Original Article

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Dentistry Section

Estimation and Comparison of Salivary Calcium, Phosphorous, Alkaline Phosphatase and pH Levels in Periodontal Health and Disease: A Cross-sectional Biochemical Study

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ABSTRACT

Introduction: In oral diagnostics there is a great challenge to determine biomarkers for screening and evaluating the disease activity. Biomarkers can also serve as a useful tool to measure the efficacy of the therapy.

Aim: To evaluate and compare the levels of salivary calcium, phosphorous, alkaline phosphatase and pH levels in periodontally healthy subjects and patients with gingivitis and periodontitis.

Materials and Methods: The present study consisted of 150 subjects aged between 20-45 years who were divided into three groups; periodontally healthy, gingivitis and chronic periodontitis. Prior to the clinical examination the demographic details, relevant information of the subject, gingival index, plaque index, Oral Hygiene Index (OHI) and pH were recorded. Biochemical assay of saliva i.e., inorganic calcium, phosphorous and alkaline phosphatase were estimated by colorimetric method. ANOVA and Tukey's test were applied for statistical analysis.

Results: The mean levels of biomarkers studied were; inorganic calcium (12.55µg/dl), phosphorous (14.50µg/dl), alkaline phosphatase (49.62µg/dl) and pH (11.65). There was a gradual increase in these levels as the condition progressed from health to gingivitis or periodontitis which was statistically significant at $p<0.001$.

Conclusion: Based on these results, it can be concluded that, the biomarkers like salivary calcium, phosphorous, alkaline phosphatase and pH can be considered for evaluating the diagnosis and prognosis of periodontal tissues in disease and health.

Keywords: Biomarker, Gingivitis, Periodontitis, Saliva

INTRODUCTION

Periodontal disease is characterized by complex host-parasite interactions that lead to gingival inflammation, loss of connective tissue attachment, periodontal ligament destruction and alveolar bone resorption. It is the second most common oral disease next to dental caries affecting 5%-30% of adult population. The natural history of periodontitis follows a discontinuous pattern of exacerbation and remission characterized by disease activity and inactivity [1].

Role of biomarkers in oral diagnostics has been a great challenge for screening, determination of prognosis and evaluating the disease activity. Current disease activity status of previously diseased sites can be determined by identification and quantification of certain biomarkers. Various possible periodontal biomarkers are micro-organism and their products, immune-inflammatory products; host cell derived enzymes released during connective tissue degradation and bone resorption. Whole saliva, gingival crevicular fluid, dental plaque and serum can be used as source of specimen for these biomarkers [2].

The primary etiological factor responsible for periodontal disease is 'dental plaque'. The inorganic components of plaque are calcium, phosphorous and other minerals. Salivary calcium and phosphorous are readily absorbed by dental plaque forming calculus which can lead to periodontitis. The concentration of Alkaline Phosphatase (ALP), an intracellular enzyme released from secondary granules of neutrophils increase significantly with increasing inflammation and plaque accumulation. The increased activity might also be a consequence of destructive processes in alveolar bone and metabolic changes in inflamed gingiva. ALP enzyme is an indicator of higher level of cellular damage [3-4].

The enzymes released from host cells can be easily obtained within the oral cavity either from Gingival Crevicular Fluid (GCF) or from whole saliva. Raised levels of various biochemical markers in GCF have predicted clinical attachment loss and alveolar bone loss. However, there are inherent problems in collecting GCF in a routine dental office setting [4]. The whole saliva is easy-to-use noninvasive diagnostic method and can be collected in large quantity, with less discomfort to patient and clinician when compared to GCF.

The aim of periodontal diagnostic procedure is to furnish useful information related to current periodontal disease activity, its extent and severity. This diagnostic information helps in formulating diagnosis, treatment plan and also provides essential information during disease monitoring phases of periodontal treatment. Hence, the present study was undertaken to assess the levels of salivary calcium, phosphorous, alkaline phosphatase and pH in periodontally healthy subjects and patients with gingivitis and periodontitis.

MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Periodontology, School of Dental Sciences, Krishna Institute of Medical Sciences Deemed University, Karad, Maharashtra, India, after due approval from the Ethical Committee (Ref.No: KIMSDU/IEC/01/2013, dated 06/12/2013). The study was conducted during the period from March 2014 to June 2015 in accordance with Declaration of Helsinki (1975), revised in 2002.

Patient selection: The sample size for the study was determined based on the pilot study and power analysis. Following initial screening a total 155 subjects were considered, five subjects who did not meet the inclusion criteria were excluded. Finally 150 subjects in the age range 20–45 years (Mean age 32.24 ± 3.74 years), were enrolled after obtaining an informed consent. Patients with known systemic diseases which can have an effect on periodontal condition like diabetes, renal disease, hepatic disease, arthritis and malignant disease and those with history of antibiotic usage during the previous six months were excluded from the study. Pregnant, lactating women and patients with less than 15 teeth were not considered.

The selected patients were divided into three groups as follows:

Group A (n=50): Comprised of healthy subjects with sulcus depth ≤3mm with no attachment loss and Bleeding On Probing (BOP) (as per gingival index by Loe and Sillness1963) [5].

Group B (n=50): Comprised of chronic generalized gingivitis subjects with sulcus depth ≤3mm with no attachment loss but presence of BOP (as per gingival index by Loe and Sillness1963) [5].

Group C (n=50): Comprised of chronic generalized periodontitis subjects with periodontal pockets >4 mm as well as clinical attachment loss with or without BOP.

Prior to the clinical examination the demographic details and relevant information of the patient were recorded using a specially designed proforma which included medical history, past dental history and oral hygiene habits. The periodontal status was also recorded using the following parameters, Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Oral Hygiene Index (OHI) and Plaque Index (PI).

Clinical and periodontal examination: A single examiner performed all the measurements at the beginning of the study, who was calibrated against a senior periodontist who represented the gold standard. The clinical parameters like PI, PPD and CAL were recorded. A disclosing agent (Alphaplac, DPI, Wallace Street, Mumbai) was used to disclose the plaque during the examination. Plaque index was measured using Turskey-Gilmore-Glickman Modification of the Quigley Hein Plaque Index, 1970. Plaque index for entire mouth was determined by dividing the total score by the number of surfaces examined, Score 0 or 1 was considered as low and score 2 or more was considered high [6].

Periodontal parameter like PPD and CAL were assessed with periodontal probe (UNC-15, Hu-friedy, Chicago, IL) and measurements were recorded on four sites per tooth of all teeth excluding third molars. Probing pocket depth was measured from gingival margin to the base of gingival sulcus. Clinical attachment loss was measured from Cementoenamel Junction (CEJ) to base of gingival sulcus.

Participants were grouped to be suffering from gingivitis or periodontitis based on the clinical findings. The participants were classified as having chronic periodontitis based on 1999 consensus for classification of periodontal disease [7].

Methodology: After a detailed dental examination the subjects were rescheduled for estimation of salivary calcium, phosphorous, alkaline phosphatase and pH. Unstimulated 5ml whole saliva sample was collected in morning from 10am to 11 am, two hours after the last meal to standardize the collection according to the circadian rhythm. The subjects were asked to rinse thoroughly with distilled water before the collection of salivary sample. Five minutes after the oral rinse the subjects were asked to swallow any residual saliva that may be in their mouth. The subjects were refrained from talking and asked to drop down the head and not to cough up mucus as saliva is collected. The subjects were then asked to let the saliva pool in their floor of the mouth to their maximum extent and then expectorate into the collecting vessel till the desired quantity was collected. The pH of saliva was determined immediately after the collection to avoid any time related pH changes. The saliva samples were transported to the laboratory for estimation within 24 hrs using standard gel coolant pack in order to maintain the temperature between 2°C to 4°C. Biochemical assay of saliva samples were carried out in the Department of Biochemistry Krishna Institute of Medical Sciences Deemed University, Karad, Maharashtra, India.

Laboratory analysis: Estimation of inorganic salivary calcium was carried out by using Arsenazo reagent {Accucare Calcium Arsenazo Iii Lab Care Diagnostics (India) Pvt. Ltd}. Estimation of salivary phosphorous by Molybdate U.V. method (Molybdate U.V. method Crest biosystem) and Estimation of salivary Alkaline phosphatse was carried out by Autoenzyme method (Auto enzyme kinetic Accurex Biomedical Pvt. Ltd.).

STATISTICAL ANALYSIS

All the data collected was statistically analyzed using Statistical Package for the Social Sciences (SPSS) software version 19 (IBM Corporation, Armonk, New York, USA). The results were expressed in means and percentages, p-value ≤0.05 was considered significant. The significance of difference in means was tested by ANOVA test. Tukey's test was used to explore the association between explanatory variables.

RESULTS

The data of 150 subjects examined was tabulated according to the variables. In all the three groups, un-stimulated saliva was collected and analyzed for inorganic calcium, phosphorous, alkaline phosphatse and pH. Periodontal status was assessed by OHI, GI, PI and CAL. Number of teeth present and number of intact teeth were also recorded.

The study population consisted of 60 males and 90 females with a mean age of 24.94 ± 2.53, 29.59 ± 5.47 and 41.96 ± 2.44 in Group A, B and C respectively [Table/Fig-1].

The comparison of salivary inorganic calcium in Group A, B and C [Table/Fig-2] revealed that mean of salivary inorganic calcium is 5.47 ± 1.39 for the Group A, 8.40 ± 1.13 for Group B and 12.55 ± 2.73 for Group C. The observed mean difference in the three groups was statistically significant p <0.001. The mean salivary phosphorous level is [Table/Fig-2] gradually increased with increase in periodontal diseases severity (3.93 ± 1.89 for the Group A, 7.68 ± 1.40 for Group B and 14.5 ± 3.82 for Group C). The observed mean difference in the three groups was statistically significant p <0.001.

	Group A	Group B	Group C	p-value
Study sample (n)	50	50	50	
Age (mean ± SD)	24.94 ± 2.53	29.59 ± 5.47	41.96 ± 2.44	< 0.001*
Gender				
Male	22 (44%)	20(40%)	18(36%)	
Female	28 (56%)	30(60%)	32(64%)	0.448¶

[Table/Fig-1]: Demographic data of the subjects.
One way ANOVA test
*Statistically significant
¶ Not significant

Group	Salivary Calcium	Salivary Phosphorous	Salivary Alkaline Phosphate	Salivary pH
Group A (n=50)	5.47±1.39	3.93 ± 1.89	20.70± 7.18	6.91± 0.56
Group B (n=50)	8.40 ±1.13	7.68 ± 1.40	33.06 ± 5.49	8.83 ±1.29
Group C (n= 50)	12.55 ±2.73	14.50± 3.82	49.62± 16.46	11.65±2.26
p- value	< 0.001*	< 0.001*	< 0.001*	< 0.001*

[Table/Fig-2]: Intergroup comparison of levels of salivary calcium, phosphorous, alkaline phosphatse and pH level between Group A, B and C.
One way ANOVA test
*Statistically significant

	Group A	Group B	Group C
Group A	-	< 0.001*	< 0.001*
Group B	-	-	< 0.001*
Group C	-	-	-

[Table/Fig-3]: Intergroup comparison of salivary calcium, phosphorous, alkaline phosphatse and pH in group A, B and C.
Post hoc ANOVA Tukey's test
*Statistically significant

Group	OHI Index (Mean±SD)	Gingival Index (Mean±SD)	Plaque Index (Mean±SD)
Group A (n=50)	0.00 ± 0.00	0.00 ± 0.00	0.03 ±0.21
Group B (n=50)	21.47 ± 6.19	1.58± 0.57	1.45 ±0.44
Group C (n= 50)	30.12± 8.77	3.45± 3.81	3.97 ±0.64
p- value	< 0.001*	< 0.001*	< 0.001*

[Table/Fig-4]: Comparison of mean oral hygiene index, gingival index and plaque Index between Groups A, B and C.
One way ANOVA test
*Statistically significant

The mean alkaline phosphatse level for group A was 20.7 ± 7.18, for Group B was 33.06 ± 5.49 and for Group C was 49.62 ± 16.46 [Table/Fig-2]. Comparison of salivary alkaline phosphatase level in Group A, B and C revealed that as the periodontitis progressed mean of salivary alkaline phosphatase level also increased. The observed mean difference in the three groups was statistically significant p <0.001.

The mean salivary pH for Group A was 6.91 ± 0.56, for Group B was 8.83 ± 1.29 and for Group C was 11.65 ± 2.26 [Table/Fig-2]. Comparison of salivary pH in the three groups was statistically significant p <0.001.

Intergroup comparison of the salivary calcium, phosphorous, alkaline phosphatse and pH levels among the three groups showed statistically significant difference (p <0.001) [Table/Fig-3].

The multiple comparisons and the mean difference of OHI, GI and PI among the three groups was statistically significant (p <0.001) [Table/Fig-4]. This suggests that as severity of periodontitis increased, values of OHI, GI and PI also gradually increased.

DISCUSSION

Saliva plays an important role in maintenance of oral health. Changes in composition and output of saliva may have detrimental effects on oral health. Patients suffering from dry mouth experience dental caries and difficulty in maintaining oral hygiene. Saliva and GCF not only play a decisive role in preventing periodontal disease but also ironically in the induction of periodontal pathology [8-9]. Recent advances in diagnosis of oral and periodontal disease are moving towards use of various biomarkers, which can be used to identify and quantify the periodontal risk.

The results of the present study revealed that the subjects in the periodontitis group had significantly higher levels of salivary calcium than gingivitis and healthy group. These results are consistent with previous studies which concluded that, a positive correlation existed between high salivary calcium content and periodontitis [1,10]. However, in contrast, in a similar study higher salivary calcium level was related to good dental health and there was no relation to periodontal bone destruction [11].

The fluctuations in dietary calcium intake and general calcium turnover may reflect in the levels of salivary calcium. In the present study we could not demonstrate the effect of diet. Literature suggests that, the affinity of salivary calcium to be readily taken up by dental plaque plays an important role in onset of periodontal diseases [10].

Comparison of salivary phosphorus levels among the three groups in the current study revealed significantly higher levels in the periodontitis group. These results are in accordance with a previous study which concluded that there was positive correlation between high salivary phosphorous content and periodontitis [1]. In contrary, another study showed that smokers with periodontitis exhibited statistically significant reduced levels of total proteins, calcium, phosphorus and magnesium as compared to nonsmokers with periodontitis [12].

Alkaline Phosphatse (ALP) is an intracellular enzyme present in most of the tissues, associated with bone metabolism. An increased activity of ALP might be a consequence of destructive processes in alveolar bone suggestive of advanced periodontal breakdown [13,14]. In the present study salivary ALP levels were significantly raised in chronic periodontitis subjects as compared to gingivitis and healthy subjects. The increase in the mean level of ALP can be due to tissue alteration as a result of host parasite reaction. During progression of the disease, enzymes are released from dead and dying cells of the periodontium, polymorphonuclear leukocytes, inflammatory, epithelial, and connective tissue cells of the affected sites [14,15]. These results are in accordance with a previous study wherein the authors concluded that salivary ALP can be used as a biomarker as it reflects the inflammation and destruction of periodontal tissues [16].

Several studies have mainly investigated the activities of ALP biomarker in the GCF, which is in much closer contact with periodontal tissues and due to this, it surely reflects the occurrences in them much better. However, the main disadvantage with GCF is the technique of sample collection which is complex and not feasible in daily practice [17-19].

The result of the present study revealed that pH of saliva was more alkaline in gingivitis and periodontitis subjects as compared to healthy controls. An alkaline pH is associated with increased proteolytic activity of organism and favors the deposition of calcium phosphate, thereby promoting plaque mineralization. This is in accordance to previous studies which suggested that subjects with increased pH have greater remineralization potential for dental plaque [10,20].

In contrary, another study identified no correlation between pH and gingivitis, but significant correlation between pH and periodontal pockets was evident, wherein with increase in pocket depth the pH of saliva was more alkaline as compared to gingivitis [21].

The results of the current study suggest that subjects with increase in salivary mineralization parameters like inorganic salivary calcium and phosphorous, high salivary pH are potentially at a higher risk of developing periodontitis. Increase in salivary calcium consequently reflects in the levels of plaque calcium and is thus readily available for remineralization [22-23].

On the contrary, subjects with decreased salivary mineralization parameters, especially inorganic salivary calcium and phosphate, low salivary pH, reduced salivary flow rate are at a higher risk of developing dental caries as their plaque is more acidogenic and demineralization of enamel occurs more readily. The role of salivary ALP as a potential biochemical marker in detecting the progression of periodontal disease is promising [24]. Recent literature exploring link between salivary biomarkers and periodontal diseases is summarized in [Table/Fig-5] [25-28].

Clinical implications and future perspectives: In traditional periodontics, the clinical criteria are mostly insufficient for detecting the sites with active disease, monitoring the treatment response and measuring the degree of susceptibility for future disease progression. Presence of specific biomarkers associated with pathogenesis of periodontal diseases makes saliva a noninvasive, valuable source of clinically relevant information about oral health and disease.

Further longitudinal research over wide geographic distribution would be required to draw definite conclusions and prove the role of saliva in detecting and estimating the periodontal disease severity.

Author (years)	Study Sample	Sample/ Marker	Findings
Megha Varghese (2015)	50	Salivary calcium	Salivary calcium levels were significantly higher in smoker group as compared to non-smoker group [25].
C. Seethalakshmi (2016)	40	Salivary pH, Incidence of Dental Caries and Periodontal Status	In diabetes mellitus patients there was significant increase in incidence of periodontitis and dental caries and reduction in the salivary pH as compared to non-diabetic subjects [26].
Ahire A et al.,(2016)	50	Salivary flow rate, pH, salivary protein and calcium concentration	A significant reduction in levels of the salivary flow rate, pH, protein and calcium after oral prophylaxis [27].
Vivek Vardhan Gupta et al.,(2016)	108	Salivary Calcium Level and pH	Salivary calcium level and pH significantly increased in smokers having aggressive periodontitis as compared to non-smoker aggressive periodontitis patients and healthy subjects [28].

[Table/Fig-5]: Studies regarding the role of salivary biomarkers in periodontal health and disease [25-28].

LIMITATION

The present study could not demonstrate the effect of diet and age wise calcium changes. The sample size of the study was small. The study results would have been substantiated if the comparison of the evaluated parameters could have been made after non-surgical periodontal therapy (Scaling and Root Planing).

CONCLUSION

It can be concluded that as periodontal disease severity increases the salivary calcium, phosphorous, alkaline phosphatase and pH levels also increases. These parameters are potential biochemical markers for the detection and progression of periodontal disease.

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