Research Article

Quantitative assessment of antioxidants and oxidative stress against tooth paste and metal-induced toxicity

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ABSTRACT

Aim: The aim of this study is to quantitatively evaluate oxidative stress against tooth paste and metal-induced toxicity. **Introduction:** Exposure to heavy metals is a common phenomenon due to their environmental pervasiveness. Metal intoxication particularly neurotoxicity, genotoxicity, or carcinogenicity is widely known. The unifying factor in determining toxicity and carcinogenicity for all these metals is the generation of reactive oxygen and nitrogen species. The toxic manifestations of these metals are caused primarily due to an imbalance between pro-oxidant and antioxidant homeostasis which is termed as oxidative stress. **Materials and Methods:** Five criteria such as estimation of lipid peroxidase, myeloperoxidase, protein carbonyl content, superoxide dismutase, and catalase ,were taken for evaluating the oxidative stress and antioxidants levels in tooth paste. **Result:** Thus, this study shows that there is a decrease in antioxidants levels and subsequent increase in oxidative stress levels in heavy metal tooth paste. **Conclusion:** In the present study, a decrease in antioxidants in heavy metal containing toothpastes shows that it can lead to localized disorder as well as systemic disorders such as cancer and cardiovascular diseases. Hence, toothpaste containing heavy metals should be avoided.

KEY WORDS: Antioxidants, Free radicals, Oxidative stress, Reactive oxygen species, Saliva

INTRODUCTION

An antioxidant is molecule that can inhibit oxidation of other molecules. Oxidation of molecules produces free radicals. These free radicals can cause harmful chain reactions that are responsible for cell damage or cell death, which can be responsible for carcinogenesis or low-density lipoprotein oxidations in cardiovascular diseases. Antioxidants neutralize these free radicals by releasing their electrons, ending the electron taking reaction.[1]

Oxidative stress is a characteristic feature in many of the diseases. The majority of free radicals that damage biological membranes are oxygen-free radicals, and these are known as "reactive oxygen species" (ROS). ROS can be formed during ultraviolet (UV) light irradiation, and by X-rays, gamma rays produced during metal catalyzed reactions are present in the atmosphere as pollutants are produced by inflammatory mediators such as neutrophils and macrophages during inflammation and are by-products of mitochondrial-catalyzed electron transport systems and various other mechanisms.[2] The imbalance between ROS production and antioxidant numbers to scavenge ROS will increase oxidative stress. ROS are oxygen-derived small molecules produced as intermediates in the redox reactions, such as ozone, superoxides, singlet oxygen, and hydrogen peroxide. Hence, ROS plays important roles in cellular signaling processes and triggers diseases.

Saliva is a heterogeneous fluid that is rich in antioxidant compounds. Since oral cavity is the first entrance of the body for the food, fluids, and inhalants, saliva is the first milieu for those environmental materials. It has been shown that saliva includes many defensive mechanisms such as secretory IgA and protein-enzymatic defense system, histatin, lysozyme, and lactoferrin. Uric acid is the major component of the salivary antioxidant system constituting 70% of the total antioxidant capacity.[3]

Antioxidants are produced from several sources including minerals, vitamins or food, and herbal supplements. These supplements can be acquired in

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capsule, liquid, or tablet forms. In dental field, there are toothpastes, mouth rinses, or oral sprays incorporating antioxidant supplements. The majority of supplements include green tea, propolis, grape seed, or pine bark extracts. Antioxidants in food are of interest for four major reasons: They can protect the food itself against oxidative damage, they can exert antioxidant effects in the human gastrointestinal tract, they can be absorbed and exert antioxidant effects in other body tissues, and they may be used in plant extracts, or as pure compounds, as therapeutic agents.

In normal, healthy individuals, the metabolic process results in balanced levels of free-radical ROS and antioxidants. If the normal oxidant/antioxidant balance is disturbed, it can result in a pro-oxidant burden, i.e., a proliferation of ROS and free radicals. The overabundance of oxidants is called oxidative stress.

Oxidative stress can lead to cell damage through microdamage to the cell membrane, protein deactivation, DNA damage, and stimulation of cell signaling molecule-induced tissue damage. Some molecules are more vulnerable to oxidation (i.e., "electron theft") than others. In particular, some of the molecules in cell walls, containing unsaturated lipids, are particularly susceptible to the attack of free radicals. Other vulnerable molecules include RNA, DNA, and protein enzymes.[4]

Oral cells are uniquely susceptible to free radical damage because the mucus membranes allow rapid absorption of substances across their surfaces.[5] In oral tissues, infection from gum disease can generate oxidative stress as an alcohol, nicotine, hydrogen peroxide, and other dental procedures and substances such as hydrogen peroxide, dental cement, and composite fillings. The increase in free radicals from oxidative stress leads to further breakdown of cell walls and oral tissue.[11]

Thus, there is a need for a balance between oxidative stress and antioxidants. As the oral cavity is susceptible to more of oxidative stress, this study aimed at evaluating the oxidative stress and antioxidant level in metal and toothpaste-induced toxicity.

A study of the concentration of heavy metals in local and foreign toothpastes showed that foreign toothpastes contained a higher concentration of these heavy metals and may pose greater health problems to the consumers.

Therefore, there is the need to study the oxidative stress level and assess quantitatively the antioxidant such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase in metal and toothpaste-induced toxicity.

MATERIALS AND METHODS

Sample Collection

A study was carried out to investigate whether there is a difference in susceptibility to chemically induced irritation of oral mucosa in local exposure to sodium laureth sulfate in toothpastes. Four different pastes differing only in detergent concentration present, or not, were used.

The 15 participants applied 1 cm of different test toothpastes to a cap splint covering the teeth and the oral mucosa of the upper jaw 2 times daily for 2 min during a period of 4 days.

Estimation of Lipid Peroxidation

Lipid peroxide content in buccal mucosal tissue was determined by thiobarbituric acid reaction as described by Ohkawa *et al*.[6]

To 0.5 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of sodium dodecyl sulfate, and 1.5 ml of TBA were added. The mixture was made up to 4 ml with distilled water and then heated for 60 min at 95°C using glass ball as condenser. After cooling, 4 ml of butanol-pyridine mixture was added and shaken well. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm. Standard and blank were treated in a similar manner. The lipid peroxide concentration was expressed as n moles of malondialdehyde formed/g tissue.

Estimation of Myeloperoxidase

The myeloperoxidase activity was assayed by the method of Krawisz *et al*.[7] The myeloperoxidase activity was measured by following the oxidation of O-dianisidine dihydrochloride by hydrogen peroxide. The reaction mixture contained all final concentrations in 1 ml, 50 mM phosphate buffer, pH 6, 0.167 mg of orthodianisidine dihydrochloride, an aliquot of the enzyme, and 0.1 µM of hydrogen peroxide. The change in absorbance at 550 nm was measured at 25°C. One unit of the myeloperoxidase activity is defined as that which would convert 1 µm of hydrogen peroxide to water in 1 min at 25°C. The activity of myeloperoxidase was expressed as U/g tissue.

Estimation of Protein Carbonyl Content

The weighed amount of mucosal tissues was homogenized in sodium phosphate buffer and in a Potter-Elvehjem glass homogenizer for 45s to get 10% homogenate. After centrifugation at 600× *g* for 10 min, the proteins from 0.8 ml of the supernatant were precipitated with 5% tri carboxylic acid (TCA) and allowed to react with 0.5 ml of 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 10% TCA, the protein was washed thrice with

a mixture of ethanol-ethyl acetate (1:1), dissolved in 0.6 ml of a solution containing guanidine hydrochloride in potassium phosphate and centrifuged, and the supernatant was used for the measurement of carbonyl content at 362 nm.[8]

Assay of SOD

Superoxide oxido reductase and SOD activity were assayed by the method of Misra and Fridovich. To tubes containing 0.5 ml of carbonate buffer, 0.5 ml of ethylenediaminetetraacetic acid solution, required amount of enzyme was added, and the final volume was made up to 2.5 ml. The reaction was initiated by the addition of 0.4 ml of epinephrine, and the increase in absorbance at 480 nm was measured in a Shimadzu UV spectrophotometer. 50% autoxidation of epinephrine to adrenochrome was performed in a control tube without the enzyme. The enzyme activity was expressed as units/mg protein.[9]

Assay of CAT

Hydrogen peroxide oxido reductase CAT activity was assayed by the method of Sinha . 0.05 ml of tissue homogenate was added to 1.2 ml of the phosphate buffer. To this, 1.0 ml of hydrogen peroxide was added to start the enzyme reaction. The decrease in absorbance was measured at 620 nm at 30 s intervals for 3 min. The enzyme blank was run simultaneously with 1 ml of distilled water instead of hydrogen peroxide. The activity of CAT was expressed as nmoles of hydrogen peroxide decomposed/min/mg protein.[10]

RESULTS AND DISCUSSION

Oxidative stress is an imbalance between the production of ROSs at cellular level. In this study, estimation of lipid peroxidationand myeloperoxidase [Graph 1] and protein carbonyl content [Graph 2] reveals an increase in oxidative stress among people who used heavy metal containing toothpaste. Estimation of lipid peroxide shows marked increase in levels of oxidative stress which is up to 70 mm/g tissue in metal and toothpaste-induced toxicity, whereas in normal toothpaste, the stress was found to be nearly half of the former, i.e., 35 mm/g tissue [Graph 1].

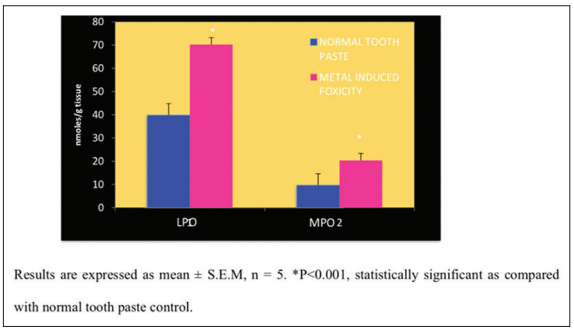
Antioxidant is a molecule which prevents oxidation of other molecules. In the present study, it is evident from the experiments conducted that there is a decrease in the concentration of antioxidants which are seen in the assay of SOD [Graph 3] and CAT [Graph 4]. In the estimation of assay of SOD, antioxidant level in metal and toothpaste-induced toxicity was noted to be 220 units/mg protein, which when compared to the normal toothpaste contained about 350 units/mg protein.

In the estimation of CAT, the level of antioxidant present in metal and toothpaste-induced toxicity was found to be 3 mm/min/mg protein. In normal toothpaste, the antioxidant level was found to be 7 mm/min/mg protein [Graph 4].

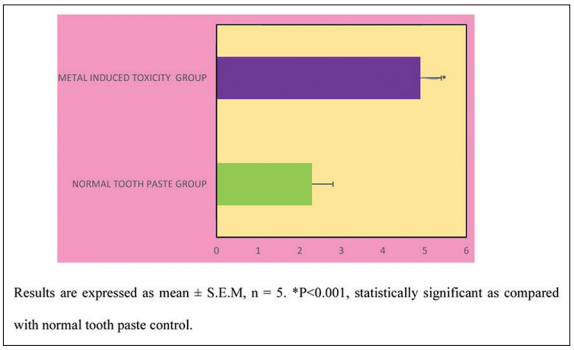
In general, antioxidants play a vital role in health. As oral cavity is more susceptible to oxidative stress, there lies an imbalance between oxidative stress and antioxidants which can induce local as well as systemic complications.

Not only does oxidative stress exacerbate inflammation in the oral tissues but also recent scientific studies have shown it as a contributing factor to systemic inflammatory diseases, including rheumatoid arthritis and cardiovascular disease.[11-13] Due to the association between gum disease, inflammation, and oxidative stress, it is critical to control gum disease and to maintain the balance between oxidants and antioxidants in oral tissues.[14-16]

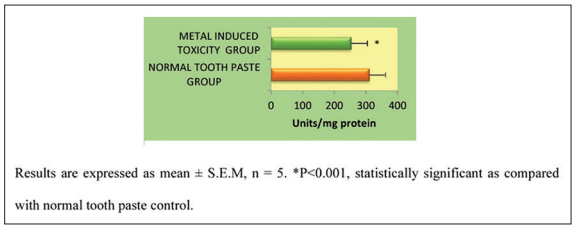
In local complication such as periodontal diseases, inflammation results from interaction between



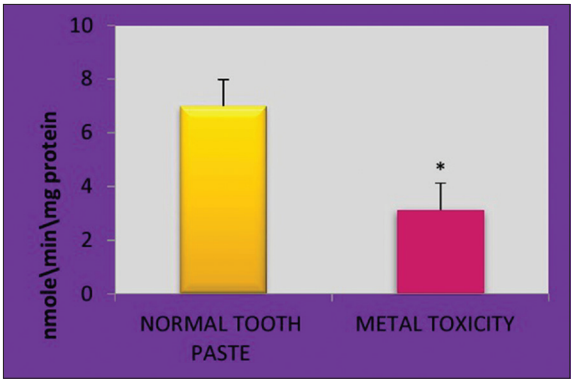
Graph 1: Estimation of Lipid Peroxidation and Myeloperoxidase Activity



Graph 2: Estimation of Protein Carbonyl Content



Graph 3: Assay of SOD



Graph 4: Assay of CAT

bacterial invasion and host inflammatory response. Free radicals and ROS are responsible for the inflammatory response. Periodontal pathogens can induce ROS overproduction and thus causes collagen and periodontal tissue breakdown. When ROS are scavenged by antioxidants, collagen breakdown can be minimized. Decreased levels of Vitamins A and C, β-carotene, and β-cryptoxanthin significantly increase the risk of gingival disease.[13] Low levels of most antioxidants are a risk factor for periodontal disease and infection. Free radicals are released as a result of bacterial clearance and killing. Periodontal tissue depends on natural antioxidants to overcome this oxidative stress and maintain homeostasis. When antioxidants are depleted, the ability of gingival tissue to overcome oxidative stress, maintain normal tissue, and control the bacterial damage appears to be compromised. Increased production of ROS necessitates an elevated need for zinc, copper, and selenium, nutrients which are involved in antioxidant defenses.[17,18]

Antioxidants show preventive and therapeutic potential in many stages of oral carcinogenesis. Researchers have recently stated that there is an inhibition of oral cancer phenotypes after antioxidant intakes.[15] The administrations of proanthocyanidins that can be found in flavonoid structures of antioxidants have an ability to reduce cell growth and proliferation of oral carcinomas1. Dietary antioxidants can protect the lipids and other membrane molecules against oxidative damage by intercepting oxidants before they try to destroy the tissues.[19,20]

Recently, it has been claimed that the imbalances in levels of free radicals, ROS, and antioxidants in saliva play an important role in the onset and development of dental caries.[21] Hence, evaluation of those factors in saliva that may increase the risk of individuals to dental caries can pave way to make recommendations that will cater specifically to the needs of an individual. Most important would be the function of salivary peroxidase system, which constitutes one of the major salivary antioxidant systems.[18] Salivary peroxidase brings the control on oral bacteria which lead to dental caries. Salivary peroxidase catalyzes the peroxidation of thiocyanate ion (SCN-) to generate oxidation products (more stable OSCN); this inhibits the growth and metabolism of many microorganisms, thereby inhibiting caries or at least slowing down the progress of caries.[19]

CONCLUSION

Thus, from this study, it is evident that there is a need for antioxidants for maintaining good health. In the present study, a decrease in antioxidants in heavy metal containing toothpastes shows that it can lead to the localized disorder as well as systemic disorders such as cancer and cardiovascular diseases. Hence, toothpaste containing heavy metals should be avoided.

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