ORIGINAL ARTICLE

Impact of Smoking on the Salivary Contents in Chronic Generalized Periodontitis Patients

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Abstract

Background: Chronic periodontitis is an inflammatory disease caused by specific microorganism, resulting in progressive destruction of the supporting tissue of the dentition. Tobacco smoking, especially cigarette smoking, may be a risk factor for periodontitis. Radiographs may be insufficient in diagnosing active disease areas, assessing medication response, or determining susceptibility to future disease progression. Saliva markers, such as alkaline phosphatase (ALP), amylase, salivary thiocyanate (SCN), and total antioxidant capacity (TAC), may be proposed as tests for diagnosis as well as prognosis of periodontitis. This study was undertaken to compare SCN, salivary amylase, and ALP and TAC level in smokers and nonsmokers with generalized chronic periodontitis.

Materials and methods: The present cross-sectional study involved 60 subjects of both sexes diagnosed with periodontitis, categorized into two groups: Group I comprised smokers with generalized chronic periodontitis (S + P), while group II consisted of nonsmokers with generalized chronic periodontitis (NS + P). Clinical metrics such as plaque index, gingival index, probing depth, and clinical attachment level were documented and evaluated. Salivary samples were examined for levels of ALP, amylase, SCN, and TAC. Statistical analysis was conducted utilizing the Student's unpaired *t*-test.

Result: The activity of salivary ALP and salivary amylase were elevated in group I (S + P) in contrast to group II (NS + P) ($p < 0.0001$). The SCN levels were higher in chronic smokers compared with nonsmokers. Whereas TAC levels were found to be in low level in group I than group II of study.

Conclusion: Significant correlation of increased salivary ALP and amylase activity was found between S + P than NS + P. SCN was reported to be positive in S + P indicating presence of SCN in saliva of periodontitis patient. Thiocyanate was found in group I compared with group II, indicating oxidant overload exceeding capacity of antioxidant. Smoking can be an accelerating factor for periodontal destruction. Patients should be evaluated for smoking history before initiating periodontal therapy subsequently followed by appropriate counseling.

Keywords: Alkaline phosphatase, Nonsmokers, Periodontal disease, Salivary amylase, Smokers, Thiocyanate, Total antioxidant capacity.

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Introduction

Periodontitis is an inflammatory multifactorial condition triggered by a particular microorganism or a group of specific microorganisms, leading to gradual destruction of the alveolar bone and connective tissues and possible tooth loss.[1] Besides the direct causative role of bacteria, cigarette smoking and tobacco chewing are regarded as significant environmental risk factors for the onset and advancement of periodontitis.[2]

Periodontal disease is still predominantly diagnosed using radiographs to detect periodontal pockets, loss of connective tissue attachment, and/or evaluation of alveolar bone loss. Conventional criteria in periodontology often fall short of identifying active disease site, assessing medication response, or assessing future disease progression.

Saliva has been used in the past few decades as a new diagnostic fluid and is an important source of clinically significant information, as it contains biomarkers that reflect distinct physiologic aspects of periodontal diseases.[3]

Tobacco smoke comprises a blend of gases from combustion along with suspended particulate matter. Carbon monoxide and hydrogen cyanide are two of the many components of the gas phase. Serum SCN and CO, which is produced as a byproduct of hydrogen cyanide, can serve as effective indicators of smoking. Each cigarette releases about 30–200 µg of hydrogen cyanide into the smoker's mouth.[4] Tobacco smoking, particularly cigarette smoking, is identified as the most important environmental risk element in periodontitis. Research indicates that smokers are two to six times more prone than nonsmokers to develop periodontitis.

Amylase is released through exocytosis by the parotid and submandibular glands when stimulated by β-adrenergic activity.[5] Salivary glands may adapt to inflammatory circumstances by increasing the synthesis of salivary amylase, hence increasing the

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defensive potential of saliva. This explains why people with chronic periodontitis have higher amylase concentrations in their saliva.[5]

Cigarette smoke includes free radicals and compounds that produce free radicals in both gaseous and particulate forms, which can interact with polyunsaturated fatty acids in cell membranes and nucleotides in DNA. Antioxidants help maintain the integrity of structures and tissues by neutralizing the harmful effects of free radicals.[6]

Enzymes are biological catalysts that carry out highly specialized biological reactions in a strictly controlled manner. An enzyme, like a chemical catalyst, works by reducing the activation energy of a reaction, causing the formation of products from the substrates. Humans have alkaline phosphatase (ALP), which is an enzyme involved in intracellular destructive processes and cellular damage. It contains bicarbonate and phosphate ions, which aid in acid buffering. Smoking reduces ALP activity by altering the pH of the oral environment, which has negative effects.[7]

During inflammation, polymorphonuclear cells (PMNs) secrete ALP as do osteoblasts and periodontal ligament fibroblasts during bone production and periodontal regeneration, respectively.[8]

The current research was conducted to assess the activity of salivary amylase and ALP and to compare the levels found in S + P and NS + P groups. Presence of salivary SCN indicates impact of smoking; hence, it was measured to check the extent of presence. Total antioxidant capacity (TAC) in smokers and nonsmokers with chronic generalized periodontitis was measured to assess the oxidant load in periodontitis patients.[9–14]

Materials and Methods

The current cross-sectional study comprised 60 patients aged between 30 and 70 years, who were randomly chosen from the outpatient department of the Dental Surgery at Gandhi Medical College and Hamidia Hospital in Bhopal, India. The patients were evenly split into two groups, with 30 individuals in each group: smokers with generalized chronic periodontitis (group I) and nonsmokers with generalized chronic periodontitis (group II).

Inclusion Criteria

- Healthy individuals without any sign of oral and respiratory diseases as determined from their physical examination and history.
- The smokers should have had a history of smoking 10 cigarettes or more per day for at least a year.
- Patients suffering from generalized chronic periodontitis (>30% of sites involved, probing pocket depths (PPDs) ≥5 mm, clinical attachment loss (CAL) >3 mm, and local factors).

Exclusion Criteria

- No antibiotics utilized in the past 3 months (as the pathophysiology of periodontitis is changed in these conditions).
- Patients undergoing consistent treatment for any systemic condition that could affect the ALP levels.
- Individuals taking multivitamins or antioxidant supplements.

Ethical Clearance

Approval for ethical consideration was secured from the Institutional Ethical Committee.

Procedure for Data Gathering

All patients who met the inclusion criteria were provided with comprehensive information about the study, and only those who signed the written informed consent, which guaranteed their confidentiality, were included. They were also presented with the option to withdraw from the study at any time without needing to provide a reason. After agreeing to participate, a translated consent form was filled out and signed by the study participants.

Assessing Clinical Parameters

For the clinical evaluation, a minimum of 18 teeth from each participant were examined, and the gingival index, developed by Loe and Silness, as well as the plaque index, created by Silness and Loe, were calculated for each group. Additionally, PPD and CAL were measured to the nearest millimeter using a standardized calibrated Williams graduated periodontal probe at four locations around each tooth in every group. One trained examiner documented all measurements while the subject was positioned in a dental chair under adequate lighting to minimize variability in measurements taken by the same examiner.

Saliva Collection and Analysis

Saliva was collected in a sterile disposable plastic container from patients who were instructed to avoid eating for 1 hour prior to sample collection. Following this, the saliva samples were promptly sent to the laboratory, where they were centrifuged at 1,000 rpm for 15 minutes, and the supernatants were preserved at (–20° C) until assay time. Amylase levels were determined using a direct substrate assay. Alkaline phosphatase was estimated using pNPP-AMP (International Federation of Clinical Chemistry) kinetic assay. Levels of SCN were analyzed using a noninvasive test in which 3 cc of saliva was added with five drops of 0.5% of ferric chloride ($FeCl_3$) and mixed. Later, few drops of concentrated HCl was added and mixed. Orange to red color development showed the presence of SCN in smokers. Further, TAC was estimated using the Kovacevic assay (Table 1).

Statistical Analysis

All the data was noted in MS Excel sheet, and using Epi Info software statistical analysis of the results was done. All variables are reported as mean ± SD (standard deviation). A p-value of less than 0.05 is deemed statistically significant. The difference in significance between groups was evaluated using Student's unpaired t-test.

Results

Table 1: Distribution of population based on demographic characteristics

S. No.	Variable	Groups	Group I	Group II
1.	Age-group	Less than 30 yrs	1	2
		31–40 yrs	9	7
		41–50 yrs	5	9
		51–60 yrs	9	6
		>60 yrs	6	6
2.	Area of living	Rural area	13	15
		Urban area	17	15

Table 2: Salivary level of ALP, amylase, and TAC in group I (S + P) and group II (NS + P)

S. No.	Salivary enzyme	Level	Group I	Group II	Fisher exact test statistic value
1.	ALP	Normal	3	0	<0.00001
		Increased	27	27	
2.	Amylase	Normal	0	30	<0.00001
		Increased	30	0	
3.	TAC	Normal	0	2	0.2051
		Decreased	30	28	

Table 3: Statistical level of study parameters in Study Groups

S. No.	Salivary enzyme	Group I	Group II	t-value	p-value
1.	ALP	675 ± 21.0	222 ± 89.9	8.7713	<0.0001
2.	Amylase	122.2 ± 10.5	67.1 ± 22.7	10.2265	<0.0001
3.	TAC	638 ± 114.2	832.7 ± 8.3	8.8273	<0.0001

Unpaired *t*-test, 95 % confidence interval

Discussion

In this research, the levels of salivary enzymes and SCN in smokers with chronic generalized periodontitis were compared with those in nonsmokers with the same condition. Periodontitis represents a complex, long-lasting inflammatory disorder affecting the oral cavity, leading to the gradual deterioration of the alveolar bone and connective tissues, which may eventually result in tooth loss. The etiopathophysiology of periodontitis, marked by the loss of connective tissue and the formation of pockets due to alveolar bone loss, is complicated and involves various bacteria along with the effects of different biological substances. As a multifactorial condition, it encompasses various risk factors that can influence the progression of the disease. Smoking is particularly recognized as a major risk factor for periodontitis. Once the disease begins, the activation of the immune response in the patient triggers the release of various metabolic byproducts at the junctions of the tooth and periodontal pockets; these include harmful cellular enzymes, cytokines, chemokines, and other proinflammatory substances that contribute to tissue damage. Many biomarkers found in gingival crevicular fluid (GCF), saliva, and serum have been evaluated as diagnostic indicators for periodontal diseases, showing high levels of specificity and sensitivity. However, it is unlikely that there exists a single independent biomarker for periodontitis due to its complex mechanisms, thus prompting ongoing research into various combinations of biomarkers in periodontal investigations. The identification of periodontitis primarily relies on clinical and radiographic findings, though salivary enzymes significantly contribute to diagnosis and the future management of periodontal health.

Variations in age-groups with periodontitis have been noted, but a pattern of older age and periodontitis was seen. This suggests that smokers in middle age are at a higher risk of developing periodontitis. However, no substantial correlation was found regarding the demographic orientation of rural and urban areas.[15,16]

The activity of ALP was observed to be increased in both groups I and II (smokers and non-smokers) (Tables 2 and 3). Alkaline phosphatase is an indicator of significant inflammation and cellular damage in the periodontium, as a larger amount of ALP is released from injured periodontium cells due to altered metabolic processes (given that ALP is generated by PMNs, osteoblasts, macrophages, periodontal fibroblasts, and plaque bacteria found in the periodontal pocket). Lubaba et al. suggested that the decline in salivary osteocalcin levels due to smoking, along with a slight increase in ALP among smokers, might explain the detrimental effects of smoking on periodontal health. Similarly, Mohammad K et al. recommended the use of both GCF and salivary ALP levels as diagnostic markers for periodontitis. Increased levels of ALP in GCF samples from smokers with periodontitis may contribute to the heightened rate of alveolar bone loss seen in smokers.[16,17]

The activity of salivary amylase was found to be significantly higher in smokers than in nonsmokers with periodontitis (Table 3). The TAC was found to be reduced in smokers when compared with nonsmokers, indicating an overload of oxidants in smokers. Smoking and periodontitis diminished the antioxidant ability of saliva and other bodily fluids. They raised enzymes associated with oxidative stress.[18–20] The activities of antioxidants are influenced by various factors, such as diet, stress, exercise, and smoking. In reality, smoking causes a disparity between reactive oxygen species and antioxidant levels, thereby resulting in oxidative stress.

Salivary SCN has been noted to be positive in periodontitis patients who are chronic smokers, indicating the presence of cyanide in the mouth (Table 3). This is because the primary origin of SCN is tobacco smoke, which is inhaled into the lungs and subsequently converted to SCN.[21,22] Borgers and Burckhard discovered that the elevated cyanide levels in tobacco smoke can linger in the mouths of smokers for a period, and the resulting contaminated saliva may misleadingly elevate the cyanide levels.[23]

Conclusion

The current research assessed and contrasted the levels of salivary SCN, amylase, ALP, and TAC in smokers vs nonsmokers with generalized chronic periodontitis. Numerous studies have focused on measuring ALP levels in GCF and saliva, but limited research exists on detecting SCN, amylase, and TAC in the saliva of smokers. The process of saliva collection is noninvasive and straightforward, rendering it a reliable diagnostic method. Furthermore, among smokers suffering from generalized chronic periodontitis, a significant correlation was observed between increased salivary ALP levels and decreased salivary amylase, SCN, and TAC levels in comparison with nonsmokers. Therefore, their concentrations could act as clinical indicators in the identification and treatment of periodontal disease.

Limitation of Study

Two major limitations in this study could be addressed in future research:

- The sample size taken for the study to be increased to obtain better insight.

- Radiographs and blood samples were not taken in this study, which may have illuminated the extent of bone loss and the comparison of blood-to-salivary values.

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