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Evaluation of salivary IL-6 and carbonic anhydrase VI as biomarkers in periodontal health and disease

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ABSTRACT

BACKGROUND & OBJECTIVE: Saliva contains proteins that may serve as biomarkers for oral diseases. Periodontitis, an inflammatory gum disease linked to poor oral hygiene, alters salivary composition. This study aimed to compare salivary carbonic anhydrase VI (CA VI) and interleukin-6 (IL-6) levels among individuals with healthy gums, mild, and moderate periodontitis.

METHODOLOGY: In this cross-sectional study, ELISA measured CA VI an enzyme involved in pH regulation and IL-6 a pro-inflammatory cytokine in saliva. Periodontal status was assessed via periodontal pocket depth (PPD) and clinical attachment loss (CAL) using a Michigan O probe.

RESULTS: The Kruskal-Wallis test showed no significant differences in CA VI and IL-6 levels across the groups ($p = 0.217$ and $p = 0.579$, respectively). However, in healthy individuals, CA VI levels were inversely correlated with PPD (Spearman's $\rho = -0.455$) and CAL ($\rho = -0.433$). In mild periodontitis, CA VI negatively correlated with PPD ($\rho = -0.467$). In moderate periodontitis, IL-6 levels showed a significant inverse correlation with CAL (Pearson's $r = -0.408$).

CONCLUSION: Salivary CA VI and IL-6 levels were not significantly different across periodontal health statuses, suggesting limited use as diagnostic biomarkers. Nonetheless, significant correlations between CA VI and periodontal parameters in healthy and mildly affected individuals and IL-6 and CAL in moderate periodontitis indicate potential roles in disease progression monitoring.

KEYWORDS: Saliva, Periodontitis, Carbonic Anhydrase, Interleukin-6.

INTRODUCTION

Salivary secretion serves oral functions by moistening mouth tissues and facilitating food particle digestion [1]. Further, some of the proteins in saliva have bactericidal activities that protect against oral infections. Studies have revealed that proteins discovered in saliva could be biomarkers for detecting various diseases [2], such as dental caries, periodontal diseases [3], oral cancers, salivary gland diseases, human immune viruses, and hepatitis.

Plaque and bacteria build upon teeth, resulting in periodontitis, a disease of gums and tissues within the oral cavity. Periodontitis increases the risk of tooth loss and alveolar bone destruction compared to healthy periodontal conditions. The reasons for developing periodontitis are lack of proper dental hygiene, having less knowledge, and ineffective brushing methods [4-8].

In periodontitis, there is hypoxia in the gingival tissues, and this leads to an increase in CA VI levels, making the rate of acid production even higher, which could be destructive to the periodontal tissues. Thus, the determination of CA VI level may be used as a diagnostic tool for periodontitis and help estimate the disease's perils [9]. Consequently, researchers have also established elevated IL-6 levels in patients with periodontitis in their gingival crevicular fluid [10].

According to Turkmen, salivary IL-6 levels were higher in the periodontitis group than in the control group. Periodontitis is caused by inflammation of the gums, which shows an increased level of IL-6 in saliva and causes bone destruction. Studies have demonstrated that increased levels of IL-6 are positively associated with bone resorption caused by osteoclast activity [11].

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Studies conducted in 2021 by Isola further confirm these findings by showing that people with periodontitis had far greater concentrations of IL-6 in their saliva than healthy individuals. Considering the available research, the present study aimed to compare the levels of CA VI and IL-6 in the saliva of individuals with healthy gums and mild and moderate periodontitis for the early detection and treatment plan of the disease. The research aimed to improve the early detection, prognosis, and therapeutic management of periodontitis.

METHODOLOGY

An observational cross-sectional study conducted over 6 months (September 2023 to February 2024) involving the comparison of biomarker levels among the three study groups was carried out at the Department of Periodontology of CMH Medical College & IOD Lahore with the permission and support of the institution's Ethical Review Committee under case # 628/ERC/CMH-LMC (12-09-2023).

Ninety subjects were recruited, with thirty subjects in each group (healthy control, mild periodontitis and moderate periodontitis)[12]. After written informed consent, 2ml of saliva samples were collected and centrifuged for fifteen minutes at 4°C and 2500 RPM, and supernatant fluid was taken and stored at -80°C for ELISA [13]. Under the guidance of a consultant periodontist, the CMH-LMC history form was used to record the following information: CAL, PPD, and DMFTs (Decayed, missing and filled teeth) [14].

Periodontitis was categorized into three groups based on CAL: mild CAL = 1 to 2 mm, moderate CAL = 3 to 4 mm, and healthy CAL = 0 mm. Descriptive analysis of the data was carried out using statistical software, the SPSS version 27. It was for the levels of CA-VI and IL-6 that the quantitative mean and standard deviation statistics were computed. As for the parameter of normality, the Shapiro-Wilks test was used. The levels of CA-VI and IL-6 were compared among the three groups (control healthy, mild and moderate) using the Kruskal-Wallis test. The data obtained in the study were compared statistically; the level of significance used in the current study was <0.05. The research excluded smokers, pregnant and lactating mothers, patients with considerable systemic disease related to periodontitis, and patients on drugs. Patients aged 20 to 50 years with mild to moderate periodontitis, possessing more than 20 permanent teeth and with no history of smoking were included in the study.

RESULTS

There were 90 participants in total, of whom 43.3% were men and 56.7% were women aged 20 to 50. The healthy, mild, and moderate periodontitis groups did not differ significantly in salivary IL-6 or CA VI (p=0.579 and p=0.217, respectively); however, there was a significant difference in PPD and CAL between the groups (Table I).

A significant correlation was observed between CA VI and PPD (Spearman's rho=-0.455, p< 0.05) in healthy individuals (see Table II). Similarly, a significant correlation was observed between CA VI and PPD (Spearman's rho=-0.467, p < 0.05) in mild periodontitis (see Table III). Also, a significant correlation between IL-6 and CAL (Pearson r=-0.408, p < 0.05) was seen in moderate periodontitis (see Table IV). There was also a significant co-relation of CA VI and CAL (Spearman's rho=-0.433, p < 0.05) in healthy individuals (Table II).

In the healthy group, no significant correlation was observed between CA VI and IL-6, nor between IL-6 and clinical parameters such as PPD and CAL. Similarly, CA VI showed no significant correlation with IL-6 or CAL in the mild periodontitis group. In the moderate periodontitis group, no significant correlations were found between CA VI and IL-6, PPD, or CAL.

Table-II: Correlation matrix for healthy group (Spearman's rho).

Parameters	AGE	CA VI	IL-6	PPD	CAL
AGE (years)	1	-0.074	-0.240	-0.079	0.219
CA VI (ng/ml)	-0.074	1	0.343	-0.455*	-0.433*
IL-6 (ng/L)	-0.240	0.343	1	0.014	-0.340
PPD (mm)	-0.079	-0.455*	0.014	1	0.437*
CAL (mm)	0.219	-0.433*	-0.340	0.437*	1

Spearman's Rho Correlation Coefficient were used and (*) p-value of less than 0.05 is regarded as statistically significant.

Table-III: Correlation matrix for mild periodontitis group (Spearman's rho).

Parameters	AGE	CA VI	IL-6	PPD	CAL
AGE (years)	1	-0.211	0.272	0.208	-0.534**
CA VI (ng/ml)	-0.211	1	0.273	-0.467**	0.035
IL-6 (ng/L)	0.272	0.273	1	-0.018	-0.074
PPD (mm)	0.208	-0.467**	-0.018	1	-0.079
CAL (mm)	-0.534**	0.035	-0.074	-0.079	1

Spearman's Rho Correlation Coefficient were used and (**) p-value of less than 0.05 is regarded as statistically significant.

Table- I: Comparison of parameters between healthy, mild and moderate.

Sr. No.	Parameters	Healthy control		Mild periodontitis		Moderate periodontitis		P-value
		n	Mean ± SD / Median (IQR)[a]	n	Mean ± SD / Median (IQR)[a]	n	Mean ± SD / Median (IQR)[a]	
1	CA VI (ng/ml)	30	234.31 (212.75-217.08)	30	239.21 (218.14-256.13)	30	231.18±24.03	0.217
2	IL-6 (ng/L)	30	594.31 (556.82-641.48)	30	590.23±42.73	30	603.33±55.61	0.579
3	PPD (mm)	30	1.23 (1.00-1.50)	30	1.1 (1.00-1.41)	30	1.60 (1.5-2.00)	<0.001*
4	CAL (mm)	30	0.00 (0.00-0.00)	30	0.42 (0.36-0.50)	30	1.47±0.54	<0.001*

[a] The values were displayed as Mean ± SD for regularly distributed variables and median (iqr) for non-normally distributed variables. The Kruskal-Wallis test generated the p-value (*a p-value of less than 0.05 was considered statistically significant).

Table-III: Correlation matrix for Moderate Periodontitis Group (Pearson's=r).

Parameters	AGE	CA VI	IL-6	PPD	CAL
AGE (years)	1	0.075	0.111	0.108	-0.290
CA VI (ng/ml)	0.075	1	-0.013	0.135	0.192
IL-6 (ng/L)	0.111	-0.013	1	-0.109	-0.408*
PPD (mm)	0.108	0.135	-0.109	1	0.191
CAL (mm)	-0.290	0.192	-0.408*	0.191	1

The Pearson Correlation Coefficient were used (r) and (*) p-value of less than 0.05 is regarded as statistically significant.

DISCUSSION

This study evaluated salivary concentrations of carbonic anhydrase VI (CA VI) and interleukin-6 (IL-6) alongside periodontal parameters—probing pocket depth (PPD) and clinical attachment loss (CAL)—to assess disease severity among individuals with healthy gums, mild, and moderate periodontitis. Some recent reports suggest that this cytokine exhibits pro-inflammatory action [8] and anti-inflammatory action in inflammation. In the same way, CA VI can demineralize and re-mineralize simultaneously, depending on the pH of saliva [9].

The patients with chronic periodontitis had significantly higher IL-6 levels of P- value < 0.05 as compared to the healthy control [10,11]. Also, higher levels of IL-6 in the patients reverse the effects of TNF-alpha and increase bone resorption through osteoclastic activity. Likewise, Bataille also provided the overall idea about the bone-resorbing activity of IL-6 [15]. Similarly, Takeuchi backed up the two fragments of interleukin-6 in the bone resorption process and the regenerative process related to an inflammation disease, rheumatoid arthritis [16-18].

Mengel reported that individuals with untreated generalized periodontitis had greater blood levels of IL-6. The increase was particularly related to greater attachment loss, emphasizing the systemic influence of periodontal inflammation on circulating biomarkers [19].

Inflammatory markers such as salivary Irisin and IL-6 were also noticeably elevated in the mild and moderate periodontitis group as indicated by statistically significant values of $p < 0.001$ and $p = 0.002$, respectively [20]. It was reported that IL-6 has major anti-inflammatory activities on infection-induced osteolysis, and a lack of IL-6 enhanced osteoinflammatory response [21].

According to Khorasani, the initial stage of caries triggers a series of oral cavity immunological reactions involving cytokines, including IL-6, IL-1, IL-8, and tumor necrosis factor (TNF)[22]. Similarly, Lo Giudice reported that periodontitis was characterized by inflammatory processes that may cause marginal inflammation due to elevated levels of IL-6 [23].

The study determined no considerable variations existed between salivary IL-6 and CA VI levels across healthy participants with periodontitis patients in varying severity ($p = 0.579$ for IL-6 and $p = 0.217$ for CA VI). The studied biomarkers lack sufficient sensitivity for differentiating between periodontitis stages from moderate to mild and healthy conditions. The evaluation of PPD and CAL between patient groups revealed important distinctions. Periodontal tissue destruction worsens progressively from mild to moderate periodontitis, leading to deeper pockets and enhanced attachment loss.

The assessment of periodontitis severity through PPD and CAL measurements provides better discrimination than IL-6 and CA VI assessments when differentiating the evolution of periodontal condition.Mean IL-6 concentrations were significantly higher in the healthy & mild periodontitis groups than moderate periodontitis group suggesting that this cytokine could be operational in the primary phase of inflammation encountered in periodontal disease. More detailed study of these observations as well as substantiation of these results could shed light on the molecular pathophysiology of periodontal diseases and contribute towards the establishment of treatments.

It was found that there were increased levels of salivary CA VI due to increased levels of supra gingival calculus and increased levels of periodontitis. In the same manner, it was found that CA VI activity was significantly higher in children with dental caries, and alpha-amylase activity was significantly higher in caries-free children (P-value < 0.001) [24]. Similarly, it was demonstrated that saliva collected from caries children has higher levels of CA VI activity than the saliva collected from caries-free children. In the same way, it was suggested that the lower levels of CA VI were associated with an increased prevalence of dental caries, particularly in individuals with poor oral hygiene [25]. Similarly, it was demonstrated that there was a noticeably greater level of CA VI activity in the biofilm of children with dental cavities. The biofilm of children without dental caries had a noticeably greater concentration of CA VI. Similarly, it was found that the caries-active group had a higher concentration of the CA VI enzyme, and total protein has a linear relationship with caries activity.

Therefore, based on the established results from this study, we noticed a considerable difference between the CA VI levels in the healthy group that was statistically significant ($p = 0.041$). Likewise, the CA VI showed a highly significant difference in mild periodontist group individuals compared to controls ($P < 0.05$). The study data reveals that subjects in the mild and moderate periodontitis groups exhibit insignificant differences in CA VI levels ($p = 0.750$).

This study had several limitations. Time and budget constraints may have influenced the selected sample size, potentially affecting the generalizability of the findings. Additionally, the cross-sectional design prevented the establishment of causal relationships between salivary biomarkers and the severity of periodontal disease. The exclusion of patients with severe periodontitis further limits the ability to assess biomarker patterns across the full spectrum of disease severity. This exclusion was primarily due to time and financial limitations, as well as the clinical difficulty in clearly distinguishing between moderate and severe periodontitis.

CONCLUSION

This study found no significant differences in salivary IL-6 and CA VI levels among individuals with healthy gums, mild periodontitis, and moderate periodontitis. However, clinical parameters specifically periodontal pocket depth (PPD) and clinical attachment loss (CAL) proved to be effective diagnostic indicators for distinguishing between stages of periodontitis. Although salivary IL-6 and CA VI were not reliable as standalone diagnostic biomarkers, significant negative correlations were observed between CA VI and both PPD and CAL in healthy individuals, between CA VI and PPD in mild periodontitis, and between IL-6 and CAL in moderate periodontitis. These findings suggest that while not diagnostically definitive, salivary IL-6 and CA VI may reflect periodontal tissue changes and could serve as supportive biomarkers in conjunction with clinical assessments.

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Asma Khan: Substantial contributions to the conception and Design of the work.
Saghir Jafri: Acquisition and Analysis of data for the work.
Mohsin Ali Cheema: Interpretation of data for the work.
Abdullah Ehsan : Drafting the work .
Syeda Ayesha Fatimah: Reviewing it critically for important intellectual content.
Tayyaba Sheikh: Final approval of the version to be published.