Original Research Article

Evaluation of the Erythrocyte Antioxidant Status in Healthy Controls and Patients with Periodontitis

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Abstract

Background: Periodontitis is an inflammatory disease of the supporting tissues of teeth caused by specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone. **Objective:** The objective of this study was to evaluate any measurable change in antioxidant enzyme status (superoxide dismutase) among healthy controls and patients with periodontitis. **Method:** A total of forty subjects consisting of twenty test subjects and twenty controls were recruited for this comparative study. The level of superoxide dismutase in erythrocytes were assessed in both test subjects and controls. **Results:**There was no statistically significant difference between age and gender of Group A and Group B. In Group A there is correlation between age and SOD level and Group B, there was no correlation between the age and SOD levels, body mass index [BMI] and SOD levels.Subjects with periodontitis(Group B) have significantly lower SOD levels when compared with those subjects without periodontitis (Group A). **Conclusion:** In the current study,Periodontal disease can be one of the factors for decreased antioxidant enzyme(SOD) in red blood cells.

Keywords: Periodontal disease, Reactive oxygen species, Superoxide dismutase.

Introduction

Periodontitis is an inflammatory disease caused by opportunistic bacteria residing in the oral cavity, leading to the loss of supporting tissues of teeth [1]. It has been observed that various invading bacteria trigger the release of cytokines such as interleukin 8 and tumor necrosis factor-α, leading to elevated numbers and activity of polymorphonuclear leukocytes (PMNs) [2]. As a result of stimulation by bacterial antigens, PMN produces the reactive oxygen species (ROS) superoxide via the respiratory burst as part of the host response to infection[2]. Patients with periodontal disease display increased PMN number and activity. It has been suggested that this proliferation results in a high degree of ROS release, culminating in heightened oxidative damage to gingival tissue, periodontal ligament, and alveolar bone [3]. Hydroxyl radical is most active in damaging important molecules such as DNA, proteins, and lipids, whereas hydrogen peroxide, even not being considered a potent ROS, is capable of crossing the nuclear membrane and also damaging DNA [4]. Antioxidant defense system is very dynamic and responsive to any disturbance taking place in redox balance of the body. Antioxidants can be upregulated and neutralize free radical formation that could take place due to oxidative stress [5]. Human body does contain an array of antioxidant defense mechanisms(nonenzymatic and enzymatic anti-oxidants) to remove harmful ROS as soon as they are formed and to prevent their deleterious effects. The nonenzymatic antioxidants include vitamins E and C, and reduced glutathione (GSH), whereas the enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and GSH peroxidase (GSHPx) [6]. The possible mechanisms by which antioxidants may offer protection against free radical damage include

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prevention of formation of free radicals, interception of free radicals by scavenging the reactive metabolites and converting them to less reactive molecules, facilitating the repair of damage caused by free radicals and providing a favorable environment for effective functioning of other antioxidants [6]. In recent years, more attention has been focused on the role of ROS and in specifically the superoxide dismutase enzyme functions in the pathology of periodontitis [7-10]. Therefore, this study aimed at evaluating any measurable change in antioxidant enzyme status (superoxide dismutase) among healthy controls and patients with periodontitis.

Material and Methods

The study participants were the subjects with and without periodontits with age ≥30 and ≤60 years, who reported to the Out Patient Department of Periodontics. Based on the inclusion and exclusion criteria subjects were divided into two groups; Group A (systemically healthy subjects without periodontitis) and Group B (systemically healthy subjects with periodontitis. Individuals who were systemically healthy, within the age group of ≥30 and ≤60 years, experienced no bleeding on probing, no probing pocket depth >3mm and no clinical attachment loss and possessing more than 20 natural teeth comprised the Group A. Whereas, individuals who were systemically healthy, within the age group of ≥30 and ≤60 years but having clinical attachment loss ≥3 mm and in 5 or more teeth comprised Group B. Any patients with history of a) any systemic illness, or a history of periodontal treatment (scaling and root planing) within past six months, b) antibiotics, anti-inflammatory and antioxidant vitamin use for six months, c) smoking or consumption of alcohol and the patients with recent significant blood loss, bleeding disorders, hepatitis or human immunodeficiency virus (HIV) infection and immunosuppressive chemotherapy, and, pregnant women and lactating mothers were excluded from this study. Blood samples were obtained by venous arm puncture and collected in heparinized tubes, and the following tests were conducted.

Body Mass Index [BMI]

Weight of subjects in kilogram [kg] and height in meter[m] were recorded with a weighing machine and a metric tape respectively. The body mass index [BMI] was calculated using the standard formula.

Estimation of superoxide dismutase (SOD) activity

2 ml of venous blood samples were collected in disposable syringes and blood samples were added in EDTA vacuttainers**.**Plasma was separated and blood cells were used for hemolysate preparation for analysis of erythrocyte SOD**.** 2 ml buffered substrate is taken as the reagent blank. Readings are noted. Four sample readings are taken at 10 seconds interval at 420 nm (double beam spectrophotometer) by adding 100 µl sample to 2ml buffered substrate. The super oxide dismutase activity was measured by the inhibition of auto oxidation of 0.2 mM pyrogyllol in 50mM TrisHCl buffer containing 1mM DTPA. The percentage inhibition of ratio of auto oxidation of pyrogyllol was intiated by addition of 50µl of hemolysate to 1ml of buffered substrate in a cuvette. Values are expressed in U/gm Hb

Calculation

Rate of oxidation of sample X 50(Percentage inhibition) X 10(dilution)/Rate of oxidation of control=U /gm Hb - Eq. (1)

Numerical data were expressed as mean ± standard deviation. Inter group comparison of age and BMI was done using Independent Sample t test, while that of gender was done using Chi Square test. Karl Pearson Coefficient of correlation was used to test correlation of age and body mass index [BMI] with SOD levels. The results were considered statistically significant when *p* value was <0.05.

Results

Comparison of gender between Group A and Group B

Group A consists of 10 males (50%) and 10 females (50%). Group B consisted of 10 males (50%) and 10 females (50%). From the table 1 below, the results of statistical analysis using Chi Square test indicated that there was no statistically significant difference between two groups in gender (p>0.05)

Table 1: Comparison of gender between Group A and Group B

Gender	Group A	Group B	Total	p - value
Male	10 (50.0%)	10 (50.0%)	20 (50.0%)	>0.05
Female	10 (50.0%)	10 (50.0%)	20 (50.0%)	

Comparison in age between Group A and Group B

The below Table 2 shows that subjects in Group A had a mean age of 38.6 ±7.34years, while that of Group B had a mean age of 41.80 ±8.52 years. Results of the statistical analysis using Independent Sample t test to compare the mean age between Group A and Group B indicated that there was no statistically significant difference between two groups (p > 0.05).

Table 2:Comparison in age between Group A and Group B

Group		n	Mean	Standard Deviation	p-value
Age (years)	Group A	20	38.6	7.34	>0.05
	Group B	20	41.8	8.52	

Correlation of age with Erythrocyte SOD levels

As per the data in the below Table 3, the p-value in group A is less than the significance level 0.05; the correlation between age and SOD level is significant in group A. Erythrocyte SOD level shows a decrease with increase in age and SOD level increases with decrease in age. The p-value in group B is greater than the significance level 0.05; the correlation between age and SOD level is not significant in group B.

Table 3:Correlation of age with SOD levels

		Group A(SOD)	Group B(SOD)
Age (years)	Pearson Correlation	- 0.507 *	0.052
	P.value	0.022	0.828

** Correlation is significant at 0.05 level.*

Comparison of Body Mass Index [BMI] between Group A and Group B

The below Table 4 shows Subjects in Group A had a mean body mass index[BMI] of 24.2±1.918kg/m^2, while that of Group B had a mean body mass index[BMI] of 24.74±1.94kg/m^2. The results of the statistical analysis using Independent Sample t test to compare the mean body mass index[BMI] of Group A and Group B indicated that there was no significant difference between two groups (p>0.05).

Table 4 : Comparison of Body Mass Index [BMI] between Group A and Group B

Group		Mean	Standard Deviation	p- value
BMI(kg/m^2)	Group A	24.22	1.918	> 0.05
	Group B	24.74	1.94	

Correlation of BMI with erythrocyte SOD Level

In the below Table 5, it is shown that in both Group A and Group B there was no correlation between body mass index[BMI] and SOD levels since the p-value is greater than the significance level 0.05; (p>0.05).

Table 5: Correlation of Body Mass Index

		Group A	Group B
BMI (kg/m^2)	Pearson correlation	0.275	0.253
	p-value	0.240	0.281

[BMI] with SOD levels

Comparison of erythrocyte SOD levels of Group A and Group B

Table 6: Comparison of erythrocyte SOD levels of Group A and Group B

SOD level (gm/Hb)	Group A		Group B		*p-value
	Mean	Standard Deviation	Mean	Standard Deviation	
	39.50	9.710	12.24	6.362	<0.001

Table 6 and Figure 1 shows comparison of SOD levels of Group A and Group B. The mean SOD for Group A and Group B were 39.50 ± 9.71 and 12.24 ± 6.362 respectively.

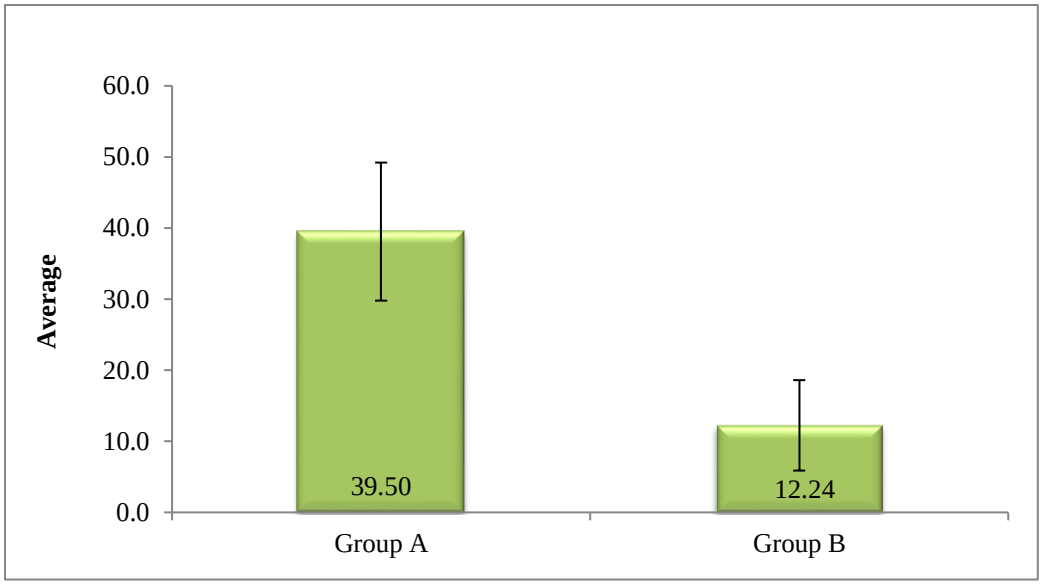

Fig 1: Comparison of SOD levels of Group A and Group B

Inter group comparison using Independent Sample t test showed that there was statistically significant difference between Group A and Group B in SOD levels ($p<0.001$).Groups consisting of subjects,with periodontitis (Group B) had significantly lower SOD levels when compared to those subjects without periodontitis (Group A).

Discussion

Reactive oxygen species (ROS) are produced in animals and humans under various physiological and pathological conditions [11]. ROS are produced continuously in living cells as by-products of normal metabolism, during the metabolism of xenobiotics and during exposure to high temperature or radiation. They also form due to the leakage of electrons from electron transport chains and the generation of superoxide or hydrogen peroxide by peroxisomal enzymes [12]. Enhanced oxidative stress in cells results in the activation of free radical scavenging enzymes to neutralize the toxic effect of ROS. SOD protects the cell against ROS by scavenging superoxide radicals and hydrogen peroxide, which cause damage to the structure and function of membrane assembly [13]. 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. Antioxidant enzymes such as SOD provide protection within cells while low-molecular-weight scavenging antioxidants are present in extracellular fluid. These include ascorbic acid, α-tocopherol, and β-carotene [13]. In the present study, we have tried to assess the antioxidant defence status of SOD in erythrocytes in patients with periodontal disease and controls. In periodontitis, progressive generation of free radicals takes place leading to increased lipid peroxidation and decrease in antioxidant enzyme defence status. Free radical-induced tissue injury has been demonstrated to be increased in individuals with periodontitis[14-16].In a study carried out by Wei et al., it was found that the levels of total oxidative status (TOS) and SOD were significantly higher in chronic periodontitis patients than in control group and after periodontal treatment [17]. However, our study did not

confirm that lowered level of SOD is associated with hyper lipid peroxidation in periodontal disease.

Conclusion

Erythrocyte SOD level were estimated since red blood cells have the greatest SOD activity as compared to periodontal ligament and it is also known that SODs have only minor activity in extra cellular fluid. The concept of the role of free radicals in the initiation and progression of periodontal disease points to the purposefulness of including bio-oxidants and other bioregulatory substances in the pharmacological prevention of these diseases thus maintaining tissue integrity. In the future, antioxidant supplementation may be used in the treatment or prevention of these chronic diseases of the oral cavity to some extent.

References

1. Bartold PM, Van Dyke TE. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000. 2013 ;62(1):203-17.
2. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nature Reviews Immunology. 2015 ;15(1):30-44.
3. Moutsopoulos NM, Madianos PN. Low-grade inflammation in chronic infectious diseases: Paradigm of periodontal infections. Annals of the New York Academy of Sciences. 2006 ;1088 (1):251-64.
4. Albertini M, López-Cerero L, O'Sullivan MG, Chereguini CF, Ballesta S, Ríos V, Herrero-Climent M, Bullón P. Assessment of periodontal and opportunistic flora in patients with peri-implantitis. Clinical oral implants research. 2015; 26(8):937-41.
5. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxid Med Cell Longev. 2013;2013:956792.
6. Poljsak B. Strategies for reducing or preventing the generation of oxidative stress. Oxidative medicine and cellular longevity. 2011:1-16
7. Akalin FA, Toklu E, Renda N. Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. Journal of clinical periodontology. 2005; 32(3): 238-43.
8. Agnihotri R, Pandurang P, Kamath SU, Goyal R, Ballal S, Shanbhogue AY, Kamath U, Bhat GS, Bhat KM. Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis. Journal of periodontology. 2009;80(4):657-62.
9. Baltacıoğlu E, Akalın FA, Alver A, Balaban F, Ünsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. Journal of clinical periodontology. 2006;33(6):385-92.
10. Akalın FA, Baltacıoğlu E, Alver A, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in pregnant women with chronic periodontitis. Journal of Periodontology. 2009; 80(3):457-67.
11. Xu Q, He C, Xiao C, Chen X. Reactive oxygen species (ROS) responsive polymers for biomedical applications. Macromolecular bioscience. 2016;16(5):635-46.
12. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cellular signalling. 2012; 24 (5):981-90.
13. Nozik-Grayck E, Suliman HB, Piantadosi CA. Extracellular superoxide dismutase. The international journal of biochemistry & cell biology. 2005;37(12):2466-71.
14. Diab-Ladki R, Pellat B, Chahine R. Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. Clinical oral investigations. 2003;7(2):103-7
15. Moore S, Calder KA, Miller NJ, Rice-Evans CA. Antioxidant activity of saliva and periodontal disease. Free radical research. 1994 ;21(6):417-25.
16. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell MolBiol Lett. 2005 ;10(2):255-64.
17. Wei D, Zhang XL, Wang YZ, Yang CX, Chen G. Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. Australian Dental Journal. 2010;55(1):70-8.

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