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Estimation of Salivary Amylase Levels in Smokers with and without Periodontitis: A Comparative Study

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Aim: ***The aim of this study is to estimate the levels of salivary amylase in smokers with and without periodontitis. Objective: To determine the levels of salivary amylase in smokers with and without periodontitis. Background: Amylases are the most important and abundant digestive enzymes in the salivary fluid produced by the parotid gland. They play an important part in early stages of carbohydrate hydrolysis. They occur in diverse quantities in various tissues of the human body. Being the most important digestive enzyme in the human salivary fluid, α-amylase is prone to alternations in response to the cell damage caused by chronic periodontitis. Conclusion: To determine whether there is any alterations in salivary amylase concentration in a smoker with and without periodontitis.***

Keywords: salivary amylase, periodontitis, smoking, saliva, biomarkers

1. Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganism or groups of specific microorganism which results in a progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession or both. There will be clinically detectable attachment loss, which is often accompanied by periodontal pocket formation and changes in the density and height of subjacent alveolar bone. The periodontal disease progression can further depend on certain modifying factors like systemic diseases, habits like smoking, usage of tobacco, nutritional deficiencies, etc.

A substantial body of evidence has demonstrated the detrimental effects of smoking to the health of the periodontium. Studies have shown the association between smoking and alveolar bone loss, tooth loss and severity of the disease progression.(1) The toxins present in cigarette smoke such as carbon monoxide, toxic substance like oxidating radicals, carcinogens and addictive psycho-active substance such as nicotine are absorbed into the lungs and also into the oral mucosa leading to the inflammatory response of the periodontal tissues.(2) As a result of these responses certain inflammatory markers are expressed in the serum, blood, and other body fluids like GCF and saliva. In India, the current tobacco smoker's population is about 14.0% of adults, where 24.3% are males and 2.9% are females. According to a study by Amano et al, periodontal diseases can be linked to more serious conditions such as cardiovascular diseases, complications of pregnancy, diabetes, etc. Axel Spahr et al through their study suggest that dental health and periodontal bone loss may be associated with coronary heart disease events even after adjustments for established cardiovascular risk factors.(3)

Amylases are the most important and abundant digestive enzymes in the salivary fluid produced by the parotid gland. They play an important part in early stages of carbohydrate hydrolysis. They occur in diverse quantities in various tissues of the human body. However, their presence is most prominent in the salivary fluid and pancreatic juice. Different types of amylases exist, including α- and b-amylase. Since the α-amylase type is a calcium metalloenzyme, it is almost inactive when calcium is not present. Most of the α-amylases are able to act at random locations along the polysaccharide chain. The final products of α-amylase reaction on a polysaccharide chain are small oligosaccharides of glucose and maltose.(4) The optimum pH of α-amylase is between 6.7 and 7.0 and it is the main digestive enzyme in the body. Being the most important digestive enzyme in the human salivary fluid, α-amylase is prone to alternations in response to the cell damage caused by chronic periodontitis. Tobacco consumption modifies several biological parameters including salivary alpha amylase. Certain studies have shown smoking increases the value of alpha amylase activity in serum and saliva whereas other studies have not shown any effect of the value of serum and salivary alpha amylase activity. It was suggested that the amylase aids against streptococcal bacterial adherence which inhibits further propagation on colonization of bacteria and may help regulate normal bacteria flora in the mouth Periodontal disease reflect the interplay between a pathogenic bacterial biofilm present on the root surface/periodontal pocket and host-derived inflammatory cells and molecules from periodontal tissue. This process results in loss of connective tissue and bone support and is a major cause of tooth loss in adults. Changes in the saliva protein composition upon development of periodontitis have been documented and there are evidences that these changes maybe a response of salivary gland to periodontitis, enhancing the protective potential of saliva (5) Studies have showed that salivary amylase output had increased in relation to the progression of the periodontal disease, increasing their salivary concentrations. The increase in salivary amylase levels might correlate with the progression of the disease would be a good indicator for the response of periodontal disease in smokers.

Therefore, the aim of this study is to estimate the levels of salivary amylase in saliva samples of smokers with and without periodontal problems, which can help in determining the progression of periodontal problems.

2. Material & Method

Study Design:

A randomized case-control study was designed. A total of 30 patients, from a Dental College in Chennai, were selected for this study. The patients were divided into two groups:

Group 1: smokers without periodontitis
Group 2: smokers with periodontitis

Inclusion criteria: The patient must be of 35 years of age or more, a chronic smoker for 10years or more and smoking at the rate of 10 cigarettes/day, and with plaque index > than 1, gingival index< than 1 for controls and > than 2 for cases. Russels periodontal index of > than 1 for cases (smokers with periodontitis)

Exclusion criteria: The patient must not have any other systemic diseases or habits and haven't taken antibiotic therapy in the last 6 months, not used any mouth washes, did not have any oral prophylaxis or any other dental treatment in the past one year. No other habits apart from smoking

Clinical parameters examined: Gingival index by Loe and Silness, plaque index by Silness and Loe by means of disclosing solution, Russellsperiodontal index by means of mouth mirror and Williams periodontal probe.

Gingival index:
0 – normal healthy tissue, 0.1-0.9 – good, 1.0-1.9 – fair, 2.0-3.0 – poor
Plaque index:
0 – excellent, 0.1-0.9 – good, 1.0-1.9 – fair, 2.0-3.0 – poor

Russels periodontal index:

Interpretation: 0 – 0.2 clinically normal supportive tissues, 0.3-0.9 – simple gingivitis, 0.7-1.9 – beginning destructive periodontal disease(reversible), 1.6-5.0 - established destructive periodontal disease (irreversible), 3.8-8.0 – terminal disease (irreversible)

Sample collection:

5 ml of unstimulated saliva was collected in a sterile container and estimated for the level of salivary amylase. The salivary amylase concentration was measured using a semi auto analyser ERBA CHEM 5 PLUS with GenX a-Amylase-ML (Proton). The method used was CNPG3 method.

The study was approved by the ethical committee. Informed consent was obtained from every patient.

Statistical Analysis:

The collected data was analysed with SPSS 16.0 version to describe about the data descriptive statistics mean and S.D were used. To find the significance difference between the (Cases & Control groups) the Unpaired sample t-test was used. In the above statistical tool the probability value.05 is considered as significant level

3. Result

Table 1and 2 shows the average values (±standard deviation) of salivary amylase activity of study population, it was found that theres an increased salivary amylase concentration in smokers with periodontitis. The results was found to be statistically significant (p=.004). Figure 1 shows the comparison levels between control and cases.

Table 1: Average values (±standard deviation) of salivary amylase activity of study population

	mean and standard deviation values	P value
smokers with periodontitis (15 cases)	164.93±73.08	.004
smokers without periodontitis (15 cases)	67.33±23.44	.004

Table 2

		t-test for Equality of Means				
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Values	Equal variances assumed	0	-97.6	19.816	-138.191	-57.009
	Equal variances not assumed	0	-97.6	19.816	-139.436	-55.764

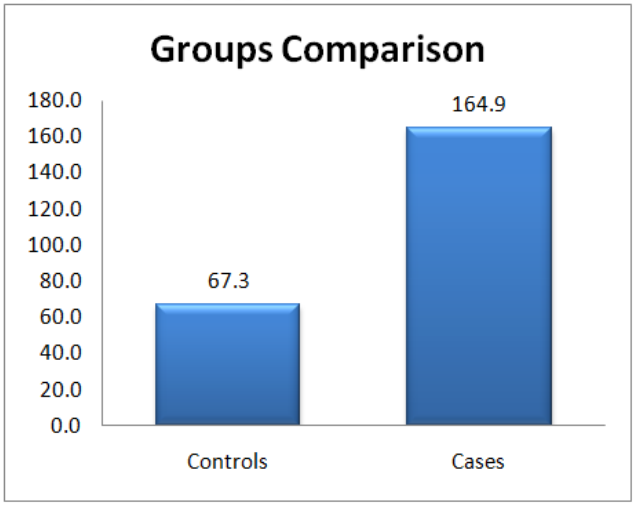

Figure 1

4. Discussion

Periodontal diseases are a group of condition affecting the supporting tissues of the tooth. The severity of periodontitis could be diagnosed on the basis of its typical clinical parameters which includes periodontal probing pocket depth, clinical attachment and gingival recession. These are multifactorial diseases where many risk factors affect the progression of the disease. Among those risk factors, cigarette smoking has been found a major risk factor associated with generalized forms of periodontitis. This is due to the various toxic products present in the cigarette. Cigarette smoke contains at least 500 potentially toxic substances such as hydrogen cyanide, carbon monoxide which has the potential to change haemoglobin to carboxyhaemoglobin, free radicals, nicotine, potent carcinogens like nitrosamine, and a variety of oxidant gases causing platelet activation and endothelial dysfunction (6) Cigarette smoking alters the microbial flora in the oral cavity thus altering the concentration of salivary constituents. Smoking reduces the blood flow thus depressing the host immune response and impairing healing. Studies have found that smokers have less success rate with open flap debridement, osseous resection, soft tissue and bone graft procedures, and guided tissue regeneration procedures. Furthermore, the rate of implant failure is significantly higher than a non-smoker (7)

The evaluation of severity of the disease which reflects disease activity in periodontal disease is very important for diagnosis and selecting clinical treatment. Studies have shown that biochemical examination is a more reliable diagnosis of periodontal disease where clinical and diagnostic test have shown that several components are increased in gingival cervicular fluid, serum, saliva and other body fluids. (8) Due to a change in the bioflora of the oral cavity in periodontits, studies have shown that there is an increase in the level of salivary amylase protein.

Hence this study was undertaken to estimate the concentration of salivary amylase in smokers with and without periodontitis. A group of 30 patients who are smokers were selected and divided into two groups, 15 of them being smokers with periodontitis and another 15 are smokers without periodontitis. The 5ml of unstimulatedsaliva sample for the estimation of salivary amylase concentration was collected in a sterile container. The salivary amylase concentration was measured using a semi auto analyser ERBA CHEM 5 PLUS with GenX a-Amylase-ML (Proton). The method used was a colorimetric method using 2-chloro-4-nitrophenyl-maltotrioside (CNPG3). Saliva can be used as a diagnostic tool, being a non-invasive method for the assessment of early detection of proteins which can serve as a diagnostic tool for the early disease progression. Amylase is an enzyme produced mainly by parotid gland which plays a role in maintaining mucosal immunity. The normal range of salivary amylase is 25-110 U/L. Based on the results obtained; this study shows that there is a significant difference in the salivary amylase concentration in smokers with periodontitis compared to smokers without periodontitis where there is increased salivary amylase concentration in periodontitis.

Components of saliva play an important role in the colonization and metabolism of bacteria in the oral cavity therefore it can be used as a reliable diagnostic tool. The collection of saliva is easy and is non invasive, thus the estimation of salivary amylase levels can be assessed for the progression of periodontal disease. This diagnostic property of saliva helps to arrest the disease in an early stage and improve the treatment modalities. Therefore, more studies can be conducted to estimate the levels of biomarkers in saliva to prevent further progression of the disease.

5. Conclusion

Based on this study, periodontitis shows a major change in concentration of salivary amylase. Smoking being an etiological factor of periodontitis can worsen the clinical parameters of periodontal health. Saliva collection being noninvasive and easy method, it can be used as a reliable diagnostic tool.

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