



A COMPARISON OF SALIVARY CALCIUM AND TOTAL PROTEIN LEVELS IN CARIES FREE AND CARIES ACTIVE CHILDREN- AN INVITRO STUDY

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Introduction: Saliva is one of the most important factor in regulating oral health. Saliva can affect incidence of dental caries by mechanical cleansing, by reducing enamel solubility by means of calcium, phosphate and fluoride, by buffering and neutralizing the acids produced by cariogenic organisms.

Aim: To estimate and compare the salivary calcium & total proteins level in caries free and caries active children.

Materials and methods: A total of 60 school children between 8-12 years of age were included in the study. They were divided into three groups of 20 each based on deft/DMFT score, Group I-Caries free (def^t/DMFT = 0), Group II-Moderate Caries active (def^t /DMFT ≤ 5) and Group III-High caries active (def^t/DMFT ≤ 10). Unstimulated whole saliva samples were collected. The intensity and colour change were noted.

Result: Statistical analysis was done by one way ANOVA, post hoc tukey and Pearson's correlation coefficient test. Statistical significance was found only between the groups of salivary total protein levels.

Conclusion: Negative correlation was found between salivary calcium and dental caries and positive correlation was found between salivary total protein and dental caries. Statistical significance was found only between the groups in salivary total protein levels.

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INTRODUCTION

Dental caries is a multifactorial oral lesion caused by the interplay between a susceptible host, fermentable substrate, microflora and saliva. *Saliva plays an important role in regulating oral health and during caries process, properties of saliva such as flow rate and composition changes.*¹

Saliva is essential for maintaining the oral equilibrium and the effects of saliva and its constituents on the oral micro-organisms influence the development of dental caries. Salivary components and its flow rate, viscosity, buffering capacity, pH etc plays a major role in initiation and progression of dental caries.¹

A variation in the ionic concentration of phosphate and calcium can alter the equilibrium of demineralization and remineralisation. The salivary proteins also can act directly and indirectly through various methods on plaque and bacteria, modulating susceptibility of the tooth to dental caries. The interaction of the different components of saliva and their preventive role against dental caries has been of

area subjected to active investigation, favouring a proper diagnosis and monitoring of the disease condition.

Thus the present study was planned to evaluate the calcium and total protein content of saliva as an effective clinical marker from the literary background. This study was undertaken with an aim to assess the level of calcium and total proteins of saliva in caries free and caries active children.

MATERIALS AND METHODS

The calcium and total protein in saliva and its relationship with dental caries experience among sixty school children were quantitatively analyzed in this study.

A total of 120 children between 8-12 years of age, were initially screened by stratified random sampling from the school, by the principal investigator. Prior to the commencement of oral examination, relevant medical and dental history was obtained from their parents.

The selected children were further divided into 3 groups according to DMFT/def^t index such as Group I-Caries free children (DMFT/def^t score=0) which included 15 boys and 18 girls, Group II-moderate caries children (DMFT/def^t score=1-5) with 18 boys and 17 girls and Group III-High Caries children (DMFT/def^t score =6-10) with 16 boys and 19 girls

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Within each group, the boys and girls were allocated separate serial numbers. Via randomized draw of lots done by one of the child, a sample size of 10 girls and 10 boys were obtained (total 20 children) for each group.

Ethical clearance was obtained from the research committee and permission was also taken from the school administrations. A written revelatory note was sent to the parents regarding the objectives of the study and written consent was received from them.

Inclusion criteria

- Children between 8-12 years.
- Good general health.
- Regular attendance to school.

Exclusion criteria

- Medically compromised children or children with physical limitations.
- Intake of antibiotics or any preventive dental treatment in the past 6 months.
- Children undergoing orthodontic treatment.
- Children with moderate or severe gingivitis or any significant soft tissue pathology

Saliva collection

The unstimulated mid - morning whole saliva samples were obtained. The samples were taken one and half hour after school had commenced so that enough time had passed after breakfast. The children were then requested to permit saliva to drool from the oral cavity into the labelled and sterile disposable containers, to determine the salivary calcium and protein levels.

Estimation of salivary proteins

The level of total salivary proteins was estimated by the procedure described by Lowry et al.² The saliva samples were treated with Folin Ciocalteau reagent. The colour change was noted spectrophotometrically at 660 nm.

Estimation of salivary calcium

The sample was treated with o-cresolphthalein complexone reagent to estimate salivary calcium. The colour change was measured colorimetrically at 470 nm. This change in colour and its intensity was directly proportional to the calcium content in saliva.

Statistical analysis

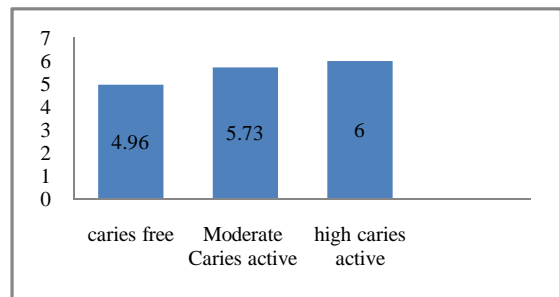
Mean and standard deviations were estimated for each group. Mean values were compared by One-way ANOVA. Multiple range test by post hoc tukey-HSD procedure was employed to identify the significant groups when P-value in One-way ANOVA was significant. In the present study, P ≤ 0.05 was considered as the level of significance.

RESULTS

Sample strata included sixty children of which 20 in group I (DMFT/deft =0), 20 in group II (DMFT/deft =1-5) and 20 in group III (DMFT/deft =6-10) by stratified random sampling. Increased salivary calcium level was noted in group III [Table/Fig1] while decreased salivary total protein level was noted in group III [Table/Fig2] An increase in the salivary calcium content as the caries progresses but there was no

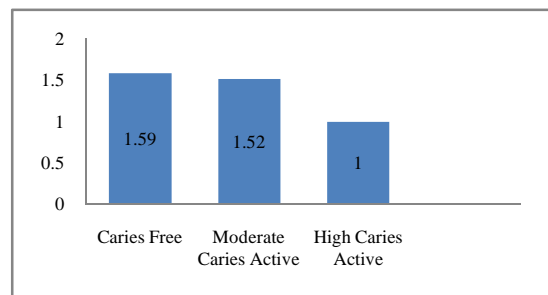
statistical significance between the groups in salivary calcium levels while significant decrease in the salivary total protein content was noted as caries progresses [Table/Fig3]. The levels of salivary total protein in group 1 was 1.59 ± 0.26 mg/dl, which was significantly higher than group 2 and group 3 children and the difference between the groups were statistically significant with p- value of 0.000 [Table/Fig4]. A negative correlation was found between calcium and total protein levels which was statistically insignificant [Table/Fig5].

Salivary calcium in mg/dl



Table/Fig1 Increase in salivary calcium levels as the caries progresses

Salivary total protein in mg/dl



Table/Fig 2 Decrease in the salivary total protein levels as the caries progresses

Parameters	Group	Mean	Standard deviation	Significance
Calcium	I	4.9600	1.07037	0.051
	II	5.7300	1.48753	
	III	6.0050	1.50280	
	TOTAL	5.5650	1.41695	
TOTAL PROTEIN	I	1.5985	.26254	0.000
	II	1.5270	.56446	
	III	1.0045	.34777	
	TOTAL	1.3767	.48481	

Table/Fig3 Mean and standard deviation of calcium and total protein levels in all the 3 groups of the sample strata in mg/dl

Dependent Variables	Group	Group	Mean difference	Std error	SIG
Calcium	1	2	-.7700	.43270	.241
		3	-1.0450	.43270	.057
	2	1	.7700	.43270	.241
		3	-.2750	.43270	1.000
	3	1	1.0450	.43270	.057
		2	.2750	.43270	1.000
Total protein	1	2	0.715	.43270	1.000
		3	.5940(*)	.43270	.0001
	2	1	-.0715	.43270	1.000
		3	.5225(*)	.43270	.001
	3	1	-.5940(*)	.43270	.000
		2	-.5225(*)	.43270	.001

Table/Fig4 intergroup comparison of the salivary calcium and total protein levels using post hoc test.

Table/Fig5 Pearson's correlation coefficient between the salivary levels of calcium and total protein.

		Calcium	Total protein
Calcium	Pearson correlation	1	-.104
	Significance	.	.430
	N	60	60
Total protein	Pearson correlation	-.104	1
	Significance	.430	.
	N	60	60

DISCUSSION

Oral cavity has a peculiar ecosystem, which performs a wide range of functions, harbours a plethora of microorganisms and is unique in accommodating exposed mineralized tissues. The saliva bathes this ecosystem, possessing a large number of components and plays a major role in the etiopathogenesis of dental caries. The calcium ions in the saliva helps to prevent dissolution of dental enamel and help in remineralisation phases as well as act as the most efficient pH buffer for regulating body fluids. In alkaline pH, calcium plays an important role in remineralisation of enamel surface via formation of hydroxyapatite crystals, whereas in acidic pH, salivary calcium takes part in preventing dissolution of enamel.³

The human body comprises more calcium than any of the other essential minerals, as much as 1200 g in a 70 Kg adult. Most calcium is settled in the form of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$.⁴

Calcium concentration in saliva is very little; the usual concentration of calcium in unstimulated whole mixed saliva is 4-6 mg/dl.^{5,6} In this study, group 1 and 2 had calcium content within the normal level but in group 3, the level was slightly higher than 6 mg/dl.

The calcium level of saliva may reflect fluctuations in both dietary calcium and general calcium turnover, but the exact mechanisms are far from clear. The present study is in accordance with Turtola L (1978), He found that salivary calcium concentration increased with increasing caries activity.⁷ but the effect of diet was not revealed.

This study demonstrates the mean of salivary calcium in children with severe caries was found to be higher than the two other groups, but this difference was not significant after statistical analysis which confirms with the results of studies by Masamurak et al, Marray and Shaw, afshar et al, Cornejo et al and Gandhi and Damle.⁸⁻¹²

Salivary proteins possess protective, antimicrobial, lubricative and digestive properties and play an important role in modulating the microbial colonization of teeth and soft tissues.¹³ The mean salivary protein level in group 3 was lower as compared to group 2 and group 1 which was in accordance with a report published by Tulungolu et al.¹⁴

In the present study, the mean salivary total protein level in children of Group-I was significantly higher than group 2 and group 3 (High caries active), suggesting that salivary protein has a protective role of total protein in prevention of dental caries.

Thus both salivary calcium and total protein can be used as a valuable tool marker for caries prevention and follow up.

CONCLUSION

In the present study, the result suggests a positive correlation between the levels of salivary calcium and dental caries and negative correlation between the levels of salivary total protein and dental caries confirming the influence of calcium and total protein levels of saliva protecting caries progression. Thus it can be concluded that

An insignificant correlation was also found between the salivary calcium and total protein levels on caries progression. Salivary calcium and total protein levels may be used as a biochemical indicator for evaluating the susceptibility of caries.

However it is suggested that further studies are to be carried out on flow rate, ph and other inorganic constituents of saliva on caries progression.

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