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Original article

The influence of cigarette smoking on blood and salivary super oxide dismutase enzyme levels among smokers and nonsmokers—A cross sectional study

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ABSTRACT

To determine the influence of smoking on blood and salivary superoxide dismutase enzyme levels among smokers, and to demonstrate the significant alterations in the levels of superoxide dismutase in association with patient age, periodontal disease status, smoking duration, and smoking frequency. This study also aimed to evaluate the use of saliva as a biological fluid for disease diagnosis.

Ninety males aged 25–56 years were selected and included 30 smokers, 30 nonsmokers with chronic periodontitis, and 30 healthy controls. Clinical parameters such as the gingival index, pocket depth, and clinical attachment loss were recorded. Blood and saliva samples were collected and superoxide dismutase enzyme levels were analyzed using spectrophotometric assay.

Superoxide dismutase enzyme levels in the blood and saliva were significantly higher in smokers than in nonsmokers and the controls ($p < 0.05$). A significant correlation existed between superoxide dismutase levels and clinical parameters. There was also a significant positive correlation between blood and salivary superoxide dismutase levels among the three groups.

Systemic and local antioxidant status is affected by periodontal disease and by the impact of smoking. The increased blood and salivary superoxide dismutase enzyme levels in smokers may be an adaptive defense mechanism to counteract the increased reactive oxygen species production induced by smoking. This study emphasizes the importance of saliva as an easy noninvasive tool in diagnosing patients who are more prone to precancerous lesions and conditions, and its importance in patient education and motivation programs for smoking cessation.

1. Introduction

Most periodontal tissue destruction is caused by an inappropriate host response to microorganisms and their products. To be more specific, a loss of homeostatic balance between proteolytic enzymes (e.g., neutrophil elastase) and their inhibitors (e.g., α_1 antitrypsin) and between reactive oxygen species (ROS) and the antioxidant defense systems that protect and repair vital tissue and cellular and molecular components are believed to be responsible.[1]

Polymorphonuclear leukocytes (PMNLs) are a particularly rich source of ROS, which in the absence of suitable antioxidants can lead to tissue damage. Stimulation by bacterial antigens causes PMNLs to produce superoxide ($O_2^{\cdot-}$) via the metabolic pathway of the "respiratory burst" during phagocytosis. Inflammatory cells such as fibroblasts, vascular endothelial cells, and osteoclasts also produce ROS. They are highly toxic to the ingested microorganisms and to the host cells.[2,3]

Because cigarette smoke contains a large amount of oxidative species, smoking increases ROS production and is a significant source of oxidative stress. Smokers are nearly four times more likely than nonsmokers to have severe periodontitis.[4]

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A variety of antioxidant defense mechanisms exist to counteract the detrimental effects of ROS *in vivo*. An antioxidant may be regarded as any substance that, when present in low concentrations (compared to the concentration of an oxidizable substrate), significantly delays or inhibits the oxidation of that substrate.[2] The human body has an array of nonenzymatic and enzymatic antioxidant (AO) defense mechanisms to remove harmful ROS and to prevent their deleterious effects. The nonenzymatic antioxidants include vitamins A, C, and E; uric acid; bilirubin; reduced glutathione; albumin; transferrin; lactoferrin; ceruloplasmin; and haptoglobin. The enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT).[2,5] A delicate balance exists between antioxidant defense repair systems and prooxidant mechanisms of tissue destruction; if the balance is shifted in favor of ROS activity, significant tissue damage ensues.[6]

Within mammalian tissues, the most significant antioxidant is SOD, which catalyses the dismutation of superoxide ($O_2^{\cdot-}$), an oxygen radical that is released in inflammatory pathways and causes connective tissue breakdown. This enzyme is released as a homeostatic mechanism to protect the tissues. It can be detected in extra- and intracellular compartments. Superoxide dismutase has been localized in the human periodontal ligament, and it may represent an important defense within gingival fibroblasts against superoxide release.[3]

Superoxide dismutase, previously known as erythrocuprein (in humans) or hemocuprein (in bovines), exists as a family of metalloproteins and is widely distributed in mammalian tissues. Erythrocytes only contain the copper/zinc SOD isoenzyme, which is coded by a gene located on chromosome 21. By virtue of their physiological role, erythrocytes are exposed to continuous oxidative stress because oxygen radicals are continuously generated by autoxidation of hemoglobin.[7]

Saliva, in addition to its lubricant properties, contains many biochemical substances, antibacterial components, and various antioxidants. It therefore could constitute a first line of defense against free radical-mediated oxidative stress.

Free radical scavenging and the antioxidant defense system have an important role in maintaining normal cellular physiology, facing diseases, and promoting immunity.[8] There are sparse previous studies that considered the effects of smoking on the antioxidant status of blood and saliva with regard to periodontal conditions, and have yielded conflicting results.[3,5,9] Hence, the present study was performed to evaluate the influence of smoking on periodontal health by estimating the SOD enzyme level in the blood and saliva of smokers and nonsmokers with chronic periodontitis. The enzyme levels in blood and saliva among healthy, smokers and nonsmokers were also compared and correlated with clinical findings.

2. Materials and methods

Ninety male participants in the age range of 25–56 years were selected by random sampling from the outpatient Department of Periodontics, P.M. Nadagouda Memorial Dental College and Hospital (Bagalkot, Karnataka, India). They were divided into three categories, based on the clinical periodontal parameters, smoking status, and inclusion criteria: Group I comprised 30 healthy individuals with no clinical and radiographic manifestations of periodontal disease; Group II comprised 30 nonsmokers with chronic periodontitis and at least 20 natural teeth and a minimum of six periodontal pockets ≥5 mm or the loss of attachment of ≥3 mm[10]; and Group III comprised 30 smokers (based on the self-reported smoking status) with chronic periodontitis. Current smokers who smoked ≥10 cigarettes per day and who fulfilled the criteria of chronic periodontitis were enrolled in the study.[3,11] All included participants were systemically healthy; had no history of antibiotic, anti-inflammatory, or antioxidant drug treatment within the previous 6 months; and had not undergone periodontal treatment for at least 6 months prior to sampling and recording.

Females; individuals with systemic diseases such as diabetes mellitus, hepatitis, rheumatoid arthritis, cardiovascular disease, and human immunodeficiency virus infection; and regular users of vitamin supplements were excluded from the study. The need and design of the study were explained to all potential participants. Only those who gave written informed consent were included in the study. Ethical clearance for the study was obtained from the ethical committee of P.M. Nadagouda Memorial Dental College and Hospital in Bagalkot.

The gingival index (Loe and Silness),[12] probing pocket depth (PD),[13,14] and clinical attachment loss (CAL)[13,14] were assessed. Blood and saliva samples were collected from all participants 48 hours after the clinical measurements in the morning after an overnight fast. The study participants were asked not to eat or drink (except water) prior to sample collection.

2.1. Collection of saliva samples

Unstimulated whole saliva samples were used in this study. After rinsing the mouth with 15 mL of plain water to remove exfoliated cells and debris, the participants were asked to allow saliva to pool in the bottom of the mouth and spit it on to ice chilled sterile polypropylene tubes. Approximately 1 mL of whole saliva was collected and centrifuged immediately at 3000 × g at 4 °C for 5 minutes. The resultant supernatant was aspirated and assayed biochemically for the estimation of the SOD enzyme level.[15]

2.2. Collection of blood samples

Two milliliters of venous blood was drawn from the antecubital vein of all participants by using a disposable syringe. It was transferred to sterile vial containing the anticoagulant EDTA.

2.3. Preparation of erythrocyte lysate

The blood was centrifuged at 2000 × *g* for 20 minutes at 25°C. The plasma and the upper layer of the red blood cell pellet, which contains the buffy coat, were removed aseptically. The red blood cell (RBC) pellet was washed three times with sterile saline (0.85 gm/100 mL) to ensure complete removal of the plasma, leukocytes, and platelets. The washed RBCs were hemolyzed by adding sterile distilled water (1:5 by volume). The lysate was then centrifuged at 800 × *g* for 15 minutes at 4°C to make the lysate ghost free. The supernatant was used as the source for the SOD enzyme estimation.[16] All samples were immediately prepared and assayed on the same day.

2.4. Estimation of SOD enzyme

The superoxide dismutase level was estimated in saliva and erythrocytic lysate by using the method of Misra and Fridovich.[17]

2.5. The principle

The ability of SOD to inhibit the autoxidation of adrenaline to adrenochrome at pH 10.2 was the basis for this assay. The superoxide ($O_2^{\cdot-}$) anion, which is the substrate for the SOD enzyme, is generated indirectly by the oxidation of epinephrine by oxygen in an alkaline pH. The SOD enzyme reacts with the $O_2^{\cdot-}$ formed during the epinephrine oxidation, and therefore slows the rate and the amount of adrenochrome formation.