



EVALUATION AND COMPARISON OF SALIVARY TOTAL ANTIOXIDANT LEVEL, pH AND FLOW RATE AMONG CARIES ACTIVE AND CARIES FREE CHILDREN.

Dental Science

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ABSTRACT

Oral hygiene, saliva and its constituents have crucial role in the development of dental caries.

AIM: To correlate the Total Antioxidant Level (TAL), pH and Flow Rate (FR) of unstimulated saliva with caries.

DESIGN: sixty children aged 6-13 years attended to the department of Pedodontics were selected. Caries Active (CA, n=30) and Caries Free (CF, n=30).

METHODOLOGY: Salivary antioxidant level estimated by Phosphomolybdenum method, pH by digital pH meter and FR measured in milliliter/minute.

RESULTS: Total antioxidant level was significantly high in CA (0.19) than CF children (0.08), pH (CA: 5.96, CF: 7.13) and FR (CA: 0.32 ml/min, CF: 0.42 ml/min) were less in caries active and more in free group.

CONCLUSION: Higher level of total antioxidant level, lower level of pH and FR among caries active children can be used as a caries risk indicator.

KEYWORDS

Caries, Salivary pH, Flow rate, Total antioxidant level

INTRODUCTION

Dental caries is the familiar chronic condition seen in oral cavity. It affects both genders with various socioeconomic status, race, and age. Other factors like oral hygiene status and saliva plays an crucial role in the formation of dental caries¹. Saliva helps for cleaning the oral mucosa and teeth, acts as a lubricant, a buffer, and act as a pool of calcium and phosphate ion, and help for remineralization². Healthy individual produce about 1.5 litre of saliva daily . Saliva containing several elements, so it can be used as a diagnostic tool³. Saliva perform as a reflector of the body's health and has many defense mechanisms such as enzymatic and immunologic defense systems. One of the foremost defense mechanism is antioxidants system^{4,5}.

Antioxidants are present in saliva which protects our body against endogenously formed free radicals (FR). Molecular oxygen is reduced to water and release of large free energy that produce free radicals and reactive oxygen species (ROS). Imbalance between FR/ROS and the antioxidants system results in oxidative stress. This oxidative stress leads to many of the oral diseases including caries. In 2000, Battino et al state that antioxidant property of saliva provides the first line of defense against oxidative stress⁶. The salivary flow rate, pH, are the other critical factors for the formation of dental caries. If the oral cavity is having balanced environment then it helps to maintain the health of tooth by providing salivary elements to construct a strongest apatite structure .

Hence assessment of those salivary components help to identify the risk of individual in formation of dental caries and also it helps to educate the individual to maintain health of their oral cavity.

METHODOLOGY

The study was reviewed and accepted by the institutional ethics panel

of A.M.E's Dental college and hospital and research centre, Raichur. Consent was taken from the patients. A total of 60 children fulfilling eligibility criteria aged 6-13 years old reported to the OPD of Pedodontics and Preventive Dentistry were selected in the study. They were split into two set; group I (caries active group, n=30) , group II (caries free group, n=30) respectively.

ELIGIBILITY CRITERIA

Inclusion criteria:

- Aged between 6 to 13 years
- For Caries active children, minimum number of decayed teeth 4
- For caries free children, decayed teeth=0
- Patients who are free from systemic conditions affecting salivary properties.

Exclusion criteria:

- Mentally and physically challenged children
- Patients on medication and patient on fasting
- Children with fluorosis and arrested carious lesion
- Children not willing to participate/non co-operative/sick.

Data recording of carious teeth:

Caries status was recorded by using WHO recommendations. Sterile mouth mirror and probe were used, and numbers of caries teeth were recorded.

Saliva collection

2ml of unstimulated saliva sample was taken in sterile plain plastic bottles. The patient was asked to sit by tilting the head downwards to collect saliva in the floor of the mouth for 1 minute which was then collected by asking patient to spit in to bottle for 5 minute. The collected sample stored in refrigerator till analysis.

Estimation of salivary total antioxidant level by phosphomolybdenum method

Phosphomolybdenum test used to estimate antioxidant capacity is based on the depletion of molybdenum (VI) molybdenum(V) by the antioxidants following establishment of a green phosphate or Mo (V) compound at an acidic pH.

0.3ml of the test sample was taken in a tube and added 3ml of prepared reagent tincture containing 0.6M of sulphuric acid, 28mM of sodium phosphate and 4mM of ammonium molybdate and incubated 95o C for 90 min. Absorbance of solution was assessed by using spectrophotometer at 695nm⁸.

Estimation of saliva pH:

For the determination of salivary pH, a digital pH meter (Erma model 035) was used. Before analyzing the sample the electrode was cleaned with distilled water and the pH meter was checked for standard calibration at pH 0.00 to 14.00. The measurement of the pH level was done immediately after the sample is collected. The electrode has a titanium tip covered with glass which can estimate pH of saliva in small quantity. The measurement was done by dipping pH meter electrode in the sample then it showed the pH value on digital display.

Assessment of saliva flow rate:

It assessed by asking children to spit saliva into the sterilized plastic bottle for the duration of 5 minutes. Then amount of saliva collected measured by using syringe and calculated for 1 minute (ml/min).

STATISTICAL ANALYSIS

Data were analyzed by the SPSSv 19 statistical package software (SPSS Inc., Chicago, IL, USA). Descriptive statistics such as mean, standard deviation and percentage was used. Comparison of mean values was carried out using student's unpaired t-test. Correlations of salivary parameters; pH, salivary flow rate and salivary total antioxidant levels with age, decayed teeth scores were evaluated by Pearson's rank correlation test. Any p-value less than 0.05 were considered as significant.

RESULT

Present study showed that positive correlation between caries and salivary total antioxidant level (Absorbance, nM) and statistically significant negative correlation were seen between caries, salivary pH and flow rate (ml/min). (Table 1)

Table:1 Correlation between decay and salivary parameters.

Teeth decayed	Pearson's correlation co-efficient		
	Salivary pH	Salivary flow rate	Salivary Antioxidant levels
Primary teeth decayed	-0.832**	-0.371**	0.241
Permanent teeth decayed	-0.394**	-0.151	0.090
Overall decayed teeth	-0.871**	0.380**	0.244

(**). 0.01 level: Correlation is significant (2-tailed). *. 0.05 level: Correlation is significant (2-tailed)

On comparison of pH, flow rate and salivary antioxidant level between 6-9 years of children, the mean pH level and flow rate (ml/min) of saliva were showing statistically significant result. The mean pH levels in caries active children were more acidic in pH (5.81) where as in caries free group it was showing alkaline pH (7.16) [p- value: 0.001]. The mean salivary flow rate was showing less in children with caries (0.11ml/min) than to caries free children (0.16 ml/min) [p-value:0.001] and TAL in caries active children was more (0.19Absorbance, nM) than TAL obtained for caries free children which was 0.09Absorbance, nM. The observed difference was statistically non significant [p-value: 0.13].

In case of 10-13 years of age group, total antioxidant level was significantly higher in caries active group (0.13 Absorbance, nM) than caries free children (0.06 Absorbance, nM) [p- value: 0.015]. The mean Salivary pH also was showing significant in caries active children (it was acidic ie 6.2) and in case of caries free group mean salivary pH was alkaline in nature (7.09) [p-value: 0.001] and mean salivary flow rate in caries active children was 0.37 ml/min and for caries free children which was 0.34ml/min, the observed difference was non-significant [p-value: 0.585]. (Table 2, figure 1, 2 and 3)

Table2: Comparing mean salivary pH, flow rate (ml/min) and antioxidant level among caries-active and caries-free individuals aged 6-9 and 10-13 years

Age	Caries Activity	N	Ph Level			Salivary Flow Rate(ml/min)			Salivary Antioxidant Level(Absorbance/ NM)		
			Mean ±SD	't' value	p value	Mean ±SD	't' value	p value	Mean ±SD	't' value	p value
6-9 years	Caries active	19	5.81 ± 0.35	-13.198	0.001*	0.29 ± 0.11	4.147	0.001*	0.19 ± 0.31	1.328	0.193
	Caries free	19	7.16 ± 0.60			0.47 ± 0.16			0.09 ± 0.11		
10-13 years	Caries active	11	6.21 ± 0.46	-5.140	0.001*	0.37 ± 0.10	0.556	0.585	0.18 ± 0.13	2.781	0.015*
	Caries free	11	7.09 ± 0.34			0.34 ± 0.17			0.06 ± 0.06		

(* statistically significant at p<0.05 , Statistical test used- unpaired student's t-test,SD- standard deviation)

Figure 1: Mean pH in 6-9 & 10-13 years of in caries-active and caries-free group.

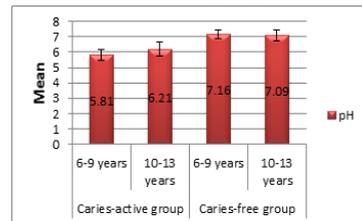


Figure 2: Mean salivary flow rate (ml/min) in 6-9 & 10-13 years in caries-active and caries-free group.

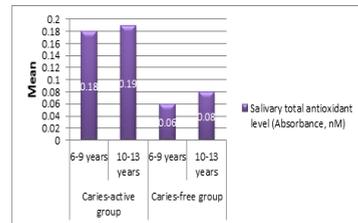
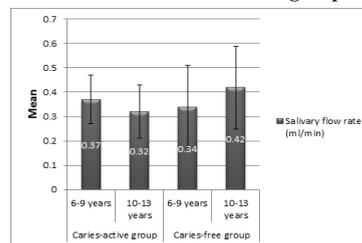


Figure 3: salivary total antioxidant level in 6-9 & 10-13 years caries active and caries free group.



Overall comparison of mean salivary pH, flow rate and antioxidant level with caries activity found to be statistically significant result. (Table 3)

Table 3: Overall comparison of mean salivary pH, flow rate (ml/min) and antioxidant level with caries activity of study subjects.

Caries Activity	N	pH level			Flow rate (ml/ minute)			Total antioxidant level (absorbance, nm)		
		Mean ±SD	't' value	p value	Mean ±SD	't' Value	p value	Mean ±SD	't' value	
Caries-active	30	5.96 ± 0.43	-12.317	0.001*	0.32 ± 0.11	-2.753	0.008*	0.19 ± 0.26	2.152	
Caries-free	30	7.13 ± 0.29			0.42 ± 0.17			0.08 ± 0.10		

(* statistically significant at p<0.05 Statistical test used- unpaired student's t-test,SD- standard deviation)

DISCUSSION

Saliva is an essential important body fluid involving several microbes and host biological constituents which can be used for the determination of caries risk. Caries risk evaluation helps to recognize

possibility of caries occurrence, i.e., number of newly formed caries lesion in a particular time interval, also possibility of differences happened in the size or progression of caries process⁹. Saliva collection is very easy, non-invasive, safe method, and low cost. In dental practice saliva acts as a biomarker for the identification of disease¹⁰.

Normal salivary flow rate has a strong protective effect against dental caries. Unstimulated saliva results will be low in severe cases, and it help to give a clear indication of increased levels of dental caries risk. In our study we have found that decreasing salivary pH level and flow rate with increasing dental caries (-ve correlation) but salivary antioxidant level was showing increasing level with increase in dental caries group. Some other studies also reported (Holbrook et al., 1993; Vehkalahti et al., 1996) parallel findings with salivary flow rate and related it to increased caries risk¹¹. So whenever flow rate increases microbial action of saliva will get reduce¹².

Result of present research revealed that saliva parameters like pH and flow rate of healthy children of the age groups 6 to 13 years showed a steady decrease with increase in caries; and was statistically significant. These results showed saliva pH and flow rate can be utilized as caries risk assessment measure. These results were agreed to previous studies conducted by chitharanjan Shetty et al¹³.

In the present study it was observed that caries-active children had significantly higher levels of total anti oxidant level (TAL) than caries-free children. Oral hygiene status of children was poor, so exposure to caries was seen to be more suggestive of increased total antioxidant level. The reason for increased total antioxidant level in caries active group could be: salivary peroxidase antioxidant system. Salivary peroxidase controls growth of bacterias of plaque which make dental caries. Salivary peroxidase speed up the peroxidation of thiocyanate ion (SCN-) to form more stable products (OSCN-); it prevent microbial growth and metabolism, so it leads to the reduction of dental caries¹⁴.

In the present study it was found that total antioxidant level was more in children between the age group of 10-13 years than 6-9 year old children. It may be due to less availability of micronutrients in 6-9 years children which may result in decreased total antioxidant level. But as the age advances the volume of consumption of food increases, this could be the reason for increase in total antioxidant level with increased age. Also, it has been seen that the immunity of an individual enhance with age proposing that total antioxidant level between the age group of 10-13 years is more. Parallel outcome were seen in research carried out by Dodwad et al found that increased total antioxidant capacity in children between the age group of 11-14 years than 7-10 year old children². Randad et al in their study found that total antioxidant capacity was more in children between the age group of 10-14 years than 5-9 year old children¹.

Based on method of action antioxidants are mainly 3 types^{15,16}:

1. Chain breaking/scavenging. E.g: Vitamins E, C, A, urate, bilirubin.
2. Preventing antioxidants: Those substances contain "thiol" group. Their function largely by segregate transitional metal ions and hampers Fenton reaction E.g.: Albumin, transferrin, lactoferrin, ceruloplasmin, etc.
3. Enzyme antioxidants: Function by catalyzing the oxidation of other molecules. Catalase, glutathione peroxidase.

In our study the overall comparison of mean salivary pH, flow rate and antioxidant level with caries activity found to be statistically significant result.

As the caries process reaches to dentin, degradation of dentin matrix starts. Reactive oxygen species (ROS) activate matrix metalloproteinases (MMPs) in dentin matrix and it tightly regulated by tissue inhibitors of metalloproteinases and alfa macromolecules in normal physiological conditions, but because of some local environmental changes of pH and ROS results in imbalance of the regulatory mechanism of oral cavity and tooth structure, it leading to tissue destruction causes the dental caries¹⁷.

CONCLUSION

Salivary flow rate, pH, and total antioxidant level are contributory factors in maintaining oral health of an individual. So we can summarize that higher level of Antioxidant Level and lower level of

pH, Flow Rate among caries active children can be used as a caries risk indicator.

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