Original Article

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Dentistry Section

Comparative Evaluation of Vitamin E and Glutathione Peroxidase Levels in Salivary Samples of Children with and without Dental Caries

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ABSTRACT

Introduction: Multiple factors influence the initiation and progression of caries. Recent research has revealed that free radicals can be a prime cause of several inflammatory oral pathologies, including dental caries by direct or indirect influence on the salivary constituents like disease mediated free radicals and antioxidants.

Aim: The aim of the present study was comparative evaluation of Vitamin E and Glutathione Peroxidase levels with the prevalence of dental caries in a group of school-going children.

Materials and Methods: The present cross-sectional clinical study was conducted in Department of Pedodontics and Preventive Dentistry, Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India from November 2016 to November 2018. Hundred children aged between 6-12 years were selected and divided into two groups i.e., caries active group (n=50) and caries-free group (n=50). Children having atleast five decayed teeth were considered as caries active and children with DMFT/deft score 0 were considered as caries-free group. A 2 mL of unstimulated saliva was collected from all the subjects and the levels of Glutathione Peroxidase (GPx) and Vitamin E were evaluated by spectrophotometric assay. Obtained imbibing values were subjected to statistical analysis using unpaired t-test and Statistical Package for the Social Science (SPSS) Version 21.0 software. The p-value <0.05 was considered statistically significant.

Results: Of the 100 participants, 52 were males and 48 were females. The mean age of the study participants was 10.21±1.4 years. Significant reduction in GPx ($p<0.001$) and Vitamin E ($p<0.05$) were noticed in caries active group when compared to the caries-free group.

Conclusion: GPx and Vitamin E levels showed an inverse relationship with the presence of dental caries. Thus these antioxidant levels can be used as biomarkers to assess the oral as well as general health. Caries activity can also be measured by these salivary factors which might be helpful in preventive dentistry.

Keywords: Antioxidants, Free radicals, Oxidative stress, Saliva, Spectrophotometer

INTRODUCTION

Oxygen is considered as an indispensable nutrient required for all mammalian energy needs. During the metabolic processes, multicellular organisms utilise the molecular oxygen, which undergoes either oxidation or reduction as concerning to the cellular activity and releases a variety of chemical species, such as free radicals [1]. In recent years, there is emerging evidence that free radicals play a significant role in the pathogenesis of disease conditions and have become an area of interest in oral health research [2]. A free radical is any molecular species capable of autonomous existence that contains an unpaired electron in an atomic orbital. The unpaired electron of a free radical makes it unstable, short-lived, and highly reactive [3]. Free radical-mediated reactions proceed as chain reaction cascade, finally damaging the living cell. Most of these free radicals that damage the biological systems are derived from oxygen and are referred to as Reactive Oxygen Species (ROS) [4].

The source of ROS in the body is both endogenous and exogeneous. The metabolic activity of mitochondria, peroxisomes, endoplasmic reticulum acts as an endogenous source. The exogenous sources are pollution, alcohol consumption, tobacco smoking, heavy metals that penetrate into the body through different routes and get decomposed or metabolised into free radicals [4]. These free radicals adversely affect various essential classes of biological molecules in the body such as nucleic acids, lipids and proteins, thereby altering the normal redox status leading to increased oxidative stress in pathological and/or inflammatory conditions [5,6]. The concept of oxidative stress-related pathology has been researched by many studies [7-9] and it has been hypothesised that this imbalance between free radicals and antioxidants could be a prime cause for oral diseases such as periodontitis, diseases involving salivary and mucosal glands, oral precancerous conditions and dental caries [10]. To counteract these deleterious effects, the body fluids and tissues possess various defense and repair systems to prevent the accumulation of toxic radicals, and one such defense system in the oral cavity is the salivary mediated antioxidant system [11].

Dentists are well versed with the myriad functions of saliva [12], including its action as a cleansing solution, ion reservoir, lubricant, and buffer. Akin to this, saliva could constitute the first line of defense against free radical-mediated diseases by circulating the antioxidants. Antioxidants are the various enzymatic and nonenzymatic compounds that counteract the potential complications of oxidants in the body. They prevent various pathological disorders by interacting with free radicals and by terminating the chain reaction [5]. The important first-line defence antioxidants are catalase, superoxide dismutase, Glutathione Peroxidase (GPx), uric acid, vitamin C, vitamin E, carotenoids and ubiquinol [6].

The interaction between free radicals and antioxidants will be better understood if one can assess the response of the individual antioxidants to a diseased condition. While previous studies have evaluated the role of antioxidants in dental caries measured the Total Antioxidant Capacity (TAC) [10,13], the present study aimed to evaluate and compare nonenzymatic vitamin E and enzymatic GPx levels in saliva of caries-free and caries active children and to determine the relationship between salivary antioxidant levels and dental caries.

MATERIALS AND METHODS

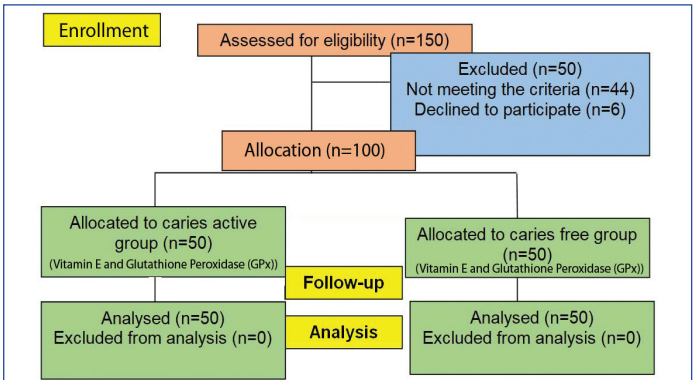
The present cross-sectional study was conducted in Department of Pedodontics and Preventive Dentistry, Sibar Institute of Dental Sciences, Guntur, Andhra Pradesh, India from November 2016 to November 2018. Before starting the study, an Institutional Ethical committee clearance was taken vide letter no (PR.61/IEC/SIBAR 2016), and informed written consent was obtained from parents/ guardians of participating children. A total of 100 children aged 6-12 years without gender differentiation were selected from 15 schools (both private and Government) located in and around the Guntur city.

Inclusion and Exclusion criteria: The inclusion criteria considered for selecting the patients were: children having atleast five decayed teeth as caries active and children with DMFT/deft score 0 as caries-free group. Children with history of any systemic illness, salivary disorders, arrested carious lesions, and presence of white spot lesions were excluded.

Sample size calculation: The sample size was calculated using G* power 3.1.9.4 software [14]. A sample of 98 was required to detect any existing difference in the antioxidant levels between caries active and caries-free groups at a moderate effect size of 0.6, α error of 5% and power 80%.

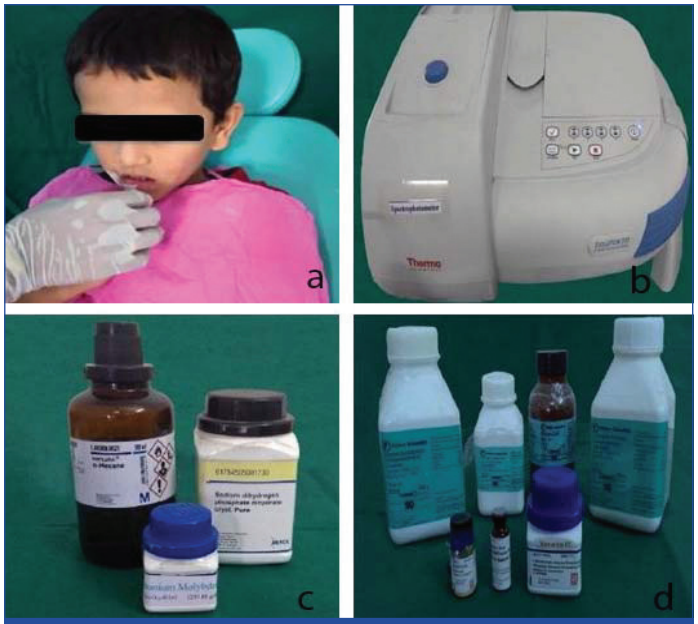
Procedure

Although the obtained sample size was 98, for the ease in statistical analysis, 100 healthy school students were considered. Further, these children were divided into two groups of 50 each, based on the dmft+/DMFT score as Group I (caries active) and Group II (caries-free) [Table/Fig-1]. For selected children, a thorough diet history was taken and oral prophylaxis was performed. The children were instructed to sit in the coachman's position [15] with head slightly down and were asked not to swallow or move tongue or lips during the period of sample collection. Approximately, 2 mL of unstimulated saliva was collected from each individual of both the groups into a sterile test tube using the drooling method, and the collected saliva was stored in a hermetically sealed case containing ice and transferred to the laboratory within 20 minutes. The samples were centrifuged at 2000 rpm for 15 minutes to separate all debris and stored at -20°C. Further, these salivary samples were tested for levels of antioxidants using Spectrophotometer [Table/Fig-2].



[Table/Fig-1]: Flowchart showing distribution of patients across various steps of the study.

Procedure for Estimation of Vitamin E: A 0.5 mL of saliva sample was added to 0.5 mL of hexane and centrifuged for 1 minute. A 0.1 mL of the above mixture was mixed in a test tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), which was incubated at 37°C for 90 minutes with vigorous shaking. The absorbance of the aqueous phase at 695 nm was measured by the spectrophotometer. In this procedure, the only requirement was a vigorous agitation to facilitate the transfer of reducing species to the aqueous phase and the subsequent formation of the green complex [16].



[Table/Fig-2]: Figure showing: a) Collection of salivary sample; b) Spectrophotometer; c) Reagents used for assessing Vitamin E; d) Reagents used for assessing glutathione peroxidase.

Procedure for Glutathione Peroxidase (GPx): The GPx activity of saliva was assayed in the system containing sample of 0.1 mL 0.0022 M hydrogen peroxide (prepared by 50 mM sodium phosphate buffer, pH 7.0) into an equilibrated mixture of 0.05 mL of saliva sample, with 2.58 mL of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.005 M Ethylenediamine Tetraacetic Acid (EDTA), 0.1 mL 0.0084 mM Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH), 0.01 mL yeast glutathione reductase (2 units), 0.01 mL 1.125 M sodium azide, and 0.1 mL of 0.15 M reduced glutathione. The absorbance was read at 500 nm for every 20 seconds and the values thus obtained were tabulated and subjected to statistical analysis [17].

The whole procedure, right from patient selection, saliva collection, biochemical analysis and recording of the values were carried by a single operator. However to avoid bias, a second operator evaluated the procedural aspect and values that were recorded. Intra Class Correlation coefficient (ICC) values were calculated to examine the reliability. The ICC values were observed to be 0.89 for vitamin E and 0.91 for GPx.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 21.0 software (IBM, ARMONK, NY, USA). Descriptive statistics, independent t-test was used in data analysis to compare antioxidant levels between caries active and caries-free groups. The p-value <0.05 was considered statistically significant.

RESULTS

Of the 100 participants, 52 were males and 48 were females. The mean age of the study participants was 10.21±1.4 years. The mean values of antioxidant levels (vitamin E and GPx) in caries active and caries-free groups were illustrated in [Table/Fig-3]. The mean values of vitamin E levels in caries active and caries-free children were 1.25±0.01 and 1.37±0.01, respectively. There was a decrease in vitamin E levels in caries active group when compared with the caries-free group. Even though the decline appeared slight, it was statistically significant with p<0.05.

The mean GPx levels in caries active and caries-free children were 0.53±0.08 and 1.62±0.14, respectively. Marked decrease in the values was noticed in caries active group when compared to caries-free group, which was highly statistically significant (p<0.001) [Table/Fig-3].

Variables	Caries active (n=50)		Caries-free (n=50)		p-value
	Mean	SD	Mean	SD	
Vitamin E (μ mole α tocopherol/mL)	1.25	0.01	1.37	0.01	0.05
GPx (Nmole NADPH oxidized/min/mL)	0.53	0.08	1.62	0.14	0.001

[Table/Fig-3]: Mean values of antioxidant levels in caries active and caries-free groups. *Independent samples t-test was performed; NADPH: Nicotinamide adenine dinucleotide phosphate hydrogen, p-value

DISCUSSION

Among the oral diseases, dental caries is the most commonly occurring disease which affects mankind and also known to be drastically influenced by the alteration in salivary parameters [18]. In the present study, children between 6-12 years were considered, as mixed dentition period could give us an idea about the status of caries risk and early initiation of caries preventive measures [19]. Unstimulated saliva was collected as it provides a more accurate account of the salivary composition when compared to the stimulated saliva [20].

Dental caries is a microbial disease with multi-factorial aetiology whose initiation and progression are due to the complex interaction between the host, microflora, diet, and time [10]. In conjunction with the bacterial challenge, the host immune response has a significant role in the progression of the disease. As part of the host immune response, host cells such as neutrophils and monocytes, phagocytose microorganisms and produce free radicals [21].

Lipid peroxidation is the oxidative degradation of lipids and forms a highly reactive peroxyl radical, resulting in cell damage as well as decreases the cellular antioxidant availability. The available evidence suggests that the initiation and progression of dental caries are related to the excessive production of lipid peroxides [22,23]. Vitamin E and GPx are the first line of defence antioxidant that scavenges the oxygen free radicals released during lipid peroxidation [24,25]. Prior epidemiological studies conducted by Rapisarda E and Longo A; and Niki E et al., have shown that supplementation of vitamin E can reduce oxidative stress and prevent many chronic diseases such as dental caries and gingivitis [26,27]. From the positive outcome of the clinical trials by Rapisarda E and Longo A suggested that there is an association between dietary intake of vitamin E and mortality of the diseases [26]. In the present study, mean value of vitamin E levels was lower in the caries active group than caries-free group.

The findings of the present study are in accordance with the study done by Rahmani M et al., in which they reported that Total Antioxidant Capacity (TAC) of saliva in children with dental caries was significantly lower when compared with those without caries [14]. Similarly, Krawczyk D et al., stated that increase in number of carious teeth resulted in a significant decrease in stimulated and unstimulated salivary antioxidant level [28]. In another study, Krawczyk D et al., reported a decrease in TAC of saliva in subjects with dental caries. The authors stated that decrease in TAC in dental caries may be related to increased activity of neutrophils and monocytes in the oral cavity. This causes production of ROS in presence of bacteria [29].

Hegde MN et al., compared the TAC of saliva in children with Early Childhood Caries (ECC) and rampant caries and stated that increase in the TAC of saliva in children with caries irrespective of type of caries. The authors concluded that the presence of infectious challenge in the form of caries or poor oral hygiene could be considered as one of the factors responsible for the increased levels of TAC of saliva [30]. These findings are also in agreement with the study results by Rai B and Saral Y et al., who observed lower vitamin E levels in oral cancer and recurrent aphthous ulcer patients, when compared to control group (p-value <0.01) [31,32]. The findings of the present study were also in accordance with the studies done by Kamodyoa AN et al., and Gopinath V and Arzreanne AR [33,34].

A study by Herbette S et al., has shown that the enzyme levels of GPx are either elevated or depleted in its activity in various diseased states [35]. Panjamurthy K et al., have reported higher plasma levels of GPx in patients with chronic periodontitis, and Hegde MN et al., observed increased levels of GPx in saliva and whole blood in caries active individuals than in the control group [36,37]. The increased levels of GPx could be due to the production of free radicals or ROS at inflammatory sites, which act as a stimulant to produce more amount of GPx at the diseased site and thereby protect the tissue from oxidative damage [38]. In contrast to the above studies, the present study showed lower GPx levels in caries active group than the control group. Assi SH and Husain RSA found lower levels of GPx in blood serum of leukemia patients and Punj A et al., reported reduced salivary GPx levels in chronic periodontitis patients [39,40]. However, the present study was only confined to dental caries in children.

From the above findings it can be said that the decreased vitamin E and GPx levels could be due to the enhanced activity of neutrophils and monocytes in the oral cavity which produce ROS in the presence of bacteria [28]. Similar view points was expressed by Rai B et al., in his study [31]. Thus, it could be stated that there is a definitive imbalance between free radicals and antioxidants in the disease process. Punj A et al., estimated antioxidant levels in saliva and serum of chronic periodontitis patients with and without ischaemic heart disease and stated that gingival tissue, serum/GCF stimulated antioxidant increase is significant compared to salivary mediated antioxidant stimulation [40]. Similarly, in the present study also a significant decrease in antioxidant levels were found in caries active group compared to caries-free group.

Limitation(s)

However, there were certain limitations in the present study; a child may have been caries active without cavitation or lesion development and thus may have confounded the results. In this study, only two individual antioxidants were evaluated. Longitudinal studies with a larger sample size are required for future research to elicit more accurate information about the interaction between free radicals and other antioxidants.

CONCLUSION(S)

The present study showed a statistically significant reduction in Vitamin E and GPx levels in caries active children than caries-free children indicating an inverse relationship between the levels of both these components with caries activity. Thus, these antioxidant levels can be used as biomarkers to assess the oral as well as general health. Caries activity can also be measured by these salivary factors which might be helpful in preventive dentistry.

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