

Research Article

Association between Dental Caries and Lipid Peroxidation in Saliva

Gargi Sarode, Anjali Shelar, Sachin Sarode, Neeta Bagul

Abstract

Aims and Objectives: To evaluate the relationship between salivary malondialdehyde (MDA) ie oxidative stress and oral health. **Materials and Methods:** Healthy adults, matched for gender and age, with (N = 30, 13 males and 17 females with mean age of 27.56 years) and without (N = 12, 6 males and 6 females with mean age of 26.1 years) dental caries were included in this study. The World Health Organization caries diagnostic criteria were used for determining the decayed, missing, filled teeth (DMFT) index and oral hygiene was assessed using the simplified oral hygiene index (OHI). Lipid peroxidation (LP) was determined by Buege and Aust method. **Results:** Simplified oral hygiene index and DMFT scores were significantly different between the groups. MDA values were significantly different ($P < 0.05$) between the groups (0.6242 ± 0.1001 and 1.699 ± 0.2088 in adults without caries and with caries, respectively). MDA values were increasing with higher OHI but were not statistically significant. **Conclusion:** Our results suggest that there is an association between presence of dental caries and salivary MDA levels.

Key words: Malondialdehyde;Energy Metabolism;Oxidation-Reduction;Lipid Peroxidation; Thiobarbituric Acid Reacting Substance;Dental Caries;Oral Hygiene Index;DMF Index.

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Introduction

Dental caries (DC) is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation. In spite of several studies on various aspects of dental caries, many aspects of its etiology are still obscure.¹

Free radicals can be defined as molecules or molecular fragments with an unpaired electron which imparts certain characteristics to the free radicals are able to produce chemical modifications and to damage proteins, lipids, carbohydrates and nucleotides in the tissues. There are various major routes in which free radicals can interact with neighboring components in cells to disturb their integrity and functions. One of these routes is lipid peroxidation (LP).^{2,3} Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism resulting in formation of malondialdehyde (MDA). MDA is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress

in cells and form advanced glycation end products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.⁴ MDA is a stable end product of peroxidation of membrane lipids and is widely used as an indicator of increased LP.⁵

Saliva is the first biological medium confronted by external materials such as food, drink, or inhaled volatile factors. Saliva constantly bathes the teeth and oral mucosa acting as an antibacterial solution, an ion reservoir, a lubricant, and a buffer. Also saliva is used for diagnostic purposes as it can be collected in a safe and patient friendly way which requires no special training. Thus normal salivary levels of antioxidants and oxidants are required to maintain the immunological balance of the oral cavity. Since DC has got immunological basis, it is hypothesized that MDA levels are in some or the other way related to dental caries. Very few studies have been carried out on salivary MDA levels in patients with dental caries.^{6,7} Thus the aim of this paper is to see the association between LP and dental-oral health.

Materials and Method

Thirty patients (M:F, 13:17) with DC in age group 17 - 50 years attending Out Patient

Department and 12 (M:F, 1:1) normal healthy subjects with age group 15 - 60 years were observed as control in this observational case control study. The WHO caries diagnostic criteria were used for determining the decayed, missing, filled teeth (DMFT) index and oral hygiene was assessed using the simplified oral hygiene index (OHI). LP was determined by Buege and Aust method.⁸ In this study, the relationship between DC and LP as an indicator of oxidative damage of oral tissue was evaluated in healthy adults with and without caries. The present study was designed in accordance with the guidelines issued and approved by the local Ethics Committee. Written informed consent was obtained from all the participants. Healthy adults, matched for gender and age, with (N = 30, 13 males and 17 females with mean age of 27.56 years) and without (N = 12, 6 males and 6 females with mean age of 26.1 years) caries were included in this study. All subjects were instructed to refrain from smoking, eating and drinking for 12 hours prior to saliva collection and to brush their teeth in the morning. Fasting unstimulated whole saliva samples were collected.

Following flushing of mouth with 100 ml of distilled water, whole saliva was collected for 5 min by the subject leaning forward and spitting saliva into test tubes. This procedure was carried out between 8 am to 9 am under the same conditions and by the same investigator adhering to the inclusion criteria. The collected specimens were soon centrifuged at 3000 rpm for 10 min at room temperature to remove microorganisms, saburra, and desquamated epithelial cells. Immediately after collection, saliva volume was measured and saliva samples were stored at -20°C until use. Mixture of one ml of saliva, 1 ml of 40% trichloroacetic acid, and 2 ml of 0.67% thiobarbituric acid was heated in boiling water bath for 10 minutes. It was then cooled at room temperature and centrifuged. The absorbance of the supernatant at 530 nm was noted and MDA concentration was calculated.

Statistical Analysis: Data were reported as means and standard deviation. Student t-test between groups and Pearson's correlation analysis was applied.

Results

Elevated salivary MDA levels were observed in patients with DC (1.699 ± 0.2088) when compared to controls (0.6242 ± 0.1001). The

study group showed a DMFT index of 5.8 and OHI of 1.866. Similarly elevated salivary MDA levels were observed in patients with poor oral hygiene when compared to controls though the difference was not statistically significant.

Discussion

DC has got multifactorial etiology which involves several factors like diet, host, bacteria, time and personal factors like oral hygiene. The first line of defense against DC is saliva. Oxidative stress-decreasing factors in dental diseases involve good oral hygiene which may affect the composition of saliva.⁹ Limited data are available about the effect of oral hygiene on salivary parameters in patients with caries. Thorough investigation of saliva is needed to study the composition and physiology of saliva as it influences the oral health.¹⁰ The balance of oxidant-antioxidant system of saliva determines the overall health of the oral cavity. Impairment of oxidant-antioxidant balance in saliva may lead to various oral pathologic conditions.¹¹

In the present study, salivary MDA levels were significantly higher in subjects with caries compared to subjects without caries (Table 1). MDA levels were higher in subjects with high OHIs but results were not statistically significant. Tsai et al.⁷ and Scully et al.¹² have shown higher salivary MDA levels in individuals with poor oral hygiene than the subjects with adequate oral hygiene. Rai et al.⁶ determined MDA in some oral diseases, such as leukoplakia, oral submucous fibrosis, candidiasis, DC and oral cancer and in healthy subjects. They reported that there were no differences in salivary MDA levels in a caries group compared to control.

	DC Free Group (N=12)	DC Group (N=30)
DMFT	0	5.8
OHI	0	1.866
MDA	0.6242 ± 0.1001	$1.699 \pm 0.2088^*$

Table 1: Dental and salivary parameters for adults with and without DC. (*P < 0.05, significantly different from caries-free group)

However, increased salivary MDA levels in periodontal and some systemic diseases, such as diabetes, osteoporosis, etc. that were related with oxidative damage, are well known. Hodossy and Celec¹³ have investigated the effects of daily dynamics, tooth-brushing and ascorbic acid

administration on salivary thiobarbituric acid reacting substance (TBARS) levels. They reported that tooth-brushing decreases salivary TBARS. In the above-mentioned studies, since DMFT and oral hygiene status of the subjects were not determined, we are not able to compare them to our results.

Although Öztürk et al.⁷ determined the relationship between DMFT, oral hygiene status and salivary MDA levels of the subjects, they did not find any significant differences in salivary MDA levels between the groups. According to them the reason behind this was teeth brushing as their subjects routinely brushed their teeth and they were also reminded to brush their teeth in the morning before saliva collection.

Thus, there is an association between the oral hygiene, DC and LP but further studies are required to establish a definite relation between lipid peroxidation and dental caries. This will be helpful in prevention of DC and establish the role of oxidant-antioxidant system in pathogenesis of DC leading to their therapeutic applications in DC.

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