Salivary Amylase as a Biomarker in Health and Periodontal Diseases

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ABSTRACT

Introduction: Amylase is a key protein secreted in the saliva as a part of oral host immune response in periodontal disease. To detect its level according to the severity of periodontal disease, the present study was conducted to evaluate and compare salivary amylase level in healthy, gingivitis and chronic periodontitis subjects.

Material and Methods: In this case-control, cross sectional study, a total of 45 subjects (15 healthy, 15 gingivitis, 15 chronic periodontitis) were divided into groups A, B and C respectively. All groups were evaluated for salivary amylase and other clinical parameters at baseline and 6 weeks after scaling. Whole saliva samples were collected and amylase evaluation was carried out by kinetic assay method. The results were analyzed by ANOVA and paired 't' test.

Results: The results of this study showed that the level of amylase in subjects with gingivitis and chronic periodontitis was significantly higher than healthy controls ($P < 0.001$). After periodontal treatment, an amylase level decreased in gingivitis and chronic periodontitis subjects.

Conclusion: The results showed that there is a significant relationship between the level of salivary amylase and periodontal disease. Its level changes as per the inflammation of periodontal disease. An amylase may be considered as an inflammatory biomarker in periodontal disease.

Keywords: Amylase, Biomarker, Protein, Periodontal Disease, Saliva

INTRODUCION

Saliva is composed of a complex mixture of secretary products (organic and inorganic) primarily secreted by salivary glands. It is an important resource for evaluating physiological and pathological conditions in humans.[1] Human saliva has a protein content of about 0.5-3.0 mg/ml, majority of which is contributed by parotid gland. These proteins plays different biological role in digestion, lubrication and host defence.[2]

The enzyme salivary α-amylase (SAA) is one of the key protein which accounts for 60% of all proteins produced by the salivary gland.[3] Action of SAA is to initiate digestion process. Besides this, SAA displays inhibitory activity against micro-organisms, plays a major role in modulation of bacterial adhesion and growth on intraoral surfaces.[4] SAA is produced locally in the oral cavity by salivary gland in response to β adrenergic stimulation by the process of exocytosis.[5]

Periodontal diseases are chronic inflammatory disorders in which numerous inflammatory and immune mediators are released in response to bacteria and bacterial products.[6] Patients with periodontal disease have differences in the protein composition of whole saliva as it is an important component of the host oral immune defense. Such protein profile reflects the nature and amplitude of the host response given to a periodontal microbial challenge.[5] Analysis of saliva may be especially beneficial in the determination of current periodontal status, as well as to identify the patients at risk for periodontal diseases.[6]

As amylase is an abundant protein found in the saliva, the present study was design

1. To evaluate and compare SAA level in healthy, generalized chronic gingivitis and generalized chronic periodontitis subjects.
2. To compare SAA level before and after the non-surgical periodontal treatment (SRP) in generalized chronic gingivitis and generalized chronic periodontitis subjects.

MATERIAL AND METHODS

The study protocol was approved by institutional ethical committee. It was explained to each patient and written informed consent was taken. This case control, cross sectional study was performed at the periodontology department of CSMSS dental college and hospital, Aurangabad. After obtaining a written informed consent, a total of 45 subjects belonging to both the sexes were enrolled and divided into three groups namely group A, B and C; so that each group had 15 subjects.

Grouping was done according to 1999 classification system for periodontal diseases and conditions[35]

- Group A: Healthy subjects
  No evidence of clinical inflammation, sulcular bleeding and clinical attachment loss.
- Group B: Generalized Chronic Gingivitis
  Presence of BOP, clinical inflammation but no evidence of clinical attachment loss.
- Group C: Generalized Chronic Periodontitis
  >30% of sites involved, moderate to severe alveolar bone loss, clinical attachment loss > 3mm and PPD ≥

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5mm; and the amount of destruction consistent with local factors.

Subjects were selected according to the following criteria:

Inclusion criteria

- Systemically healthy subjects
- Subjects with age group between 30 – 60 years

Exclusion criteria

- Pregnancy
- Use of antibiotics within past six months
- Smokers
- Patients who received periodontal treatment within past six months

The following clinical and biochemical parameters were assessed

Clinical perameters

1. Oral hygiene index-simplified (OHI-S) (Green and Vermilion, 1964)
2. Plaque index (PI) (Turesky Gillmore Glickman modification of Quingly Hein, 1970)
3. Sulcular bleeding index (SBI) (Muhlemann H.R, 1971)
4. Periodontal pocket depth (PPD)
5. Clinical attachment level (CAL)

Biochemical parameter

1. Salivary amylase level

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.[7]

Estimation of salivary amylase

SAA level measured using $CNPG_3$, kinetic assay method using commercially available reagent kit (span, India).

Assay principle: α-amylase hydrolyses 2-chloro-p-Nitrophenyl - D – maltotriose ($CNPG_3$) to release 2 chloro-Nitrophenol (CNP), Maltotriose, and glucose. The rate of increase in absorbance due to formation of CNP is measured at 405nm and is proportional to the α-amylase activity in the sample.[8]

$$5\ CNPG_3 \rightarrow 3\ CNP + 2\ CNPG_2 + 2\ glucose + 3\ maltotriose$$

To perform the test, 1000 uL of amylase monoreagent was taken into the the sterile glass tube using calliberated micropipette. 20 uL of salivary supernatant was pipetted into the same test tube and mixed well. This reaction gave yellow colour due to the presence of amylase. After waiting for 60 seconds, level of SAA was checked with an autoanalyzer.

Procedure

At baseline, the above mentioned clinical and 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.

RESULTS

Results of the present study were divided to show-

1. Comparison of groups A, B and C at baseline by ANOVA

Group	Mean SAA ± SD	P Value	Result
A	48.629 ± 7.07	<0.001	Significant
B	77.967 ± 5.12		
C	120.36 ± 19.61		
SAA- Salivary α amylase; SD- Standard deviation			

Table-1: Comparison of mean SAA for groups A, B and C at baseline by ANOVA test

Group		Mean SAA ± SD	P value	Result
B	Baseline	77.967 ± 5.12	<0.001	Significant
	After SRP	50.435 ± 6.29		
C	Baseline	120.359 ± 19.61	<0.001	Significant
	After SRP	58.857 ± 8.14		
SAA- Salivary α amylase; SD- Standard deviation				

Table-2: Intra-group comparison of mean SAA for group B and C, at baseline and after SRP using 't' test

Group	Mean SAA ± SD	P Value	Result
A	48.629 ± 7.07		
B	50.435 ± 6.29	0.466	Not Significant
C	58.857 ± 8.14	0.001	Significant
SAA- Salivary α amylase; SD- Standard deviation			

Table-3: Comparison of mean SAA for group B and C (after SRP), with group A (At baseline) using 't' test

Group			Mean ± SD	P Value	Result
C	PPD	Baseline	4.099 ± 0.56	<0.001	Significant
	PPD	After SRP	3.055 ± 0.52		
C	CAL	Baseline	4.466 ± 0.55	<0.001	Significant
	CAL	After SRP	3.365 ± 0.47		

PPP- Periodontal pocket dept; CAL- Clinical attachment level; SD- Standard deviation

Table-4: Intra-group comparison of mean PPD and CAL for group C, at baseline and after SRP using 't' test

Group	Mean OHI –S ± SD	
	Baseline	After SRP
A	0.113 ± 0.11	-
B	1.68 ± 0.51	0.12 ± 0.03
C	3.02 ± 0.76	0.59 ± 0.27

OHI-S (Oral hygiene index-simplified); SD- Standard deviation

Table-5: Comparison of mean OHI-S for group A, B and C at baseline; and before and after SRP

Group	Mean PI ± SD	
	Baseline	After SRP
A	0.104 ± 0.09	-
B	0.693 ± 0.42	0.087 ± 0.04
C	2.06 ± 0.47	0.469 ±0.22

PI- Plaque index; SD- Standard deviation

Table-6: Comparison of mean PI for group A, B and C at baseline; and before and after SRP

Group	Mean SBI	
	Baseline	After SRP
A	0.022 ± 0.02	
B	0.791 ± 0.44	0.031 ± 0.01
C	2.419 ± 0.62	0.78 ± 0.42

SBI- Sulcular bleeding index; SD- Standard deviation

Table-7: Comparison of mean SBI for group A, B and C at baseline; and before and after SRP

test.

2. Intra-group comparison for group B and C at baseline and after SRP using 't' test.
3. Comparison of group B and C after SRP with group A at baseline using 't' test.

Data were presented as mean ± standard deviation.

1. Comparison of groups A, B and C at baseline
   Levels of SAA in groups A, B and C are shown (Table-1). Increased for mean SAA level was notice from group A to group C (P< 0.001).

2. Intra-group comparison for group B and C, at baseline and after SRP
   The mean SAA level was reduced in both group B and C, 6 weeks after the periodontal treatment (P< 0.001) (Table- 2).

3. Comparison of group B and C, after SRP with group A at baseline
   Comparison of SAA value of group B (After SRP) with group A (at baseline), showed non significant difference (P>0.001). But for group C, significant difference obtained (P-0.466) (Table-3).

Other recorded parameters PPD, CAL (Table- 4); OHI –S (Table -5); PI (Table- 6) and SBI (Table -7) also showed co-relation with SAA level.

DISCUSSION

Early diagnosis, treatment and prevention of progressive periodontitis are of critical importance because of the irreversible nature of this disease. Conventional periodontal diagnostic methods include assessment of clinical parameters are inherently limited in that only a historical perspective, not current disease status or prediction of future disease can be determined.[9] To overcome such disadvantages of conventional techniques, new era of biomarkers came into existence. The concept of a biomarker arose from the recognition of the appeal of being able to monitor health status, disease susceptibility, progression, resolution, and treatment outcome with respect to a number of common medical conditions.[10]

The present study was mainly focused on SAA level. Hence recorded SAA parameter has been discussed in detail further. When compared groups A, B and C statistically significant increased for mean SAA level was noticed from group A to group C (P< 0.001). This indicated that as inflammatory condition of periodontal disease increases, SAA level also increases. Many studies have compared SAA level in health and chronic periodontitis; and found significant increased of SAA in diseased condition. Henskens YMC (1996)[11] compared SAA level in 25 healthy and 25 chronic periodontitis subjects by enzyme-linked immunoabsorbent assay technique. Results recorded SAA level in healthy subjects and in chronic periodontitis subjects which was 76±43 U/ml and 155±103 U/ml respectively. Study by Hady H (2012)[6], Goncalves L da R (2010)[12] also shown higher SAA level in chronic periodontitis. Sanchez (201)1[5] obtained SAA level in health 89.63±11.0 U/ml, moderate periodontitis 122.52±6.8 U/ml, and severe periodontitis 136.94±11.2U/ml. Goncalves L da R (2011)[13] compared SAA in health and gingivitis, and showed abundant proteins including amylase in whole saliva of gingivitis subjects compared to healthy subjects.

Studies carried by Hyun C K (2010)[14], Kejriwal S (2014)[15], Hernandez-Castaneda A A et al (2015)[16] compared healthy, gingivitis, chronic periodontitis subjects for SAA level and supported the findings of present study. Study by Swati Kejriwal 2014[15] recorded SAA level by kinetic assay method and found significantly higher values in gingivitis group 95.7047 U/ml, in periodontitis group 125.01 U/ml as compared to the healthy group 77.2113 U/ml as seen in

present study.

Three reasons are stated in the literature to support the findings that SAA level increases in periodontal diseases which are as follows: i) The increased levels may be due to the response of salivary glands to inflammatory diseases like gingivitis and periodontitis resulting in increased synthesis and secretion of α-amylase so as to enhance the oral defense mechanism.[11,17] ii) Studies showed that α-amylase is a major lipopolysaccharide binding protein of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis and interferes with bacterial adherence and biofilm formation.[18] Thus, the high concentration of salivary α-amylase seen in present study suggests it to be an important defense molecule essential for the innate immunity in the oral cavity.[15] iii) The increased levels could partly be also due to an increased leakage of plasma proteins into saliva due to inflammation.[19]

It was observed that mean SAA level was reduced, 6 weeks after the SRP in group B and C. The study by Henskens 1996[20] compared SAA in chronic periodontitis patients before and after SRP. Re-evaluation was done 6 months after SRP. Results showed that the baseline SAA level was 150±89 U/ml which was reduced to 134±90 U/ml. Study by Sanchez A 2013[17] showed similar difference in SAA values of groups healthy, mild, moderate and severe periodontitis at baseline and 3 months after the SRP. After the periodontal treatment, inflammatory and bacterial load reduces on periodontium, which reduces the immune response, which further leads to decrease in secretion of amylase. Therefore SAA level was decreased after the periodontal treatment.

As stated earlier, scaling was performed as a non-surgical treatment modality which led to complete resolution of inflammation and created a healthy condition in gingivitis subjects. Therefore SAA level in group B (After scaling) was almost equal to the SAA level in group A (at baseline). Due to the inability of SRP treatment to completely eradicate the microbial and inflammatory component from deeper periodontal pockets found in chronic periodontitis, SAA level in group C (After scaling) was higher than group A (At baseline). This illustrated that complete eradication of bacterial and inflammatory component by open flap debridement would have reduced SAA level to the normal range.

The above studies have shown different range of SAA level, such wide variation in the concentration of SAA for the same parameter could be due to differences in the assay techniques.[21] Though there is variation in the level of amylase, its value increases in the periodontal disease as seen in the present study.

Saliva analysis is undoubtedly a valuable approach to identification of salivary markers for clinical diagnosis of periodontal diseases. Periodontal surveillance and disease diagnosis has been greatly advancing via the use of rapid point-of-care oral diagnostics i.e. assessing many biomarkers at single time. Such process helped to link together novel therapeutics to emerging diagnostic disease biomarkers. This approach should accelerate clinical decision-making and monitoring of episodic disease progression in a chronic infectious disease such as periodontitis.[22]

It is highly unlikely that a single biomarker will prove to be a stand-alone measure for predicting periodontal disease activity. A combined analysis of proteomic, genomic, microbial and other indicators are required to identify the set of biomarkers with the most favorable combination of sensitivity, specificity, reproducibility and relations with established disease diagnostic criteria, and reproducibility.[23]

The present study evaluated salivary amylase as biomarker which is highly specific and sensitive, which can be used along with other biomarkes for the clinical analysis and epidemiologic purpose.

There were few limitations of the conducted study:

i. Only SRP was performed as a treatment modality in the chronic periodontitis subjects before SAA detection. If thorough removal of inflammatory component by open flap debridement could have been done, it could reduce the amylase upto normal level.
ii. Microbiologic analysis was not performed in the present study. SAA prevents biofilm formation by preventing attachment of A. actinomycetemcomitans and P. gingivalis. Microbial analysis for A. actinomycetemcomitans and P. gingivalis along with amylase could have been led to better understanding of this concept.
iii. Present study analyzed SAA level 6 weeks after SRP. Repeated analysis of SAA for longer period of time might have showed stability or fluctuency in the level of SAA.
iv. Along with SAA, other biomarkers analysis might have led to the better understanding of progression of periodontal disease.

CONCLUSION

SAA level increases as the severity of the periodontal disease increases. After periodontal treatment level of SAA decreases in both chronic gingivitis and chronic periodontitis subjects. Level of SAA was found to be correlated with the clinical parameters recorded in the study.

In conclusion, present results have documented that SAA can be used as a biomarker to link new emerging diagnostic technique with novel therapeutic approach.

Best results in terms of early diagnosis of periodontal disease and patient's response to the therapy will be obtained if other biomarkers are evaluated along with amylase.

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