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SYNERGISTIC EFFECT OF SMOKELESS TOBACCO AND CHRONIC PERIODONTITIS ON SALIVARY CATALASE – A CROSS SECTIONAL STUDY

Periodontology

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ABSTRACT

Background: Smokeless tobacco(ST) imperil periodontal health and also produces imbalance between reactive oxygen species(ROS) and antioxidants(AO) such as catalase(CAT) resulting in oxidative stress(OS). Hence, our study hypothesis is to investigate and compare salivary catalase levels in healthy, gingivitis, CP and CP with ST and to compare and correlate salivary catalase with clinical parameters.
Methods: Hundred subjects were divided into 4 groups of 25 each, healthy, gingivitis, CP and CP with ST. Saliva samples of subjects were collected and analyzed for catalase using UV spectrophotometer at 240nm. Statistical analysis was performed using ANOVA and tukey's post hoc analysis.
Results: Salivary catalase levels were found to be significantly lower in chronic periodontitis with smokeless tobacco followed by chronic periodontitis, gingivitis and healthy subjects ($p<0.001$).
Conclusion: Salivary catalase is valuable tool in periodontal disease diagnosis, prognosis, acts as preventive, therapeutic and in early planning of treatment modalities.

KEYWORDS

catalase, periodontitis, smokeless tobacco.

INTRODUCTION

In normal physiology, equilibrium is upheld among reactive oxygen species (ROS) activity and antioxidant(AO) shielding capacity. When there is disproportion, caused either by an augmented ROS production or diminution in AO shielding activity, oxidative stress results. One of the causes of generation of oxidative stress is tobacco consumption, which generates free radicals. The alkaline conditions perceived in betel nut chewing errands formation of free radicals. [1]

It is widely accepted that free radicals in diseases like rheumatoid arthritis, chronic obstructive pulmonary disease, AIDS, atherosclerosis and more recently periodontal disease play an important role in onset and progression of disease. Emerging support for conviction that oxidative stress may be vital part of enigma of the pathogenesis of periodontal disease. Periodontal disease, contemplate an inflammatory disorder that injures tissue through intricate communication among periodontopathic bacteria and host defense systems.[2-4]

Periodontal pathogens and their products trigger host defense mechanisms and stimulate neutrophils which releases ROS and results oxidative killing of bacteria in biofilms. However, release of these ROS outside of phagocytes is unfavorable for surrounding tissues, which leads to pathogenesis of various inflammatory diseases, through harmful oxidative reactions. [2, 5]

These oxidative products are detected in various body fluids such as blood, saliva and gcf. Saliva contains various antioxidants which contribute almost 70% of total radical-trapping antioxidant capacity. Saliva contains non-enzymatic antioxidants such as uric acid, albumin, glutathione, vitamins A,C, etc. and enzymatic such as superoxide dismutase, glutathione peroxidase, catalase, etc. which neutralize free radicals. [3, 6]

Catalase (CAT) is an present in both aerobic and anaerobic organisms. It is located in all major sites of hydrogen peroxide (H_2O_2) production in cellular environments such as peroxisomes, mitochondria. Catalase and along with several peroxidases catabolize H_2O_2 enzymatically into water and oxygen,helps in protecting cells which are generated within from H_2O_2. Catalase plays an important role by acting as an adaptive response to cells by withstanding various tolerance to oxidative stress. [7]

Since, first line defense antioxidants are superoxide dismutase, glutathione peroxidase and catalase, to our knowledge there are no studies which showed relationship between different forms of smokeless tobacco - gutka, snuff users, betel quid with CP and catalase. Hence, the present study aimed to investigate and compare levels of salivary catalase level in healthy, gingivitis, CP and CP with smokeless tobacco subjects and to compare and correlate the levels of salivary catalase with clinical parameters.

METHODOLOGY

Study Population

The power of this study was 80%, at 95% confidence interval and margin of error being 5%, a sample size of hundred participants was derived. Subjects, males and females, in age group 25 to 65 years were randomly selected from outpatient section of Department of Periodontics, P.M.N.M. Dental College, Bagalkot, Karnataka, India. The study was approved by institutional ethical committee. All potential participants were clearly explained regarding need and design of the study.

A written duly signed informed consent was obtained from the participants and were divided in to 4 groups of 25 each as, Group I: who had no systemic diseases and showed absence of clinical and radiographic signs of periodontitis and with atleast 20 teeth present. Group II: subjects with plaque present at gingival margin, change in color, contour, bleeding on probing (BOP) on provocation. Group III: subjects with BOP, CAL $\geq$3 mm at > 30% sites in the mouth, atleast 20 teeth present. Group IV: subjects who are current tobacco users, who reported snuff or chewing tobacco use at least 20 times and who

reported using snuff or chewing tobacco at time of interview.[8, 9]

Subjects with history of any antibiotic/anti-inflammatory/ corticosteroid therapy for 6 months prior to study, any known systemic disease or conditions, history of smoking, alcohol consumption, vitamin/minerals or antioxidants supplements intake during the last 3 months, and periodontal therapy 6 months prior to study and pregnant/lactating women were excluded from the study. [3, 10]

A short-structured case history was recorded to obtain age, sex, occupation, cigarette smoking and drug history. It included details of smokeless tobacco consumption, dietary supplements such as vitamins, drug intake, alcohol, medical history etc. [10]

Determination of clinical parameters

Full mouth periodontal examination was performed by a single examiner after proper grouping of the subjects. The periodontal parameters like gingival index (GI) (Loe and Silness 1963) [11], plaque index (PI) (Loe and Silness 1964) [12], bleeding on probing (BOP) (Muhlemann H R and Son S1971) [13], pocket probing depth (PPD) [14] , clinical attachment level (CAL) were assessed using a Williams periodontal probe by a single examiner.

Saliva Sample Collection

About 5ml of unstimulated saliva was collected from each subject into eppendorf tubes on same day. To minimize circadian influences, samplse were collected between 09:00 a.m to 11:00 a.m at least 1 hour after eating or washing mouth. During collection subjects were comfortably seated and instructed to rinse mouth thoroughly with water,head tilted slightly down. Saliva was allowed to pool in the floor of mouth for 5minutes by leaning forward. Subjects were then told to spit into 3ml eppendorf tube every 60 seconds for 10 minutes or when the subject experienced an urge to swallow the fluid accumulated in floor of the mouth. [5, 10]

Biochemical Analysis

The samples were centrifuged at 3000g for 5 minutes and the clear supernatant was gently pipetted out into another clean eppendorf tube and stored at -20°C until analyzed. Catalase activity was assessed according to method of Claiborne (1985) [15] The assay mixture consisted of 1.95ml phosphate buffer (0.05M, pH7.0), 1.0ml hydrogen peroxide (0.019M), and 0.05ml saliva sample (10%, w/v) in total volume of 3.0ml. Changes in absorbance were recorded at 240nm. Catalase activity was calculated in terms of nM H2O2 consumed/mg protein. [10,15]

Statistical Analysis

The data collected was analysed using computer software IBM SPSS statistics 20.0. Descriptive and inferential statistics, ANOVA and tukey's post hoc analysis were used. Data were expressed as mean and standard deviation. A $p<0.001$ were considered statistically significant.

RESULTS

A total of 100 subjects, 47(47%) males and 53(53%) females, age ranging 25 to 65 years, were enrolled in this cross-sectional biochemical study. Age and sex matching was not possible for the four groups, as subjects were recruited for study as and when they came to college outpatient department for dental examination.

The demographic variables of study groups are presented in [Table 1]. There was significant difference with respect to age and no significant difference with gender between groups. Highly significant mean clinical parameters i.e GI, PI, BOP, PPD & CAL was found to be higher in CP with smokeless tobacco subjects followed by CP, Gingivitis, Healthy ($p<0.001$). An antioxidant marker, catalase in unstimulated saliva, showed highly significant difference with lower catalase levels in CP with smokeless tobacco subjects followed by CP, gingivitis and healthy subjects($p<0.001$),[Table 2]. Also, catalase levels demonstrated direct positive correlation and were statistically significant ($p<0.001$). [Table 3].

Table 1: Intergroup comparision of demographic variables (Mean±SD) using ANOVA test

Variables	Healthy	Gingivitis	CP	CPSL
Age (years)	30.12±7.5	33.48±8.91*	45.04±11.23*	43.12±11.60*
Gender(Male/Female)	8/17	10/15	13/12	16/9

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**- shows significance difference from control group)

Table 2: Comparision of clinical parameters (Mean±SD) using ANOVA test in all 4 groups.

Variables	Healthy	Gingivitis	CP	CPSL
GI	0.37±0.22**	1.34±0.69**	2.4±0.23**	2.46±0.44**
PI	0.33±0.22**	1.34±0.69**	2.3±0.45**	2.4±0.23**
BOP	0.3±0.26**	1.51±0.69**	2.58±0.23**	2.76±0.39**
PPD (mm)	2.12±0.33**	2.32±0.47**	6.36±1.07**	7.28±1.1**
CAL (mm)	0.00±0.00**	0.00±0.00**	7.6±1.10**	7.92±1.22**
Catalase(Unit /ml protein)	27.69±5.9**	25.06±5.4**	22.08±4.4**	19.86±3.6**

(mm- millimeter) ($p < 0.05$ - Significant, $p < 0.001$ - Highly significant**- shows significance difference from control group)

Table 3: Intergroup comparision of catalase levels between all 4 groups using tukey's post - hoc analysis

Variables	Healthy	Gingivitis	CP	CPSL
Healthy	-	0.248	<0.001**	<0.001**
Gingivitis	0.24	-	0.157	0.002*
CP	<0.001**	0.157	-	0.397
CPSL	<0.001**	0.002*	0.397	-

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

DISCUSSION

Periodontal disease is the major outcome, whenever immune reaction occurs between pathogenic bacteria and host body with sub-gingival dental plaque playing major role for inflammation within periodontium. Various factors play vital role in progression of periodontal disease like interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), β interferon, and tumor necrosis factor-α (TNF-α) which are being translocated and facilitated by H2O2 along with phosphorylation of NF-K B-IK B complex activating NK-KB NF. Tissue damage increases due to increase in concentration of MMP(matrix metalloproteinases) and osteoclasts activation by NF-kβ and AP-1, hence leads to overproduction of lipid peroxides, inflammatory mediator and oxidised proteins. In turn macrophages, neutrophils and fibroblasts accumulate to generate more ROS and hence more tissue destruction forming vicious cycle. [16,17]

Among all markers for periodontal disease, host derived enzymes in saliva appear to be best indicators as found by Kaufman and Lamster [18] and by Kinney et al.[19] Although stimulation of saliva flow increase, saliva volume yet it leads to disruption of AO concentration. Hence in the present study unstimulated saliva samples were collected. [6]

Bleeding on probing can be expected from patient with inflammation and pocketing which leads to contamination of saliva samples. In periodontal disease, reduced salivary antioxidant and increased oxidative damage occurs as repored by Shulley and Langley-Evans[5] where saliva sample was taken after dental examination. One factor that affected study findings is leakage of antioxidants from plasma component into saliva. To avoid this, in current study all samples were collected prior to dental examination. [10]

Studies on catalase activities in relation to periodontal tissues or the oral cavity are scanty and the results are conflicting. Ellis et al.[20] found a significant and progressive reduction in catalase activities in saliva and within gingiva adjacent to deeper pockets. Marton et al. [21]showed that catalase activities were similar in periapical granuloma and healthy gingiva.

Catalase levels were found to be lower in study groups when compared to healthy group in the present study. This could be due to, in healthy conditions, cellular respiration in the mitochondria seemed to be more. As amount of ROS generated is less, the amount of antioxidants required to counteract it is less thus large amount of AO is preserved. The current results demonstrate that the antioxidant capacities are significantly lower in gingivitis and periodontitis sites and agree with report of Thomas B et al. [22]

The quantity of ROS piles up as destruction progress from gingivitis to periodontitis and can lead to much tissue destruction directly or indirectly. [3] Depletion of defensive antioxidants such as catalase released by various phagocytes leads to further accumulation of ROS. Free radical activity and destruction of protective antioxidant species can be the cause of low catalase levels in patients with CP in this study

which was in accordance with study by Punj A et al. [23] and which was against the study done by Thomas B et al. [24]

Chronic periodontitis with smokeless tobacco are more prone to oxidative stress due to increasing load of free radicals in body due to chemical carcinogens present in tobacco. Our study showed that tobacco habit significantly reduced catalase levels in chewers emphasizing that tobacco habit has a definitive impact on antioxidant enzyme status which might be an outcome of synergistic effect of antioxidant diminution due to ongoing free radical activity and destruction of protective AO species by ST and CP which was in accordance with study by Sirisha et al. [25]

Salivary catalase have important therapeutic implications in terms of the use of antioxidants in periodontal therapy to reduce oxidative stress in periodontal diseases thereby preventing periodontal tissue destruction which will enhance the prognosis.

Limitations of the present study been smaller sample size and various other differences such as race and population variations don't allow generalizing these findings on to the entire population. Therefore, further studies with long term interventional studies with larger sample size and on a multi-center level including population of various races are required to consolidate findings of the present study. All these limitations warrant further studies of prospective, interventional, and experimental study designs.

CONCLUSION

Smokeless tobacco results imbalance between AO and ROS, thus this study suggests that periodontitis along with smokeless tobacco leads to significant changes in AO enzyme activity in saliva. Smokeless tobacco habit coupled with periodontal lesion would probably have synergistic effect in lowering the bodies antioxidant status paving way for an enhanced oxidative stress. Hence, an early intervention of tobacco habit and its related oxidative stress is recommended.

A chair side test for measurement of salivary catalase is reliable, easy and inexpensive. Hence, a chairside test of catalase can be a valuable tool in periodontal disease diagnosis, prognosis, acts as preventive, therapeutic and in early planning of treatment modalities.

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