

## Original Research

### Salivary Amylase as a Biomarker in Health and Periodontal Diseases

Rohi Rashid

Private Practitioner, Jammu, India

#### **ABSTRACT:**

**Background:** Saliva is an accessible biofluid that contains components derived from the mucosal surfaces, gingival crevices, and tooth surfaces of the mouth. Research on the composition of the saliva and the presence of periodontal and other disease markers became intensive again thanks to the development of laboratory nanotechnologies that pushed detection limits of various metabolites, signal molecules, hormones, and other substances by several orders of magnitude. Traditional clinical measurements, such as probing pocket depth, bleeding on probing, and clinical attachment loss, which are used for periodontal diagnosis, are often of only limited usefulness because they are indicators of previous periodontal disease rather than present disease activity. **Aim of the study:** To study on salivary amylase as a biomarker in health and periodontal diseases. **Materials and methods:** The present study was conducted in the Department of Periodontics of the Dental Institutions. A written informed consent was obtained from the patients explaining them the protocol and procedure of the study before starting the study. A total of 30 subjects were included in the study and were grouped into 3 groups of 10 subjects in each group. Group 1 consisted of individuals who are healthy and have no evidence of clinical inflammation, sulcular bleeding and clinical attachment loss. Group 2 consisted of individuals with presence of BOP, clinical inflammation but no evidence of clinical attachment loss, indicating that they have generalized chronic gingivitis. Group 3 included individuals with generalized chronic periodontitis confirmed by bone loss, clinical attachment loss  $> 3\text{mm}$  and  $\text{PPD} \geq 5\text{mm}$ ; and the amount of destruction consistent with local factors. At baseline, biochemical parameter was recorded in groups A, B and C. Thorough full mouth scaling was done in group B; and scaling and root planning was done in group C. Subjects were given careful instructions regarding self-performed oral hygiene measures. All the parameters again assessed in group B and C, after 6 weeks after the periodontal therapy. **Results:** We observed that mean SAA levels dropped significantly in group A, B and C after receiving SRP indicating that severity of periodontal and gingival disease are related to SAA levels. The results on comparing were found to be statistically significant. **Conclusion:** Within the limitations of the present study, it can be concluded that SAA levels elevates with increasing of severity of the periodontal disease. After periodontal treatment, the level of SAA drops. Thus, SAA level can be used as a biomarker for gingival and periodontal diseases.

**Key words:** salivary amylase, periodontal, gingival.

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**Corresponding Author:** Dr. Rohi Rashid, Private Practitioner, Jammu, India

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#### **INTRODUCTION:**

Saliva is an accessible biofluid that contains components derived from the mucosal surfaces, gingival crevices, and tooth surfaces of the mouth. Saliva also contains microorganisms that colonize the

mouth and other exogenous substances and so can potentially provide an insight into the relationship of the host with the environment.<sup>1</sup> Research on the composition of the saliva and the presence of periodontal and other disease markers became intensive

again thanks to the development of laboratory nanotechnologies that pushed detection limits of various metabolites, signal molecules, hormones, and other substances by several orders of magnitude.<sup>2</sup> In addition to molecular methods, analytical chips are also available that may, by merely changing the detection plates, detect a series of various chemical substances.<sup>3</sup> Enzymes, specific and nonspecific proteins, antibodies, and other substances are among the potential salivary biomarkers of periodontal and certain distant tissue diseases. And so saliva became the topic of interest among experts in proteomics, research of sequential composition of individual proteins. Periodontitis is a multifactorial chronic non-reversible inflammatory disease affecting the supporting structures of dentition, initiated and propagated through a complex interaction between periopathogens and the host defense system.<sup>4</sup> It starts with a microbial infection, followed by a host mediated destruction of periodontal tissues caused by hyper activity of leukocytes and generation of cytokines, eicosanoids and matrix metalloproteinases. Traditional clinical measurements, such as probing pocket depth, bleeding on probing, and clinical attachment loss, which are used for periodontal diagnosis, are often of only limited usefulness because they are indicators of previous periodontal disease rather than present disease activity.<sup>5,6</sup> Hence, the present study was planned to study on salivary amylase as a biomarker in health and periodontal diseases.

**MATERIALS AND METHODS:**

The present study was conducted in the Department of Periodontics of the Dental Institutions. The ethical clearance for the study was approved from the ethical committee of the hospital. A written informed consent was obtained from the patients explaining them the protocol and procedure of the study before starting the study. A total of 30 subjects were included in the study and were grouped into 3 groups of 10 subjects in each group. Group 1 consisted of individuals who are healthy and have no evidence of clinical inflammation, sulcular bleeding and clinical attachment loss. Group 2 consisted of individuals with presence of BOP, clinical inflammation but no evidence of clinical attachment loss, indicating tha the y have generalized chronic gingivitis. Group 3 included individuals with

generalized chronic periodontitis confirmed by bone loss, clinical attachment loss > 3mm and PPD ≥ 5mm; and the amount of destruction consistent with local factors.

**Collection of saliva for amylase estimation**

Salivary sample collection was performed in the morning between 9.00-11.00 a.m. with study subjects sitting upright in a comfortable position. Participants were instructed not to brush their teeth, or eat, or drink two hours before the time of saliva collection. After rinsing mouth with water to wash out exfoliated cells, subjects were asked to wait for 5 minutes and were asked to spit out or swallow saliva that was already present in the mouth before sample collection. Samples of unstimulated saliva (1 ml) were collected by allowing saliva to passively flow into sterile tube (without stimulation). Analysis of sample was done immediately after collection. Saliva sample was centrifuged at 3000 rpm for 20 min. The upper part was drawn and used for amylase determination. Estimation of salivary amylase SAA level measured using CNPG3, kinetic assay method using commercially available reagent kit (span, India).

Salivary amylase level was assessed. At baseline, the biochemical parameters were recorded in groups A, B and C. Thorough full mouth scaling was done in group B; and scaling and root planing was done in group C. Subjects were given careful instructions regarding self performed oral hygiene measures. All the parameters again assessed in group B and C, after 6 weeks after the periodontal therapy.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student’s t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

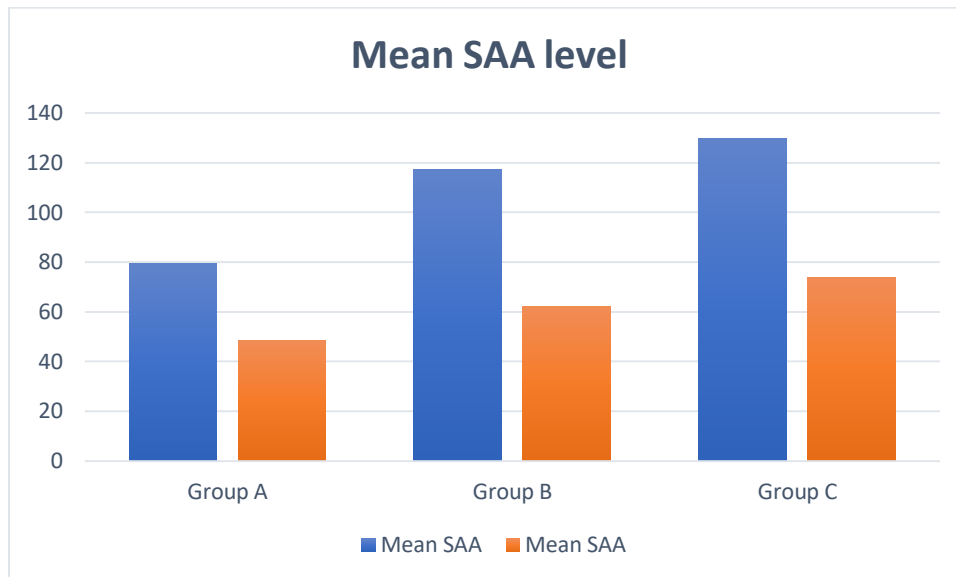
**RESULTS:**

Table 1 shows the comparison of mean SAA for Group A, B and C at baseline and after SRP. We observed that mean SAA levels dropped significantly in group A, B and C after receiving SRP indicating that severity of periodontal and gingival disease are related to SAA levels. The results on comparing were found to be statistically significant (Fig 1).

**Table 1: Comparison of mean SAA for Group A, B and C at baseline and after SRP**

		Group A	Group B	Group C	p-value
Mean SAA	At baseline	79.32	117.36	129.65	0.002
	After SRP	48.36	62.21	73.650	

Figure 1:



**DISCUSSION:**

In the present study, we observed that the level of SAA increases with increasing severity of periodontal and gingival disease and drops significantly after treating the respective disease. This indicates that SAA level can be effectively used as a biomarker for indication of gingival and periodontal disease when clinical symptoms are not much evident. The results were compared to previous studies from the literature. Totan A et al illustrated the influence of periodontal disease on the level of salivary AST, alanine aminotransferase (ALT) and ALP. All clinical periodontal examinations were performed by the same periodontist. All patients included in the study presented a probing depth >5 mm, bleeding on probing and alveolar bone loss >40%. Salivary AST, ALT and ALP activities were measured using DiaSys analysis kits from Diagnostic Systems. The methods were adapted for saliva. Salivary AST activity in patients with periodontal disease was significantly increased (median 81.75+/-23 U/L) compared with controls (15.25+/-10.5 U/L). Salivary ALT activity was not significantly modified in saliva from patients with periodontal disease compared with the control group. Our results showed a significant (p<0.01) increase in salivary ALP activity (34.38+/-1.5 U/L) in patients with periodontal disease compared with controls (6.6+/-4.2 U/L). Their results revealed that periodontal destruction such as periodontal pockets, gingival bleeding and suppuration are related to higher ALP and AST levels in saliva. Salivary AST could be used as a useful marker for monitoring periodontal disease. The increase in salivary ALP activity in periodontitis demonstrated could be associated with alveolar bone loss, a key feature of periodontal disease.

Luke R et al estimated the levels of enzymes AST, ALT, ALP and BUN and to correlate the level of estimated enzymes with that of clinical parameters in the saliva of Healthy subjects, Gingivitis patients and patients with chronic periodontitis. The study included a total of 40 male subjects within the age group of 21 to 50 years, and examined the activity of enzymes AST, ALT, ALP and BUN in saliva spectrophotometrically and compared their values between healthy subjects, gingivitis and chronic periodontitis patients. Clinical parameters like OHI - S (Oral hygiene index - Simplified), SBI (Sulcus Bleeding Index), PPD (Probing Pocket Depth), CAL (Clinical Attachment Level), and PI (Periodontal Index) were recorded. Obtained results showed statistically significant increases of activity of AST, ALT, ALP, and BUN in saliva from patients with periodontal disease in relation to gingivitis and control groups. There was also an increase in periodontal parameters with an increase in salivary enzymes. This study shows that the salivary enzyme activity can be used as biomarkers to determine periodontal tissue damage, which may be useful in diagnosis, prognosis and evaluation of post therapy effects in periodontal disease.

Haririan H et al determined CgA and AA in saliva and serum in periodontal health and disease to assess their potential relationship to periodontitis. Patients with aggressive (AgP) (n = 24) and chronic periodontitis (CP) (n = 34) as well as healthy control (CO) (n = 30) individuals participated in this study. CgA and AA were determined in saliva and serum with enzyme-linked immunosorbent assay and an adapted clinical amylase test; salivary cortisol was determined using mass spectrometry. Clinical parameters of periodontal disease

were evaluated, and their possible correlations with stress-related biomarkers were assessed. Significantly higher CgA levels were found in the saliva of patients with AgP compared with those in patients with CP and CO individuals. Salivary cortisol levels were higher in the AgP group compared with those in patients with CP. No differences in serum CgA levels and salivary and serum AA activities were found among all groups. A positive correlation was revealed between salivary AA activity or salivary CgA levels and the extent of periodontitis. The results suggest an association of CgA and cortisol levels as well as AA activity in saliva with periodontitis, especially a significant relationship of salivary CgA and cortisol to AgP. Rai B et al explored the associations among periodontal disease, psychologic factors, and salivary markers of stress, psychoneuroimmunologic variables, and health behaviors. One hundred periodontitis patients were selected, and participants provided information on general health, chronic stress, and demographics. Stress markers (chromogranin A, cortisol,  $\alpha$ -amylase, and  $\beta$ -endorphin) were measured from saliva. A dentist assessed the presence of dental plaque on lingual and buccal surfaces, the gingival index, and the number of remaining teeth with periodontal disease. Stress and salivary stress markers were significantly correlated with clinical parameters of periodontal disease. Neglecting to brush teeth during stress was associated with missing teeth. After adjusting for stress variables, salivary cortisol and  $\beta$ -endorphin were significantly associated with tooth loss and periodontal clinical parameters. This study suggests that stress might be associated with periodontal disease through physiologic and behavioral mechanisms. In making diagnoses of psychiatric patients, the association between salivary stress markers and periodontal disease needs to be included.

#### CONCLUSION:

Within the limitations of the present study, it can be concluded that SAA levels elevates with increasing of severity of the periodontal disease. After periodontal

treatment, the level of SAA drops. Thus, SAA level can be used as a biomarker for gingival and periodontal diseases.

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