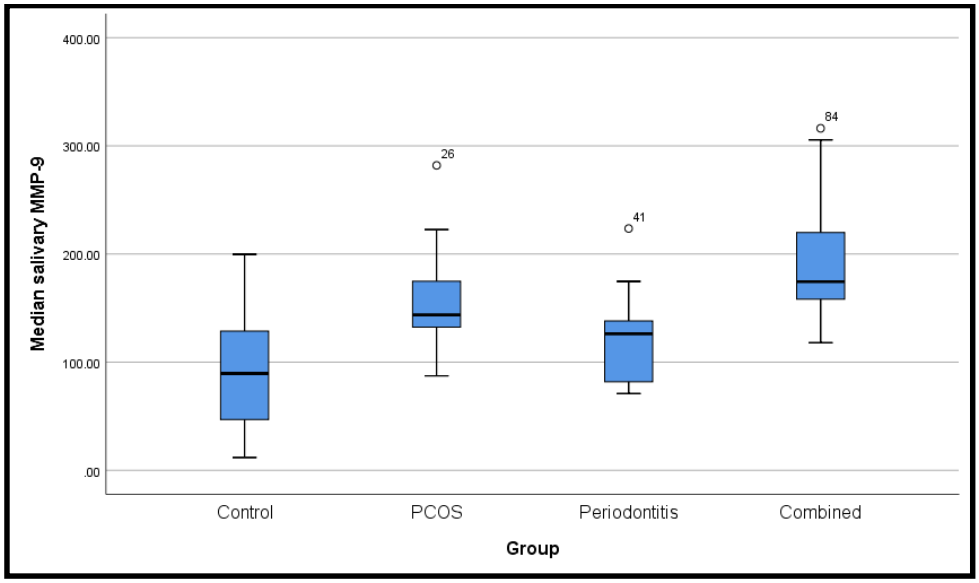

Figure 1: Comparison of median bleeding on matrix metaloproteinase-9 (MMP-9) among patients and control subjects

There no significant correlation between matrix metalloproteinase -9 (MMP-9) in saliva and clinical parameters in control and PCOS groups (p > 0.05), see table 5. Furthermore, there has been no significant correlation between salivary matrix metalloproteinase -9 (MMP-9) and clinical parameters in periodontitis and combined groups (p > 0.05), see table 6

Table 5: Correlation between salivary matrix metalloproteinase -9 (MMP-9) and clinical parameters in control and PCOS groups

Characteristic	Correlation index	Control	PCOS
Plaque index	*r*	-0.382	0.320
	p	0.276	0.119
Bleeding on probing	*r*	-0.404	0.157
	p	0.247	0.453

Table 6: Correlation between salivary matrix metalloproteinase -9 (MMP-9) and clinical parameters in periodontitis and combined groups

Characteristic	Correlation index	Periodontitis	Combined
Plaque index	*r*	-0.022	0.027
	p	0.917	0.897
Bleeding on probing	*r*	0.252	-0.190
	p	0.225	0.363
PPD	*r*	0.042	0.016
	p	0.840	0.939
CAL	*r*	0.064	-0.061
	p	0.761	0.773

In the present study, it has been shown that MMP-9 salivary level was greatest in combined group followed by PCOS category then by periodontitis category and then by control group indicating that both PCOS and periodontitis are associated with increased level of this salivary marker and that its association with PCOS is more than that with periodontitis.

An investigation of serum and salivary concentration of neutrophil elastase, myeloperoxidase and matrix metalloproteinase (MMP)-9 in systemically healthy individuals in the absence of presence of gingival inflammation and women with polycystic ovary syndrome was performed. The results of the later study pointed to that MMP-9 in saliva was greater in the systemically healthy individuals with gingival inflammation in comparison to women with PCOS with healthy periodontal tissue while MPO and MMP-9 serum concentrations were higher in females with gingival inflammation and PCOS compared with women with PCOS and healthy periodontal tissues. Therefore, our results are inconstant with respect to MMP-9 (10).

In addition, it has been shown that clinical signs of gingival inflammation correlate positively with level of MMP-9 (10) an observation that is in line with our

observation with respect to periodontitis. In another case-control study, by Maboudi *et al* (12), in which 87 women were grouped into three categories: 26 women who were healthy, 26 women with PCOS with no inflammation of gingiva and 26 females having PCOS and inflammation of gingiva. Measurement of the concentration of MMP-9 in serum was done and it was found that serum MMP-9 was higher in a significant way in patients with PCOS than in control category.

Only a few number of researches have since further investigated the connection between PCOS and periodontal illness that was first reported by Dursun et al. (13). Because periodontal infections are contagious, they cause a short-term localized inflammatory response in case of inflammation of periodontium. According to Ozçaka et al (14), PCOS can affect inflammation of gingiva by changing IL-17. This local inflammation is made worse in PCOS individuals by systemic inflammation. Affected lipid profiles, which are common in PCOS patients, among other things, cause the release of pro-inflammatory cytokines like TNF, which aids in the pathogenesis of periodontal disease (15, 16).

In this regard, Ozçaka *et al* (17) found that PCOS patients with gingivitis had a greater inflammatory response than did healthy individuals with gingival inflammation, with lower saliva, serum and gingival crevicular fluid concentrations of IL-6 and higher salivary concentrations of TNF in the earlier category. Porwal et al. found that PCOS females had greater levels of periodontium breakdown and inflammation than females free of PCOS, indicating a higher prevalence of and propensity for developing inflammation of periodontium in the earlier category (9).

It is suggested that a great concentration of highly specific CRP in the blood stands for a higher risk of ischemic heart status (18). In women with periodontal disease and PCOS, a rise in concentration of systemic highly sensitive CRP has been noticed (9, 19), suggesting the existence of a synergistic causal process which may be drive by periodontal tissues. These reactions are linked to the production of other molecules, including neutrophil elastase and MPO, which are both produced by neutrophils and play crucial roles in the early host inflammation reaction to infections of periodontal tissues (10). According to previous reports (10, 20) these mediators encourage and amplify local and systemic inflammation, MMP activation, bone resorption and attachment loss. Additionally, chronic periodontal inflammation in PCOS females has been linked to an increase in blood and salivary markers of oxidative stress as well as a drop in concentrations of total antioxidant status, all of which promote oxidative stress (11).

The relationship between gingival inflammation, serum antibody responses and salivary oral microbiota in PCOS was initially examined by previous authors (21). They noted notable variations between controls and PCOS women who had disease in periodontium in the microbiological components. Patients with PCOS who had gingivitis had increased levels of F. nucleatum and P. gingivalis in their saliva as well as serum antibodies to S. oralis, P. gingivalis and P. intermedia. PCOS may substantially impact the makeup of the oral microbiota as a systemic endocrine disorder

and hence contribute to the heightened systemic reaction to particular microbial community members (8).

Materials and Methods

The present study embraced a total of 85 females having an age range of 20 to 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 period of the study. The research was carried out during the time period from January 2022 to July 2022. The study participants' salivary samples were taken between 9:00 am and 1:00 pm. 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. Full mouth PI, full mouth BOP, PPD, and CAL were the clinical parameters that were measured for the entire dentition that was already present. ELISA method was used to measure salivary metaloproteinnase-9.

Analysis, summarization and presentation of data in this study were based on the use of Microsoft Office Excel 2010 and statistical package for social sciences (SPSS) version 23. The test of normality, Shapiro-Wilk test, was used to evaluate the distribution of quantitative continues variables. Accordingly, not normally distributed variables were presented in the form of inter-quartile range and median. The difference in mean rank of variables between study group and reference group was done using Mann Whitney U test. The difference in mean ranks among more than two groups was carried out using Kruskal Wallis test which was followed by using Dunns' test. Correlation between any two variables was based on the use of Spearman correlation test. The level of significance was regarded at p-value of less than or equal to 0.05.

Conclusion

PCOS is associated with more severe periodontal inflammation and tissue destruction in comparison with non-PCOS women. Salivary matrix metalloproteinase-9 is useful in predicting periodontitis in women with or without PCOS.

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