Association of Salivary Lipids and Early Childhood Caries in an Indian Subpopulation: A Preliminary Study

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***Aim**: This preliminary study aimed to estimate and correlate the relationship between salivary flow rate and levels of salivary triglycerides, cholesterol and total lipids in children with and without early childhood caries. **Study design:** Ninety children aged 3 – 6 years were divided into three groups of 30 each based on their decayed missing filled tooth (dmft) score, group I (dmft score = 0), group II (dmft score ≥4 and ≤ 9) and group III (dmft scores ≥ 10). Whole unstimulated saliva was collected in a sterile graduated cup over a period of 5 minutes and was quantitatively analyzed for levels of salivary triglycerides, cholesterol and total lipids. Data obtained was subjected to statistical analysis by one way ANOVA, Post-Hoc tukey and Pearson's correlation test. **Results:** Salivary flow rate was 1.20±0.36, 1.01±0.37 and 0.86±0.31 ml/min in group I, II and III respectively. The mean levels of salivary triglycerides in group I, II and III was 3.57±0.43mg/ml, 6.11±1.70mg/ml and 6.03±1.73 mg/ml, respectively. The mean levels of salivary total lipids were higher in group II and III, ie 22.51±2.87 mg/ml and 22.68±2.54 mg/ml respectively. The mean level of salivary cholesterol was highest in group III (8.03±2.91 mg/ml). Salivary triglycerides and total lipids showed a significant positive correlation with dmft scores of children (p≤ 0.001). Salivary cholesterol also had a positive association with dental caries experience of children but was not significant. There was a negative correlation between salivary flow rate and levels of salivary triglycerides, cholesterol and total lipids. **Conclusion:** Children with ECC (group II and III) had lower salivary flow rate and higher levels of salivary triglycerides and total lipids compared to caries free children (group I). Levels of salivary cholesterol did not differ between caries free and children with ECC.*

***Keywords:** ECC, salivary flow rate, triglycerides, lipids*

INTRODUCTION

Early childhood caries (ECC) is a particularly virulent form of caries that affects the primary teeth of infants and children.[1]ECC begins early in life, progresses rapidly and often goes untreated. It is a multi-factorial disease.[2]

Saliva is a complex biofluid that has important functions in oral homeostasis and therefore its composition is related to systemic and oral physiological conditions.[3]Saliva plays a very important role in defense mechanism against dental caries. Physiological, pathological and environmental factors can cause changes in salivary composition that can be correlated to dental caries susceptibility. Many salivary constituents like proteins, carbohydrates, lipids, and ions play an important role in health and disease. Local and systemic disorders may disturb or interrupt these complex balanced functions, which can lead to mucosal and tooth damage.[4]

Saliva has emerged as an important biofluid for oral research. The ease of collection, storage, transportation and non invasive sampling makes saliva particularly useful for research in children, where repeated sampling is needed.[5] The salivary pellicle is an

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organic membrane composed of glycoprotiens and lipids derived from saliva.Salivary lipids could play an important role in the dynamics of dental caries due to its glycoprotein and lipid content.[6]

Salivary lipids are mostly of glandular origin, although cholesterol and some fatty acids are believed to come directly from serum. The most frequent lipids in saliva are neutral lipids, glycolipids, and phospholipids. Lipids originate from three origins they are through serum element transudation, through cells exfoliation and of glandular origin.[7] Salivary lipids are associated with proteins, especially to high molecular weight glycoproteins and to proline-rich proteins (PRPs). [6]

The role of salivary lipids in the etiology of dental caries is not well established.[8]Though, few studies affirm a positive association of salivary triglycerides and total lipids to caries experience in adults there is disconcordance in the data relating parotid and submandibular salivary cholesterol levels to dental caries.[9-11]Studies have also reported higher levels of salivary cholesterol, triglycerides and total lipids in enamel and cementum pellicles of caries susceptible adults.[12]There is lack of information on relationship of salivary lipids and caries in children. The present study was conducted to determine the relationship between salivary lipids and early childhood caries (ECC).

MATERIALS AND METHOD

The present study was carried out on 90 healthy children aged 3 to 6 years visiting the Department of Pedodontics and Preventive Dentistry, The Oxford Dental College, Bangalore,India. The study was approved by the institution's Ethical review board. Healthy and cooperative children (Frankel behavior rating of 3 and 4) with only primary dentition were selected for the study. Only those children with a capacity to expectorate were included. Prior written informed consent was obtained from the parents of children included in the study. Children with any systemic or underlying medical /metabolic condition and on any medications were excluded from participation in the study. The World Health Organization (WHO) caries diagnostic criteria (2013) were followed to diagnose and record dental caries.[13] The children were then divided into three groups of 30 each. group I children with dmft score zero , group II children with dmft score ≥4 and ≤ 9 and group III children with dmft scores ≥ 10.

Method of saliva collection

Each child was made to sit in Coachman position[14]wherein child is seated on the edge of dental chair,the head and neck bent down with arms resting comfortably on the knee/thigh.Lips are only slightly apart and unstimulated whole saliva was allowed to passively drool into a sterile graduated cup. Saliva collection was done in the morning between 10 and 11:30 am over a period of 5 minutes. The eyes were kept open except for blinking during the 5-minute collection period.It was ensured that the child did not eat or drink anything for 1 hour prior to the saliva collection. Salivary flow rate was estimated as the amount of saliva expressed in one minute and was denoted as milliliter per minute (ml/min). The collected saliva was transferred to sterile eppendorftubes® and placed in an ice box at 4°C and transported immediately to the laboratory.

Biochemical analysis of saliva

Lipids in saliva were extracted by Folch method.[7]In this method, chloroform:methanol in 2:1 ratio are added to the sample to increase the volume by twenty fold and filtered. The solution was centrifuged at low speed (2000 revolution per minute) to separate the solution into two phases. The upper phase was removed by siphoning and lower chloroform phase containing lipids was used for further analysis.

A fully automated bichromatic analyzer was used for estimation of levels of salivary cholesterol, triglycerides and total lipids using a commercially available MERIL diagnostic kit (MERIL diagnostics private limited, Vapi, Gujarat,India). Salivary total cholesterol and triglycerides were estimated by cholesterol oxidase (CHOD)[15] method and glycerol phosphate oxidase peroxidase (GPOP)[16] method, respectively. Salivary total lipids in milligrams per deciliter (mg/dl) were calculated by the sum of levels of salivary triglyceride, cholesterol, low density lipoprotein and high density lipoprotein in the salivary sample.

Salivary total lipids (mg/dl) = salivary triglycerides + salivary cholesterol + salivary Low Density Lipoprotein (LDL) + Very Low Density Lipoprotein (VLDL).

The levels of salivary LDL and salivary VLDL were calculated using the Ferguson formula which is as follows:

- Salivary Low Density Lipoprotein (mg/dl) = Total cholesterol- (VLDL)–(HDL)
- Salivary Very Low Density Lipoprotein (mg/dl) = Triglyceride/5

Statistical method

The data obtained was tabulated and subjected to statistical analysis using post-hoc Tukey, one way analysis of variance (ANOVA) and Pearson's correlation coefficient.Statistical Package of Social Sciences (SPSS) for windows version 18.0, Armonk, NY: IBM Corp., USA was used to perform statistical analyses.

RESULTS

The mean age of children in the groups did not differ. Among the three groups, the mean salivary flow rate was significantly higher in group I (caries free children) ($P<0.001$) (Table 1). There was no statistically significant difference in the levels of salivary cholesterol of the children between the groups. The mean levels of salivary triglyceride of children in group II and group III were significantly higher ($p<0.001$) compared to that of group I. The mean levels of salivary triglyceride was higher in group II compared to group III, with no significant difference.The mean levels of salivary total lipids and triglycerides were higher in children with dental caries (group II and III) which was significantly different from caries free children. ($p<0.001$) (Tables 2 and 3).There was a significant positive association between dental caries and salivary triglycerides and total lipids ($p\leq 0.001$) (Table 4). Salivary flow rate showed an inverse relation with levels of salivary triglycerides, cholesterol and total lipids. (Table 5).

Table 1: Mean salivary flow rate of children

Groups	Salivary flow rate (ml/min) (Mean ±SD)	p value
Group I	1.20±0.36	<0.001*
Group II	1.01±0.37	
Group III	0.86±0.31	

*p< 0.001 significant

Table 2: Mean levels of salivary cholesterol, triglyceride and total lipids

Salivary lipids (mg/ml)	Group I	Group II	Group III	P value
Cholesterol	7.27±0.56	7.10±2.23	8.03±2.91	0.211
Triglycerides	3.57±0.43	6.11±1.70	6.03±1.73	<0.001*
Total Lipids	19.23±1.18	22.51±2.87	22.68±2.54	<0.001*

*p< 0.001 significant

Table 3: Pairwise comparison of mean levels of salivary lipids.

Salivary lipids	Group I vs II	Group I vs III	Group II vs III
Cholesterol	0.949	0.364	0.221
Triglycerides	<0.001*	<0.001*	0.974
Total Lipids	<0.001*	<0.001*	0.959

*p< 0.001 significant

Table 4: Correlation between dental caries and levels of cholesterol, triglyceride and total lipids in saliva.

Salivary lipids	r value	p value
Cholesterol	0.124	0.246
Triglycerides	0.546	<0.001*
Total Lipids	0.448	<0.001*

*p< 0.001 significant

Table 5: Correlation between salivary flow rate and levels of cholesterol, triglyceride and total lipids in saliva.

Salivary lipids	r value	p value
Cholesterol	-0.142	0.145
Triglycerides	-0.546	0.375
Total Lipids	-0.448	0.514

DISCUSSION

Various studies have demonstrated that the caries process is controlled largely by natural protective mechanisms that are inherent within saliva.[4,5,17] The flow rate, pH, buffering action and remineralizing capacity of saliva are recognized as critical factors that affect and in some ways, regulate the progression and arrest of dental caries. Salivary pellicle is an organic membrane composed of glycoproteins and lipids derived from saliva.[18]

Lipids in saliva are a reflection of their plasma levels.[19]Alterations in the levels of salivary lipids have been observed in certain conditions like type-Idiabetes mellitus, salivary gland disorders and xerostomia.[20,21]Thus, healthy co-operative children without any systemic diseases or metabolic disorders were included in the study. Parvinen *et al* reported significant difference in salivary flow rate to type of dentition so, children with primary dentition only were included.[22]As a clinical tool, saliva has many advantages over serum.[4]

Lipids in saliva comprise of neutral lipids, glycolipids and phospholipids. The neutral lipids consist of free fatty acids, cholesterol, cholesterol esters, monoglycerides, diglycerides and triglycerides. Phospholipids consist of phophotidylcholine, phosphsphatidyl ethanolamine and sphingomyelin. The glycolipids consist of glycerogluco-lipids and glycosphingolipids. Neutral lipids are usually evaluated in serum lipid profile of individuals.[23] Lipids in saliva correspond to their plasma levels therefore, in our study triglycerides, cholesterol and total lipids were evaluated.[24]

The normal salivary flow rate of children is 0.7-1ml/min. The mean salivary flow rate in our study was significantly higher in caries free children compared to children with caries.The salivary flow rate of caries free children was found to be similar to earlier reports on 3-6 year old children.[25,26]An optimal or higher flow rate of saliva keeps the mouth moist and helps in faster rate of oral clearance. Also, higher flow rate aids in buffering action and neutralization of acidic pH. Lower salivary flow rate favors bacterial colonization and thus creates an acidogenic condition on tooth surface.[18]In this study, there was a negative correlation between salivary flow rate and levels of salivary triglycerides, cholesterol and total lipids, which were similar to results obtained in previous study.[11]The limitations of the present study, are that other physical parameters of saliva like pH and buffering capacity were not evaluated.

Children with ECC had significantly higher mean levels of salivary triglyceride and total lipids compared to children who were caries free. Similar results have been reported in adults.[18,11,21] Significantly higher levels of salivary triglycerides were reported in parotid[11] and submandibular saliva of caries susceptible adults compared to caries resistant individuals.[9] A recent study on children in India also found a positive association between salivary triglycerides and dental caries.[21]

The probable reasons for higher levels of salivary lipids in children with ECC could be due to higher frequency of consumption of sweets and fatty foods.[27,28] Children with higher caries demonstrate higher colony forming units of bacterial counts in saliva. Higher bacteria load has been found to be a source of lipids in saliva.The lipid rich cell wall of bacteria and their byproducts could have contributed to the lipid levels, though minutely in these children.

Higher levels of salivary triglycerides, cholesterol and total lipids lead to higher concentration of lipids in plaque, which in turn retards the diffusion of lactic acid from plaque.[10,29] It could be one of the probable reasons for significant association observed between level of salivary lipids and dental caries.

Furthermore, lipids enhance the glucosyltransferase enzyme activity which potentiates the cariogenic potential of oral microorganisms.[10] Studies have demonstrated that salivary lipids can also facilitate the penetration of oral mucosa by lipophilic substances and are capable of altering the interaction of calcium with salivary glycoproteins. The elevated level of lipids in saliva facilitates adherence of bacteria in the oral environment, by modifying the attractive hydrophobic forces between lipophilic molecules present on bacterial cell surfaces.[30]

In the present study, the mean salivary cholesterol levels were marginally lower in caries free children compared to children with ECC. Similar findings have been reported in the literature. [10,21]

The observations of this preliminary study on the association of salivary lipids and early childhood caries needs to be further researched. Studies on salivary lipids and other variables such as diet, cariogenic microflora and body mass index need to be carried out.

CONCLUSION

The results of this study found a positive association between early childhood caries and salivary lipids. Children with ECC (group II and III) had lower salivary flow rate and higher levels of salivary triglycerides and total lipids compared to caries free children (group I). Levels of salivary cholesterol did not differ between caries free and children with ECC.

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