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Comparative Evaluation of Salivary Malondialdehyde Levels in Children with Different Caries Status- A Cross-Sectional Observational Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Early childhood caries is a major public health problem especially in young children. ECC affects the quality of life of young children by affecting the chewing ability of children due to the development of pain and swelling, and this may indirectly affect the nutritional status of a child. The present study was done to assess the level of salivary malondialdehyde in children with and without Early childhood caries. The main objective of the study is to determine the salivary malondialdehyde levels and to compare it with the three groups of children with different caries experiences.
Materials and Methods: It is cross-sectional observational research carried out at Saveetha Dental College and Hospitals. Children between 3-6 years were examined and 20 caries-free, 20 children with ECC as well as 20 children with S-ECC were recruited for the study. The caries status was assessed using dmfs and the severity of caries was assessed using pufa index. Salivary samples were collected and analysed for salivary malondialdehyde levels.

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Statistical Analysis: The data was entered and analysed using SPSS software version 20.0. One-way ANOVA was done to determine the difference in malondialdehyde levels between the groups. "Post-hoc Tukey" test was done to measure the intergroup significance. A "P value of < 0.05" was measured as statistically "significant" and P < 0.001 was deemed as statistically "highly significant". Pearson's correlation was done to correlate pufa values with malondialdehyde levels.
Results: The results demonstrated that there was a statistically significant difference in salivary malondialdehyde levels among caries-free, ECC as well as S-ECC children. There was a positive correlation between the pufa score and salivary malondialdehyde levels.
Conclusion: 1. There is a significant difference in the salivary malondialdehyde levels among caries-free, ECC as well as S-ECC children.
2. There was a positive correlation of salivary malondialdehyde levels and pufa score in ECC and S-ECC children.

Keywords: Saliva; early childhood caries; salivary malondialdehyde.

1. INTRODUCTION

Early childhood caries (ECC) is considered as a major public health problem more than a century, and still remains as a dominant disease in children younger than 6 years of age. ECC affects the quality and value of life of the children. ECC affects the quality of life of children by affecting the chewing ability of children due to the development of pain and swelling, and this may indirectly affect the nutritional status of a child. ECC is defined as "the presence of one or more decayed (non-cavitated or cavitated lesions), missing teeth (due to caries), or filled tooth surfaces in any primary tooth in a child 71 of months age or younger". [1,2] ECC follows a distinct pattern of affecting the primary teeth, primarily affect the smooth surface of maxillary incisors followed by occlusal surface of molars. Initially it appears as a white spot lesion and if not treated leads to formation of cavitated lesion.

The microbial flora of the oral cavity is highly diverse, with various bacterial and fungal species. The normal oral flora of the human mainly consists of streptococci and anaerobic Gram negative bacteria [3,4]. These oral microorganisms play a major role in preventing the colonization of pathogenic microbes; whereby maintains the oral health of the individual. The disruption of the normal flora can trigger or influence the occurrence of oral diseases such as dental caries. Among the oral flora, Streptococcus mutans and Streptococcus Sobrinus is considered as a key pathogenic organism in the etiology of ECC [5]. As ECC being a multifactorial disease, other etiological agents such as diet, socio-economic status, salivary proteins, salivary anti oxidants, salivary free radicals also plays an equally important role in initiation of ECC [6-9].

Saliva being a biological fluid, maintains the integrity of the teeth and oral structures. The whole saliva is composed of majority of secretion (90%) from parotid, submandibular and sublingual glands and 10% of secretion from minor salivary glands and gingival crevicular fluid [10]. Saliva helps in flushing of food debris and bacteria from the oral cavity and also maintains acid base balance through buffering action. Since saliva is considered as a mirror of the body's health and can be collected in a safe, non invasive technique, it can be used as a substitute to plasma samples [11]. Saliva is used as a diagnostic tool to assess the systemic disease such as, diabetes, anemia and chronic kidney disorder [12-14].

Free radicals are molecular fragments with an unpaired electron, which produce chemical changes and cause damage to healthy cells of body such as damage to body's protein, lipids and neucleotides. Reactive oxygen and reactive nitrogen species are the byproducts of free radicals [15]. These free radicals are considered to have a dual role, exhibit both toxic as well as beneficial effects and hence the balances between these effects are important in maintaining body's health. Any imbalance between the formation and removal of free radicals leads to development of oxidative stress. When the imbalance shifts towards one side, oxidative stress develops and leads to cell damage [16,17]. These oxidative stress biomarkers are stable in saliva and can be found at detectable concentrations and hence the level of the oxidative stress markers in saliva are crucial and reflect distinct oxidation pathways associated with caries and periodontitis [18] Lipid peroxidation is defined as oxidative degradation of lipid and is one of the major pathway in which free radicals affect the integrity of cell and cause

cell damage. The stable end product of lipid peroxidation is the formation of malondialdehyde (MDA) and it is used as an indicator of increased lipid peroxidation. Oxidative stress causes lipid peroxidation and tissue damage [19,20]. Few studies have been carried out to evaluate the relationship between increased MDA levels and dental caries. Sarode G et al found a positive association between dental caries and increased MDA levels [21]. Ahmadi-Motamayel F et al evaluated serum and salivary MDA levels in a group of 15-19 year individuals. The author found a significantly higher levels of serum and salivary MDA levels in caries active individuals than caries-free individuals [22]. Similarly, a study was done to correlate the level of MDA and ECC in children, and the author inferred an increased level of MDA in children with ECC [9]. To date, there are no much study conducted to evaluate the role of salivary MDA and ECC, and there are no studies evaluated the level of MDA in children with different caries status.

Hence the present study was undertaken to compare the levels of MDA in children between 3-6 years of age with different caries status. The main objective of the study is to determine the correlation between salivary MDA levels to compare it with the three groups of children with different caries experiences.

2. MATERIALS AND METHODS

2.1 Type of Study

2.1.1 Patient selection

The study recruited children between 3-6 years of age to participate in the research. A meeting was held with the parents/caregivers regarding the protocol of the study prior to the beginning of the study.

2.1.2 Inclusion criteria

Children of both genders between 3 - 6 years old.

Parents who accepted to participate in the research with an informed consent .

2.1.3 Exclusion criteria

Uncooperative children who don't allow the collection and/or examination of saliva.

Children with pharmacological treatment and/or systemic diseases.

The research recruited 78 children between 3-6 years of age to participate in the study. However, 18 children were excluded due to non - cooperation during saliva collection. Finally 60 children, were divided into 3 groups, with 20 children in each group. Group I – Caries free (dmfs=0), Group II- ECC (dmfs=1-3), and Group III- S-ECC (dmfs>3).

The awareness among parents/care givers about ECC was assessed by providing a questionnarie regarding oral hygiene practices of infants, bottle feeding practices, nutritional status and vitamin supplements. Oral health education was given to parents/care givers after obtaining their basic knowledge and attitude from the questionnaire. One-to-one education was provided for 15 min.

All children were examined by 2 calibrated pediatric dentist (DR and MR). Initially, instructions were given to pediatric dentists regarding diagnostic criteria using clinical images. Ten children were examined by 2 pediatric dentists separately and re-examined one week later for the calculation of intra-examiner kappa values. Kappa coefficient was calculated between the examiners and the value was found to be 0.8, indicating a good agreement between the examiners. The CPI (community periodontal index) explorer, disposable mirror, disposable glove and dental chair light were used to diagnose ECC. The caries status was recorded based on criteria of the World Health Organization Oral Health Survey Methods for Field Studies [23]. The oral health status of the children was measured using the decay–missing–filled tooth surface (dmfs) index. The dmfs index was calculated based on presence of one or more decayed, missing due to caries, or filled tooth surfaces in any primary tooth and the mean dmfs score was calculated. The pufa index was recored based on pulp involvement, ulceration, fistula, abscess score to measure the extent of the caries lesion and the mean pufa score was calculated [24]. For anterior teeth, 4 surfaces were examined and recorded, namely labial, lingual/palatal, mesial and distal. For posterior teeth, 5 surfaces were examined and recorded, such as, labial, palatal or lingual, mesial, distal and occlusal. The dental examination was completed within 10 min and an assistant recorded the clinical findings.

2.1.4The pufa index scoring system are as follows

p/P: Pulpal involvement is considered when the opening of the pulp chamber is visible or when the coronal structures have been destroyed by the carious process and only roots and root fragments are left. No probing was performed to diagnose pulpal involvement.

u/U: Ulceration due to trauma from sharp pieces of a tooth is recorded when sharp edges of a dislocated tooth with pulpal involvement or root fragments have caused traumatic ulceration of the surrounding soft tissues, e.g., tongue or buccal mucosa.

f/F: Fistula is recorded when a pus-containing swelling related to a tooth with pulpal involvement is present.

a/A: Abscess is considered when a pus-containing swelling related to a tooth with pulpal involvement is present.

2.2 Saliva Collection

Prior to saliva collection, the children were instructed not to eat or drink anything for 2 hours. To avoid circadian variation, the salivary samples were obtained between 10.00 to 11.00 am. The children were instructed to rinse their mouth with water prior to saliva collection. The children were seated in a well-ventialated and calm room. Children were instructed to spit saliva into a sterile container and a 5ml of unstimulated saliva was obtained. Sampling was performed by two dental examiners. During saliva collection, aseptic precautions were strictly followed. Attention was given to placing the saliva collection tube inside the lips to collect saliva and to the removal and placement of the test tube cover.

2.3 Laboratory Procedure

The salivary samples were transferred to the laboratory immediately and centrifuged at 3000 rpm for 24 min at 4°C to remove desquamated epithelial cells and micro organisms. Following centrifugation, the samples were stored at -20°C until use.

2.4 Reagents Used

Chemicals Tris HCl–KCl, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA) and 1,1,3,3 tetraethoxypropane (TMP) were usesd.

2.5 Biochemical Procedure

The level of MDA was estimated in the saliva of children, as stated by Stalnaya and Garishvili et al in 1977 [25]. Briefly, 0.3 ml of saliva was mixed with 3 ml of 0.025 M Tris–HCL and 0.175 M KCl buffer (pH 7.4). Then, 2.5 ml of diluted saliva was mixed with 1 ml of 17% (w ⁄ v) TCA and centrifuged at 4000 rpm for 10 min. The precipitate was pelleted by centrifugation and the supernatant was reacted with 1 ml of 0.8% (w⁄v) TBA in a boiling water bath for 10 min. It was then cooled at room temperature and centrifuged. The MDA obtained from lipid peroxidation reaction reacts with TBA to yield a yellow fluorescent product. The absorbance of 2 ml colored layer of the supernatant was recorded at 530 nm using a spectrophotometer. The absorbance of the supernatant at 530 nm was noted and MDA concentration was calculated. The results are expressed as micromoles per millilitre (μmol ml^{-1})

2.6 Statistical Analysis

The data was entered and analysed using SPSS software version 20.0. One-way ANOVA was done to determine the difference between MDA levels between the groups. “Post-hoc Tukey” test was done to measure the intergroup significance. A “P value of < 0.05” was measured as statistically “significant” and P < 0.001 was deemed as statistically “highly significant”.Pearson’s correlation was done to correlate pufa values with MDA levels.

3. RESULTS

The mean & standard deviation value for salivary MDA is depicted in Table 1. In “Group I (caries-free) the mean” levels of MDA were found to be 0.22 +/-0.24 μmol ml^{-1}, Group II (ECC), the mean value of MDA was 0.82+/-0.11 μmol ml^{-1} and Group III (S-ECC), the mean value of MDA was 0.91 +/-0.13 μmol ml^{-1}. The mean MDA levels in the study groups (P < 0.05) were statistically significant. Post hoc Tukey test revealed that Group III had a considerably greater mean salivary MDA value than that of Groups I and II. However, the mean salivary MDA level from Group II & III was not significantly significant (Table 2).

Table 3 shows the correlation of pufa score and the salivary MDA levels. There was a positive correlation between the pufa score and MDA levels (0.68, 0.88), indicating that when there is an increase in the pufa score there is an increase in salivary MDA levels as well.

Table 1. Comparison of salivary MDA levels between the groups

Groups	N	Mean	SD	p-value
Group I (Caries-free)	20	0.22	0.24	
Group B (ECC)	20	0.82	0.11	
Group C (Severe ECC)	20	0.91	0.13	0.03*

p-value was evaluated using ANOVA. p-value < 0.05 – Significant

Table 2. Intergroup comparison of Salivary MDA levels

Groups	Group I (Caries-free)	Group B (ECC)	Group C (Severe ECC)
Group I (Caries-free)	-	0.05*	0.05*
Group B (ECC)	0.05*	-	0.21
Group C (Severe ECC)	<0.05*	0.21	-

p value < 0.05 – Significant – Post- hoc Tukey test

Table 3. Correlation of pufa score with Salivary MDA levels of ECC and S ECC group

Groups	Mean pufa score	MDA value	Pearson correlation value
Group II (ECC	6.1	0.82	0. 68
Group III (S ECC)	9.7	0.91	0. 88

4. DISCUSSION

The American Academy of Pediatrics states that young children are continuously affected by dental and oral infection. ECC is a preventable disease and it can be prevented if diagnosed at an early stage. If left untreated it can affect the quality of life of young children by affecting the chewing ability by causing pain, bacteremia, premature loss of teeth and damage to the succedenous permanent tooth. Saliva remains as a potential diagnostic tool of dental caries and importance should be emphasized on salivary parameters to diagnose ECC at an early stage and to incorporate preventive measures in clinical practice.

Free radicals affects the integrity of oral mucosa and the anti oxidants of saliva is considered as an regulators in maintaining the integrity of oral mucosa during physiological and pathological condition. [26]. Free Radicals namely Reactive oxygen species (ROS induce lipid peroxidations and affects the cells [27]. Lipid peroxidation is a process which begins with the interaction of ROS with the polyunsaturated fatty acids. This leads to formation of oxidative stress and damage to cell integrity. Uncontrolled production of lipid peroxidases can result in oxidative stress, with significant damage to cell integrity [28,29]. Since the outcome of oxidative stress is lipid peroxidation , numerous markers have been used to monitor this process. MDA is considered as one of the main product of lipid peroxidation and its values were found to increase during oxidative stress [29,30]. Since, saliva plays a major role in preventing dental caries through its defence action and these oxidative stress can alter the composition of saliva, this study focussed on comparing the level of MDA levels in children with different caries status.

ECC is an inflammatory disease caused by the toxins produced by pathogenic bacteria and leads to break down of collagen due to the activation of matrix metallo proteinase. This mechanism promotes the initiation of lipid peoxidation and alter the level of MDA and progress to initiation of ECC. MDA is the prime by-products of lipid peroxidation, which is produced in the host tissue cells in the saliva, leading to compromised host immune response. [22] This altered immune response weaken the level of antioxidant level of saliva, which in turn reduce the control of dental plaque formation. leading to ECC [31].

Kumar D et al, Dowad R et al and Mahjoub S et al assessed Total antioxidant capacity of saliva in children with and without ECC and reported an increase in TAC in children with ECC as compared to caries free children [8,32,33]. Since

only few studies were carried out in evaluating the level of lipid proxidation in children with ECC and at present no study has compared the MDA levels in children with different caries status, the present study focussed towards assessing the level of MDA in children with different caries status.

In the current study, there was a significant difference in the level of MDA between the groups. The MDA levels were found to be increased in S ECC children followed by ECC and caries free group. The results were similar to the study done by Subramanyam D et al where the author reported an increase in MDA levels in children with ECC [9]. An interesting study by Ahmadi-Motamayel F et al. Who compared the serum and salivary MDA levels and stated that there was a significant increase in serum and salivary MDA levels in caries active group as compared to caries free group [22]. Similarly, Sarode G et al compared the salivary MDA levels and oral hygiene status of adults and stated that there was an increase in MDA levels with an increase in oral hygiene score [21].

The present study compared the pufa score of ECC and S ECC group with MDA levels. The results showed that there was a statistically significant difference in pufa score and MDA levels in ECC and S ECC group. As pufa score will reflect the depth of caries status, this correlation gives us a view of the severity of caries and salivary MDA levels. Since no study have assessed the pufa score with MDA levels, direct comparison of the current study result s with the previous study is not possible.

The limitation of the study includes smaller sample size and selection of samples from a same geographic location. Hence, further studies are needed with larger sample size with sample selection from different geographic location to assess the exact role of MDA levels in children with ECC.

In summary it can be concluded that MDA levels can be utilized as a caries diagnostic marker in children with ECC. The study further stated that, children with higher pufa score had an increased level of salivary MDA levels. In addition, this study is one of the first research done to compare the Salivary malondialdehyde levels in children with different caries status. The results of this study will be relevant for clinicians in managing caries in their pediatric patients.

5. CONCLUSION

The following conclusion may be formed in the light of available evidence.

1. There is a significant difference in the salivary MDA levels among caries-free, ECC as well as S-ECC children.
2. There was a positive correlation of salivary MDA levels and pufa score in ECC and S-ECC children.

CONSENT AND ETHICAL APPROVAL

The present study obtained Ethical approval from the Human Ethical Committee of Saveetha Dental college and Hospitals, Chennai. The Ethical committee reviewed and approved the research protocol. The parents/care givers who provided a written informed consent were included in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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