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Original Research

A cross sectional study to find a relationship between serum catalase and glutathione peroxidase among chronic periodontitis patients

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ABSTRACT

Background: Periodontal disease is a very common observation among dental patients these days. Till date, no study has been done correlating the levels of serum catalase and glutathione peroxidase among chronic periodontitis patients who are tobacco chewers.

Purpose: To find a relationship between catalase and glutathione-peroxidase levels in Chronic Periodontitis (CP) patients who are non-tobacco users, smokers and smokeless tobacco (gutkha) users & periodontally healthy subjects.

Materials and Methods: This cross-sectional study included 120 subjects ranging in age from 18 to 60 years. Participants were divided into four groups: 30 healthy subjects (H) (group-A), 30 subjects with chronic periodontitis (CP)(Group-b), 30 smokers with CP (CPS)(Group-c) and 30 smokeless tobacco users with CP (CPSTP)(Group-d). The clinical parameters included pocket probing depth (PPD), clinical attachment level (CAL) & gingival index (GI). Catalase (CAT) and glutathione peroxidase (GPx) levels were estimated using UV-spectrophotometry, and the data were analyzed using SPSS software, Mann-Whitney U test and Pearson correlation coefficient tests.

Results: Higher serum levels of CAT and GPx (an antioxidant parameter) were found in healthy subjects as compared to periodontitis subjects who were gutkha chewers and smokers. Pairwise comparison by the Mann-Whitney U test showed a significant difference in the mean levels of GPx among all groups ($p < 0.05$). The mean serum levels of CAT were significantly lower in group (b) as compared to other groups (b<d<c<a). The clinical parameters GI, PD and CAL among the four groups were also statistically significant ($p < 0.05$).

Conclusion: The study results suggested that serum levels of both catalase and glutathione peroxidase were found to be decreased in chronic periodontitis patients who used to smoke and chew gutkha .

Keywords: Antioxidants, Biological Markers, Catalase, Glutathione Peroxidase, Chronic Periodontitis, Saliva, Smokeless tobacco, Smoker.

INTRODUCTION

Periodontal diseases result from the complex interaction between pathogenic bacteria & the host immune inflammatory responses[1]. Pathogens such as gram negative species, motile rods & spirochetes have the ability to invade gingival tissues. The interaction between pathogenic bacteria & the host immune response is accompanied by an increase in cytokine expression & immunological activity in gingival tissues[2].

The etiology of periodontitis is multifactorial in nature. Smoking is associated with 2.6 - 6 times increased prevalence of periodontal diseases compared to non-smokers[3]. Among the risk factors, smoking and chewing tobacco are the most prevalent ones which are also the most common oral abusive habits in today's life style[4].Smoking stimulates the oxidative burst of neutrophils,

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increases reactive oxygen species production and leads to lipid peroxidation and alterations in protein carbonyls in plasma. In smokeless tobacco users, the primary alterations are white mucosal lesions and gingival recession at the site of tobacco placement, which occur in 50-60 percent and 25-30 percent of smokeless tobacco users respectively[5].

Another most common form of tobacco consumption in the southern Asia, especially in India is in the form of smokeless tobacco products (STP). It is composed of crushed arecanut, tobacco, catechu, paraffin, slaked lime & sweet or savory flavorings. This form of tobacco has many oral effects, including leukoplakia, oral cancer, and loss of periodontal support (recession), alveolar bone loss and staining of teeth and composite restoration[6].

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. Catalase (CAT) protects the cell from hydrogen peroxide generated within them. CAT plays a crucial role in the acquisition of tolerance to oxidative stress during the adaptive response of cells. Glutathione is essential to the glutathione peroxidase (GPx) antioxidants enzyme system which also removes hydrogen peroxide [7,8].

Since free radicals have been implicated in the pathogenesis of various human diseases, this has prompted interest in evaluating cellular levels of antioxidant agents. CAT is an antioxidant enzyme with high specific activity present in all aerobic cells. In erythrocytes, catalase and GPx jointly protect hemoglobin from oxidative damage [9,10].

OBJECTIVE

No studies have as yet analysed the comparative evaluation of serum catalase and glutathione peroxidase levels in patients with chronic periodontitis who are smokeless tobacco users. Taking this into consideration and the adverse effects of tobacco consumption, the present study was conducted to investigate and correlate the serum catalase and glutathione peroxidase levels in healthy (H) , chronic periodontitis (CP) subjects, smokers with CP (CPS) and smokeless tobacco chewers with CP (CPSTP) and also to correlate the serum levels with the clinical periodontal parameters.

MATERIALS & METHODS

120 systemically healthy subjects aged in between 18 to 60 years were randomly selected from the out-patient department, Department of Periodontics, P.M.N.M Dental College, Bagalkot. Patients were equally divided into four groups as clinically healthy periodontium (Group a), chronic periodontitis(CP) subjects (Group b), smokers with CP (Group c/CPS) & tobacco chewers with CP (Group d/CPSTP).Written informed consent was obtained from all recruits. The research project was approved by the ethical committee of P.M.N.M. Dental College and Hospital, Bagalkot. The selection of patients was made according to the criteria approved by the 1999 International Workshop for the classification of periodontal diseases and conditions.

INCLUSION CRITERIA

- All individuals were systemically healthy.
- Healthy group included 30 individuals with clinically healthy periodontium, GI=0 (absence of clinical inflammation), PPD $\leq$ 3 mm and CAL=0.
- Chronic periodontitis group included 30 subjects characterized by at least 30% sites with PPD $\geq$ 5 mm, GI >1 and CAL $\geq$3 mm.
- Tobacco chewers with chronic periodontitis (CPSTP) were enrolled if they regularly chew smokeless tobacco at least 1 sachet daily for atleast 12 months.
- CP subjects who had smoked $\geq$100 cigarettes over their lifetime were considered under CPS group.

EXCLUSION CRITERIA

- History of any antimicrobial and anti-inflammatory therapy for 6 months prior to study.
- Subjects who had undergone any periodontal therapy for 6 months prior to study.
- Pregnant women or lactating mother.
- Subjects with vitamin supplements within previous 3 months.
- History of regular use of mouth wash within previous 3 months.

Clinical measurements

Gingival index (GI) (Loe & Silness -1963), probing pocket depth (PPD), clinical attachment level (CAL) were recorded using the Williams graduated periodontal probe at four sites around each tooth (mesiobuccal, midbuccal, distobuccal and midlingual), excluding the third molars. One calibrated examiner obtained all the measurements so as to reduce intra-examiner variability. Collection of serum samples was carried out in a standardized manner where in samples were collected at the same time duration (9.00- 11.30 am).

Periodontal Parameters: Depending upon the gingival index (GI), probing depth (PD) and CAL measurements, study subjects were divided into 4 groups:

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Group a (n= 30): Periodontally healthy subjects characterized by GI=0 (absence of clinical inflammation), PD ≤ 3 mm and CAL=0,
Group b (n= 30): Subjects with CP characterized by at least 30% sites with PD ≥ 5 mm, GI >1 and CAL ≥4 mm,
Group c (n=30): Smokers with CPS
Group d (n=30): Gutkha chewers with CPSTP
Serum sampling
Around 4 ml of blood was drawn between 08:30 and 11:00 am in fasting state, from the antecubital fossa by venipuncture using 20- gauge needle with a 5ml syringe. The blood sample was allowed to clot at room temperature, and, after one hour, serum was separated from blood by centrifuging at 3000-3500 rpm for five minutes. It was immediately transferred to a plastic vial and stored at -70o C until further examination was carried out.[11]
Spectrophotometric analysis

Assay of serum catalase and glutathione peroxidase levels: For catalase estimation, 50 mM PBS was prepared by adding 1.71 gram of KH_2PO_4 and 2.5 grams of NaH_2PO_4 in 250 ml of distilled water. Also, 0.7 mM of H_2O_2 was also prepared. Later on, 1.95 ml of PBS and 1 ml of H_2O_2 was added to 50 microlitre of serum and readings of absorbance were recorded at 240 nm at 0 and 1 minute. One unit (U) is equal to 1 mmol of H_2O_2 decomposed/min .
For glutathione peroxidase analysis, the main reagent was made by mixing 8.00 mL of KH_2 PO_4 buffer (100 mmol/L; 1 mmol EDTA/L; pH 7.4), 4.00 mL of glutathione reducatse (5000 U/L,), 2.00 mL of reduced glutathione (2.5 mmol/L), and 2.00 mL of NADPH (2.5 mmol/L). The main reagent (200 mL) and the sample (25 µL of 1:200 serum plus 10 µL of H_2O) were added to the cuvette and the absorbance at 340 nm was monitored for 200 s (step A). Then 10 mL of *tert*-butyl hydroperoxide (25 mmol/L) were added as start reagent. The absorbance was monitored for another 225 s (step B). The final reaction volume was 250 mL. The difference in absorbance per minute between steps B and A was used to calculate the enzyme activity by using a molar absorptivity of NADPH at 6.22 $x10^3$ L mol^{-1} cm^{-1}.The unit is mmol of NADPH oxidized/min .

Statistical Analysis: SPSS software (version 19) was used for analysis. Kruskal Wallis ANOVA, Mann-whitney U and Wilcoxon matched pairs test were used to evaluate the mean GI, PD and CAL scores among all the four groups. Statistical analysis for CAT was done by ANOVA and Tukeys multiple post hoc procedures. For GPx , Kruskal Wallis, Wilcoxon matched pair test and Mann-Whitney U tests were used.
Relationship among serum VB12 and FA with clinical parameters in H, CP, CPS and CPSTP subjects was analysed by Kruskal Wallis test, Wilcoxon matched-pair test and Mann-Whitney U test. Multiple comparisons, Wilcoxon Signed Ranks Test and Pearson correlation were done to correlate the levels. All statistical analysis was performed using Statistical Package for Social Scientists (SPSS) software. (version11)

RESULTS

The present cross-sectional study was carried out among 120 subjects and categorized into four groups based on their history and clinical presentation .The mean gingival index, clinical attachment level, probing pocket depth scores in healthy (H) group, chronic periodontitis (CP), chronic periodontitis who are smokeless tobacco chewers (CPSTP) and chronic periodontitis who are smokers (CPS) are shown in Graph 1. The differences in the values of GI, PPD and CAL among the four groups of patients were statistically significant ($P < 0.05$) (Table 1).

Table 1: Study population: Periodontal and serum parameters (Mean ± SD): Gingival Index (GI); Probing Depth (PD); Clinical Attachment Level (CAL); Catalase (CAT)(U); Gutathione peroxidase (GPX) (µ mol)

Characteristics	Group A	Group B	Group C	Group D	P Value
Age (yrs)	37 ± (5.1)	41 ± (4.9)	32.5 ± (5.6)	54 ± (4.2)	
Male/Female	80/20	70/30	100/0	100/0	
GI Score	0.93 ± (0.44)	1.86 ± (0.50)	1.83 ± (.53)	1.40 ± (0.56)	<0.001*
PD (mm)	1.63 ± (0.41)	5.53 ± (0.57)	5.2 ± (0.40)	5.4 ± (0.56)	<0.001*
CAL (mm)	0	6.13 ± (0.86)	6.03 ± (0.76)	5.9 ± (0.84)	<0.001*
CAT (U)	36.62 ± (6.2)	18.86 ± (3.56)	27.17 ± (4.83)	23.06 ± (4.64)	
GPX (mg/ml)	1901.7 ± (340.6)	1220 ± (215.03)	724.44 ± (125.6)	434.2 ± (70.27)	

The differences between the four groups were significant ($P< 0.001$) in terms of GI, PD and CAL scores (Table 1). The mean scores of all the parameters (GI, PD, CAL) were significantly higher among groups b, c and d compared to Group a ($p < 0.001$). Likewise, pairwise comparison of GI, PD and CAL between group a and other groups (b/c/d) by Mann-Whitney U test were highly significant ($p = 0.000$) [Table 2]. With respect to GI, pairwise

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comparison between group d and groups (a, b) showed significant difference ($p < 0.001$ and $p < 0.003$ respectively). While considering PD, significant difference was seen between groups b and c ($p < 0.014$) [Table 2].

Table 2: Pair wise comparison of GI, PPD and CAL among four groups by Mann-Whitney U test.

Parameters	Group Comparison	P Value
Gingival Index	Group a vs b/c/d	P = 0.000***
	Group b vs c/d	P = 0.793 / 0.001***
	Group c vs d	P = 0.003**
Pocket Depth	Group a vs b/c/d	P = 0.000***
	Group b vs c/d	P = 0.014** / 0.323
	Group c vs d	P = 0.141
Clinical Attachment Level	Group a vs b/c/d	P = 0.000***
	Group b vs c/d	P = 0.758 / 0.288
	Group c vs	P = 0.440

*Statistically Significant *** P< 0.001, **p<0.01, *p<0.05

The results of our study showed that the mean serum levels of CAT were significantly lower in group b as compared to other groups b<d<c<a). Mean serum GPx levels were found to be decreasing in sequential order from group a to group d (Table 1). Upon pairwise Tukey's post hoc comparison after applying ANOVA test, significant difference was seen in the mean values of CAT among all groups ($p < 0.05$). Similarly, significant difference in the mean levels of GPx was found among all groups ($p < 0.05$) except between group c and d ($p = 0.198$) [Table 3].

Table 3: Pair wise comparison of four groups with CAT and GPx levels by ANOVA and Tukey's Post–hoc multiple comparisons.

Groups		Other groups	CAT	GPx
Group a	v/s	Group b	p=0.000***	p=0.000**
		Group c	p=0.000***	p=0. 000***
		Group d	p=0.000***	p=0. 000***
Group b	v/s	Group c	p=0. 000***	p=0.005***
		Group d	p=0.007**	p=0.000**
Group c	v/s	Group d	p=0.008	p=0.198

P value is significant in all the groups except for GPx in Group III v/s IV
Statistically Significant when ***p≤ 0.001, **p<0.01, *p<0.05

According to Pearson correlation test, serum CAT and GPx levels are directly proportional to each other in groups a and b but inversely proportional in groups c and d [Table 4].

Table 4: Pearson co-relation between CAT and GPx groups

	R value	P value
Group I	0.039	0.838
Group II	0.098	0.607
Group III	-0.117	0.538
Group IV	-0.049	0.799

DISCUSSION

Antioxidants are present in all body fluids and tissues, and protect against endogenously-formed free radicals, usually produced by leakage of the electron transport system [12]. The strongest indication implicating ROS in periodontal destruction of the connective tissues during periodontal diseases arises in considering PMN infiltration as a key event of host response against bacterial invasion [13].

Smoking and smokeless tobacco have shown to impair various aspects of innate and acquired host immune responses. There has been an increased prevalence of chewing tobacco owing to its free accessibility, lower price and also rising education concerning well-known hazards of smoking in India. Adults currently using smokeless tobacco are twice more likely to have severe active periodontal disease than adults who never used smokeless tobacco [14].

In consideration of the strong evidence in favor of smoking as a major risk factor for destructive periodontal disease, the possible risk associated with the use of smokeless tobacco products is worthy of

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attention. In this backdrop, an attempt has been made in the present study to investigate the possible association between smokeless tobacco and smoking with the serum levels of enzymatic antioxidants such as catalase and glutathione peroxidase in chronic periodontitis subjects [10].

It has been reported that the gingival blood flow is increased in smokeless tobacco users [15]. Exposure of keratinocytes and monocytes to aqueous extracts of smokeless tobacco increases production of inflammatory mediators, such as prostaglandin E2 (PGE2) and interleukin–1 (IL-1) and increases keratinocyte proliferation. This may be an explanation for the more severe periodontal conditions and disturbance in antioxidant levels in gutkha chewers. The primary periodontal alteration in smokeless tobacco users is localized gingival recession (25-30 %) [16]. The tobacco specific nitrosamines (TSNAs) are metabolites of nicotine and are major carcinogens. Chronic inflammation may promote the carcinogenic effect of these nitrosamines through the generation of ROS [17].

Smoking may have an adverse effect on fibroblast function, chemotaxis of neutrophils, immunoglobulin, production and induction of peripheral vasoconstriction [18]. Nicotine metabolites concentrate in the periodontium and cause functional alterations in phagocytosis and the oxidative burst [19]. The direct exposure to cigarette smoke represent only a portion of total oxidative stress and contributes to additional endogenous oxidant formation through effects on inflammatory immune response [20].

The results obtained in the current study demonstrate that the highest value of serum CAT and GPx is found in healthy subjects. The obligatory use of body-reserve of antioxidants to detoxify the excess of free radicals in smokers and smokeless tobacco users therefore results in alteration in antioxidant levels. The antioxidant disturbance may be further enhanced by their lower intake of both supplemental and dietary antioxidants [15].

It was observed that there was no significant correlation between CAT and GPx with age, in any of the groups when seen group wise. Although, there exists positive correlation between CAT and GPx (Pearson co-relation) (Table 4).

Studies have shown that nicotine increases ROS in a time and concentration-dependent manner. Barr and co workers have reported that as low as 0.1 μM concentration of nicotine induces ROS by approximately 35%. Bagchi et al. reported that ST extract produces oxidative tissue damage [21]. The induction of oxidative stress in the body by nicotine and the subsequent depletion of antioxidants may be one of the mechanisms for the tissue damage, including periodontium .

The current study results were found consistent with the findings as reported by M. K Reejamol who showed the effect of cigarette smoking on periodontal damage in terms of antioxidants in gingival tissue [22]. Thus, it reveals that smoking increases the level of free radicals in periodontal tissues, which in turn may augment the level of periodontal destruction. Ellis and collaborators analyzed gingival tissues from patients with severe periodontal disease and showed that the activity of catalase was decreased [23]

Biju Thomas et al have found that levels of glutathione, catalase and selenium are significantly lower in serum of diabetic patients with periodontitis and also in healthy individuals with periodontitis but are highest in healthy controls showing that the serum levels are inversely proportional to inflammation and tissue destruction [24].

The most unique finding of our study is that serum CAT and GPx levels were higher in gutkha chewers as compared to smokers group.

CONCLUSION

The present analysis may well be of great importance for further understanding the relationship between serum and anti-oxidants. Regarding the use of the measured biochemical parameters as biomarkers, results suggest that measured serum antioxidants can be accurate for evaluation of periodontal status. It becomes imperative to consider smokeless tobacco as harmful as smoking for periodontitis.

Ethical Approval: Ethical approval is obtained from Institute Ethical Committee and Review Boards.

More studies with a result in positive direction can set these two anti-xidants as biomarkers for oral health in future. Special educative methods should be designed depicting the role of smokeless tobacco on systemic health other than oral cancer. . It becomes important to consider smokeless tobacco chewing as a major risk factor for periodontitis as both tobacco chewing and periodontitis are more prevalent in india. Future researches to assess smokeless tobacco as a definitive risk factor for periodontitis should be carried out.

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