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Original Research Article

Comparative Evaluation of the Effect of Topical Fluoride Agents on the Physicochemical Properties of Saliva in Caries Free and Caries Active Children

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Abstract:

Introduction: The physicochemical properties of saliva play a major role in the development of dental caries. Application of fluoride helps in preventing dental caries in both extrinsic and intrinsic pathway. However, their effects on the physicochemical properties of saliva have not been studied extensively. High caries risk group predisposes to early childhood caries. Thus, the purpose of this study was to evaluate the effect of topical fluoride agents on the physicochemical properties of saliva in caries free and caries active children.

Aim: To evaluate and compare the effect of topical fluoride agents on the physicochemical properties of saliva in caries free and caries active children among selected North Indian population.

Materials and Methods: 80 children of 6 to 12 years age group were included in the study. They were divided into two groups of 40 children each. Group I children with active caries (DMFS/defs > 5) Group II - children without caries (DMFS/defs=0). They were further divided into subgroups each. Subgroup A and B for caries active children and subgroup C and D for caries free children. Each subgroup includes 20 children. Subgroup A and C received 2 % neutral sodium fluoride gel and subgroup B and D received 5 % sodium fluoride varnish. The saliva samples were collected at two different time i.e. first, at the base line and second after the application of topical fluoride agent. Subsequently, they were analyzed for various physicochemical properties. Statistical analysis of the results was done using Student 't' test to compare the mean values between caries free and caries active groups.

Results: At baseline level the difference in pH, flow rate and total protein of saliva in caries free and caries active group were found to be statistically significant. After intervention with fluoride the difference between the two groups in respect to total protein level became statistically insignificant. In caries free group, application of fluoride gel and varnish showed statistically significant difference in all the salivary parameters. However, the total antioxidant capacity with fluoride varnish was statistically insignificant. In caries active group, application of fluoride gel and varnish showed statistically significant difference in the salivary flow rate, pH and buffering capacity. However total antioxidant capacity with fluoride gel was statistically significant. The difference in total protein level after application of fluoride varnish or gel was statistically insignificant. The result also showed that both fluoride gel and fluoride varnish were almost equivocal in their efficacy in both the groups.

Conclusion: Overall, caries active children showed lower flow rate, pH and buffering capacity in comparison to caries active children. Whereas the total protein and total antioxidant were higher in caries active children. Thus, it can be concluded that application of fluoride either varnishes or gels overall augment the physicochemical properties of saliva which is considered to be one of the major factors in the development of dental caries.

Keywords: Saliva Flow Rate, pH, Buffering Capacity, Protein, Total Antioxidant Capacity.

Introduction

Saliva is a complex exocrine secretion produced by a number of specialized glands which discharge into the oral cavity of the glands of mammalian vertebrates. The total volume of saliva produced each day in an adult range from 500 - 1500 ml with an average volume of 1ml at any given time (Lentner, 1981)[1]. Saliva consists of approximately 99.5% water and 0.5% solid. Mixed saliva, consists mainly of the secretions of submandibular (65%), parotid (23%), and sublingual (4%) glands, the remaining 8% being provided by the minor numerous glands. Saliva is critical to the preservation and maintenance of oral health. Saliva, like blood, removes waste, supplies nutrition, cleans and protects cells. It has multi-functional characters, it helps in digestion, buffering, lubrication and viscoelasticity, has anti-bacterial, anti-fungal and anti-viral properties, apart from that it helps in taste, speech articulation and mineralization of teeth. The mucus in saliva lubricates and binds the masticated food into a slippery bolus that (usually) slides easily through the esophagus without inflicting damage to the mucosa. Saliva also coats the oral cavity and esophagus, and food basically never directly touches the epithelial cells of those tissues Therefore saliva is a determinant factor in oral health.

Dental caries is a multifactorial disease caused by demineralization of enamel by the action of acids from the bacterial metabolism. Incorporation of oral hygiene habits and various carious preventive measures have been taken up to prevent the occurrence of dental caries. Many studies go in line with the fact that fluoride helps to prevent the caries formation by increasing the resistance of remineralised areas to secondary acid attack and increasing the rate of repair of carious enamel lesions by enhancing remineralization. Total or whole saliva is a complex mixture of fluids from the salivary glands, oral mucosa transudate, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products. In addition whole saliva contains contributions from non-glandular sources such as gingival crevice.

Hypo salivation is one of the factors for dental caries. The amount of saliva secreted in the oral cavity, its constituents and propertied like pH and buffering capacity have a role in the incidence of dental caries. Evidence shows that saliva is detected from birth and the flow rate gradually increases with age and slows down after 29 years of age, this could be due to a maturation of the central control mechanism and progressive replacement of salivary gland tissue by fat and atrophy of acinar cells. It is also shown that the salivary flow rate fluctuates with the circadian cycle. It has been suggested that the unstimulated flow rate may be at its maximum in the mid-afternoon and diminishes considerably during sleep; which allow populations of bacteria to build up in the mouth that result in dragon breath in the morning. Flow has a caries-preventive effect by influencing the clearance rate of the substrate. The higher the flow rate, the faster the clearance rate. The constant flow of saliva eliminates food debris and bacteria by swallowing. When stimulated by chewing or by moderate strength citric acid, the whole saliva flow rate increases from the resting value of around 0.3–0.65 ml/min to around 1.5–6.0 ml/min with great individual variations. Chewing or mastication in any degree brings forth "activated" saliva, which is more alkaline than cresting saliva. Salivary flow is increased in response to both gustatory (taste) and mechanical (chewing) stimuli (Kerr, 1961). There is now general agreement that saliva is more often slightly acid than basic. From an examination of available data, it appears that the pH range of saliva is between pH 5.0 and pH 8.0. Eisenbrandt also reported that a significant cycle changes of the pH of the saliva occurred during the day; the lowest pH was at 9 A.M. and the highest at 5 P.M. In controlled experiments, Mathur found that the pH of saliva changed with the first morsel of food taken into the mouth, that there was a rise in pH during eating followed by a decided drop, and that saliva returned to its original pH value after some time.

The buffer capacity of both unstimulated and stimulated saliva is due to the bicarbonate (HCO3), the phosphate and the protein buffer systems. The most important buffering system in saliva is the carbonic acid / bicarbonate system. The saliva bicarbonate increases the pH and buffer capacity of saliva, especially during stimulation. Various methods have been used to measure the salivary buffer capacity. Values obtained for buffer capacity in different studies are not comparable. However, final pHs under 3.5 for unstimulated saliva and 4.0 for stimulated saliva are considered low. The buffering capacity of saliva greatly influences the pH of plaque surrounding the enamel, thereby inhibiting or promoting caries progression. It is generally accepted, that certain salivary components especially proteins have protective effects against dental caries. These proteins act directly and indirectly through various methods on plaque and bacteria modulating susceptibility of the tooth to dental caries.

All biological species produce free radical which is any atom with at least one unpaired electron in the outermost shell and is capable of independent existence. They are very unstable and react quickly with other cells, to gain stability causing damage to the cells they react with. Antioxidant donates one of their own electrons and protects the cells against potential harmful effects caused by neutralization of free radicals. Saliva also presents an antioxidant action due to its enzymatic and non-enzymatic components.

In the saliva, uric acid is the predominant antioxidant accounting for more than 85% of the total AOA, albumin and ascorbate providing minor contributions. Fluoride is recognized to affect oxygen metabolism by inducing the production of reactive oxygen species and inhibiting the activity of several antioxidant enzymes in several tissues and cells.

The effects of topical fluoridated products on the physicochemical properties of saliva have not been studied extensively.

Hence the purpose of this study is to evaluate the effect of different types of topical fluoride agents on the physicochemical properties of saliva in caries free and caries active children.

Aim: To evaluate and compare the effect of topical fluoride agents on the physicochemical properties of saliva in caries free and caries active children among selected North Indian population.

Objectives:

- To evaluate the effect of fluoride varnish on the flow rate, pH, buffering capacity, total protein and total antioxidant capacity in caries free and caries active children.
- To evaluate the effect of fluoride gel on the flow rate, pH, buffering capacity, total protein and total antioxidant capacity in caries free and caries active children.
- To compare the efficacy of fluoride varnish and gel on the physicochemical properties of saliva in caries free and caries active children.

Materials and Methods

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, I.T.S Dental College Hospital and Research Centre, Greater Noida.

Ethical clearance was obtained from the ethical committee (IEC/PEDO. /19/12) of I.T.S Dental College, Greater Noida and enclosed in the annexure.

Sample Selection:

Caries active children between the age group of 6-12 years, attending OPD of Department of Pedodontics and Preventive Dentistry, I.T.S-Dental College Hospital and Research Centre, Greater Noida, were included as subjects in the study. Samples for caries free were taken from those children who were attending OPD for routine examination.

Prior to the collection of saliva from the children, an informed consent was obtained from their respective parents/guardians and detailed case history was recorded. All the children were clinically examined with the aid of a mirror and explorer. The presence of decayed, missing and filled surfaces was scored in the permanent (DMFS) as well as in the primary (defs) dentition according to WHO criteria.

Inclusion Criteria

- Patients between the age group of 6 to 12 years.
- Patients with a minimum of 5 DMFS/defs score for the caries active group and 0 for the caries free group.
- Cooperative patient.
- Patients whose parents/guardians gave consent

Exclusion Criteria

- Patients who were physically or medically compromised.
- Patients on medication.
- Patient who had undergone any type of preventive therapy to combat caries process.
- Patients with fluorosis or hypoplastic enamel/dentin
- Patients suffering from any systemic disease.

Study Design: 80 children of 6 to 12 years age group were included in the study. They were divided into two groups of 40 children each -

1. Group I - children with active caries (DMFS $\geq$ 5)
2. Group II - children without caries (DMFS=0)

These two groups were further divided into sub groups as follows:

Group I: Caries active children:

- Subgroup A (20 children):

In whom 2% sodium fluoride gel was applied.

- Subgroup B (20 children):

In whom 5% sodium fluoride varnish was applied.

Group II: Caries free children:

- Subgroup C (20 children):

Includes children in whom 2% sodium fluoride gel was applied.

- Subgroup D (20 children)

Includes children in whom 5% sodium fluoride varnish was applied.

Stimulated salivary samples were collected at two different time intervals from each participant. First at base line and second one hour after the application of topical fluoride agent.

The saliva samples were then analysed for various physicochemical properties. The results obtained were subjected to statistical analysis.

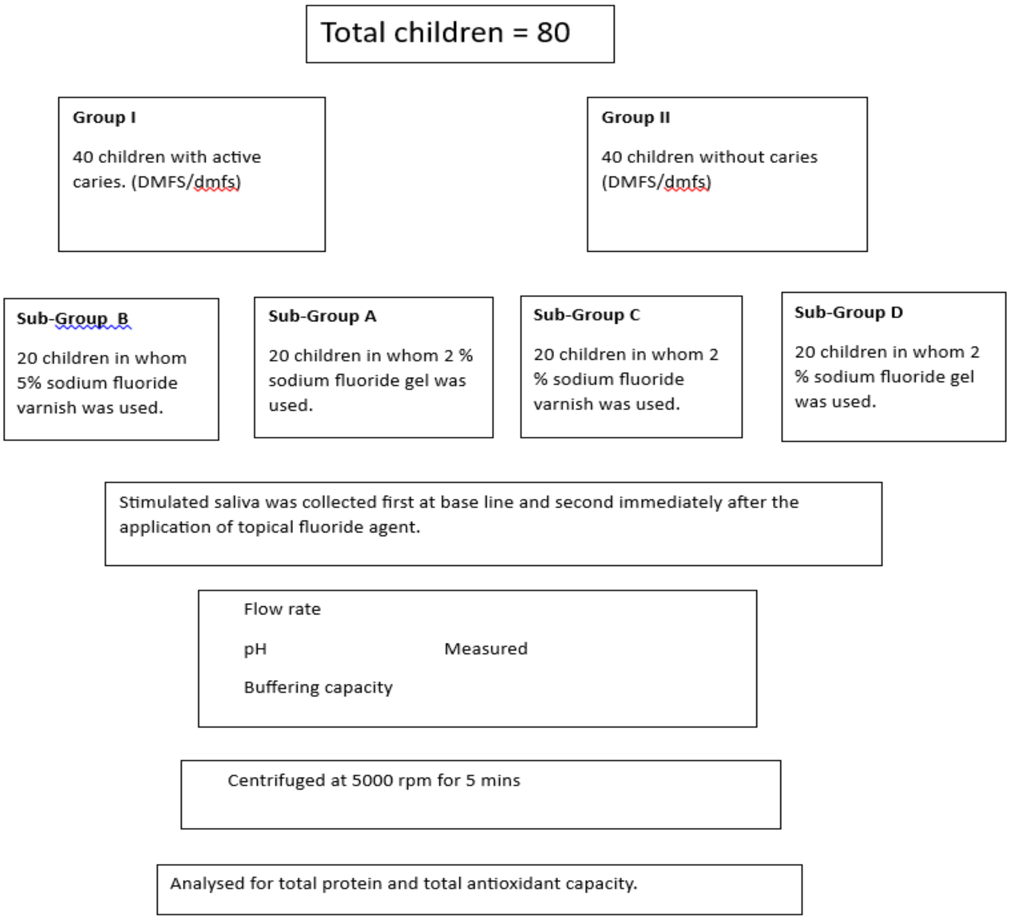


Chart 1:

Following Materials and Armamentarium Were Used In the Study

1. Screening Instruments. [Fig 1]

- Sterile mouth mirror
- Sterile explorer
- Sterile kidney trays
- Sterile gloves

2. For Salivary Sample Collection [Fig 2]

- Sterile test tubes with stoppers
- Disposable syringes

3. For Measuring Saliva {Fig –3}

- Micropipette
- Plastic cylinders

Materials:

Reagents

- Buffering capacity – HCl Hydrochloric Acid
- Protein –
- Total antioxidant capacity -

Solution:

- Sodium phosphate
- Sodium benzoate
- NaOH- Sodium Hydroxide
- EDTA- Ethylene Diamine Tetra acetic Acid
- $Fe\{NH_4\}$ SO_4 - Ammonium Iron Sulfate
- H_2O_2 -Hydrogen Peroxide
- Acetic acid
- Thiobarbituric acid
- Uric acid

Equipments: -

- Manual pH meter
- Semi Auto analyzer
- Weighing machine
- Centrifuge machine
- Spectrophotometer

Methodology:

Collection of Saliva: On the day of collection, participating children were instructed not to eat or drink anything for at least one hour before the collection of saliva sample. To control the circadian variations, samples were collected between 10 am – 12 am. Children were asked to rinse their mouth with water thoroughly 10 minutes before collection of saliva to avoid the contamination of food debris. They were made to sit in an upright, relaxed position on a dental chair. They were then made to chew a sterile rubber band for 1 min. During this time, any saliva produced were either spitted out or swallowed. Chewing of the sterile rubber band is continued for further 5 min during which any saliva produced every minute was collected in a sterile plastic container. Flow rate, pH and buffering capacity were measured immediately after collection of the saliva sample and the remaining saliva sample was transferred to sterile test tube which was then carried to the laboratory for analysis of total protein, & total antioxidant capacity of saliva.

Salivary Analysis: After collection of saliva samples, the saliva was analyzed and the following were estimated

Estimation of flow rate of saliva: The weight of the sterile plastic container was measured before and after the 5 minutes period of collection of stimulated saliva. Then the flow rate was calculated in gms/min which is approximately equivalent to mL/min using the following formula:

$$\text{Salivary flow rate} = \frac{\text{Weight of container with saliva} - \text{Weight of empty container}}{\text{Collection period}} = \_\_\_ \text{g/min or ml/min}$$

Estimation of pH & buffering capacity:

pH: pH of the collected saliva was then measured for each sample by using a manual pH meter and the values were recorded.

Buffering Capacity: The estimation was carried out as per the method described by Ericsson modified for smaller volumes. 0.5 ml of saliva from the sample was added to 1.5 ml of 5mmol/L HCl and the mixture was shaken vigorously and allowed to stand for 10mins at room temperature. The final pH was measured by using manual pH meter.

The saliva was then transferred into a sterile glass test tube and centrifuged for ten minutes at 5000 rpm. It was than stored at – 35 ºC until further analysed

Estimation of total protein of saliva: Biuret method was used to estimate the total protein of the saliva sample. Commercially available Liquixx Total Protein Kit (Erba Mannheim, Transasia Bio-Medicals Ltd) was used.

Principle: the peptide bonds of the protein react with copper II ions in alkaline solution to form blue violet complex, (biuret reaction). Each copper ions complexing with 5 or 6 peptide bonds. Tartarrate is added as a stabilizer whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The color formed is proportional to the protein concentration and is measured at 546nm (520-260nm).

Reagent preparation: Ready to use reagent is used.

Reagent composition:

Reagent 1: total protein reagent

Copper II sulphate	19 mmol/l
Potassium sodium tartarate	43 mmol/l
Potassium iodide	30 mmol/l
Sodium hydroxide	600 mmol/l

Total protein standard

Protein standard	6.0 g/dl

Assay procedure

Pipette into tubes marked	Blank	standard	Test
reagent	1000µl	1000µl	1000µl
Distilled water	20µl		
standard		20µl	
test			20µl

It is than incubate for 10 mins at 37º C. absorbance of the standard and each test was read at 546 nm (520-560nm) against reagent blank.
Calculation

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (g/dl)}$$

Estimation of total antioxidant capacity of saliva: {Fig-}

Total salivary antioxidant levels were estimated by using a spectrophotometer method.

Principle: A standardized solution of Fe- EDTA complex reacts with hydrogen peroxide by a Fenton –type reaction, leading to the formation of hydroxyl radicals {OH}. These reactive oxygen species degrade benzoate, resulting in the release of TBARS.

Antioxidants from the added sample of human fluid {saliva} cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the AOA.

Solutions:

1. Sodium phosphate buffer: 100mmol/litre, pH 7.4
2. Sodium benzoate: 10mmol/litre
3. NaOH:-50mmol/litre
4. EDTA:-2mmol/litre in phosphate buffer
5. Fe-EDTA complex: prepared freshly by mixing equal volumes of solutions
6. H_2O_2: 10mmol/litre
7. Acetic acid: 20%
8. Thiobarbituric acid {TBA}: 0.8% {wt/vol} in 50mmol/litre NaOH
9. Uric acid: 1mmol/litre in 5mmol/litre NaOH
10. Fe{NH4}SO4: 2mmol/litre

Analytical procedure:

Each sample (A1) should have its own control (A0, sample blank) in which the Fe–EDTA mixture and H2O2 should be added after 20% acetic acid.

For each series of analysis a negative control (K1 and K0) should be prepared (at least in triplicate), containing the same reagents as A1 or A0, except that serum (or other human fluid) is replaced with phosphate buffer.

Standards containing 1 mmol/litre uric acid (UA1 and UA0) are used for calibration.

Pipette into tubes (in millilitres):

	A1	A0	K1	K0	UA1	UA0
Saliva	0.01	0.01	-	-	-	-
Uric acid	-	-	-	-	0.01	0.01
Buffer	0.49	0.49	0.50	0.50	0.49	0.49
Na-benzoate	0.50	0.50	0.50	0.50	0.50	0.50
Acetic acid	-	1.00	-	1.00	-	1.00
Fe-EDTA	0.20	0.20	0.20	0.20	0.20	0.20
H2O2	0.20	0.20	0.20	0.20	0.20	0.20
Incubate for 60 minutes at 37°C, then add:						
Acetic acid	1.00	-	1.00	-	1.00	-
TBA	1.00	1.00	1.00	1.00	1.00	1.00

Incubate for 10 minutes at 100°C (in a boiling water bath) then cool in an ice bath. Measure absorbance at 532 nm against deionised water.

Calculation

Antioxidant activity should be calculated as follows:

(CUA) (K − A)

AOA (mmol/litre) = ----------------------------

(K − UA)

Where,

K = absorbance of control (K1 − K0)

A = absorbance of sample (A1 − A0)

UA = absorbance of uric acid solution (UA1 − UA0)

CUA = concentration of uric acid (in mmol/ litre).

The data collected were subjected to statistical analysis.

Statistical Analysis: Results are presented as mean ± standard deviation values. Students't' test was used to compare the mean values between caries free and caries active groups.

A 'P' value of 0.05 or less was considered for standard significance.

Formula Used For Analysis:

$$\text{Mean, } \bar{x} = \frac{\Sigma x_i}{n}$$

$$\text{Standard deviation, SD} = \sqrt{\frac{\Sigma (x_i - \bar{x})^2}{n-1}}$$

$$\text{Standard Error, SE} = \frac{SD}{\sqrt{n}}$$

Student's t-test (Unpaired)

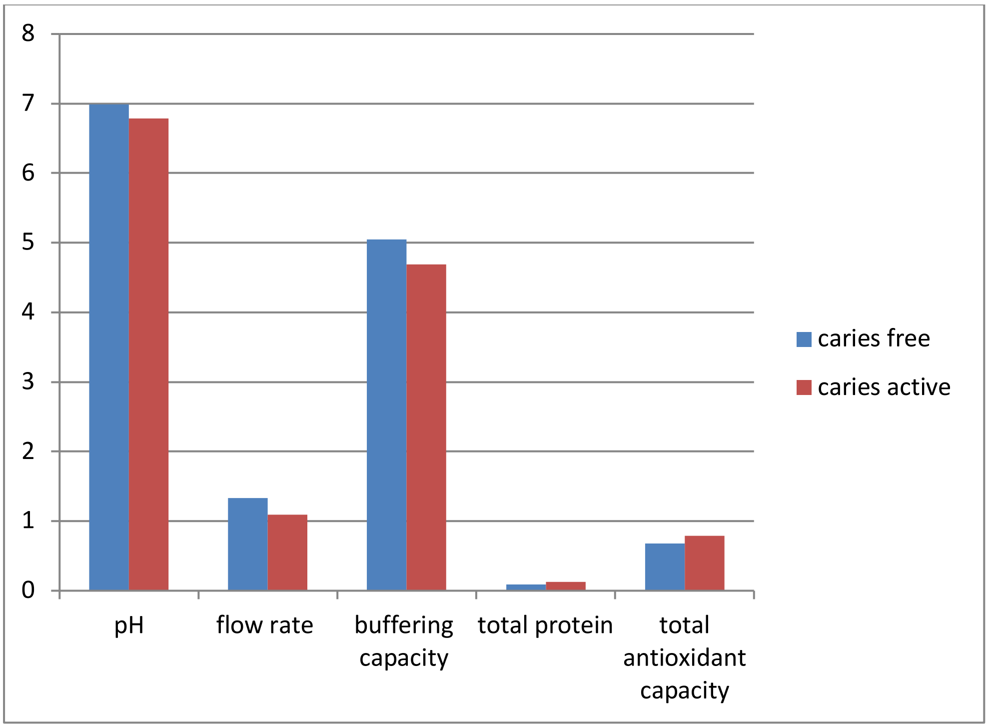
$$t = \frac{\text{Difference in means}}{\text{Standard Error of difference}}$$

$$= \frac{\bar{x}_1 - \bar{x}_2}{\text{Combined SD x} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Table 1: Physicochemical Properties of Saliva at Baseline Level for Caries Free and caries Active Group.

Variables	Caries free (n=40)	Caries active (n=40)	t-value	p-value
	Mean ± Std. Dev.	Mean ± Std. Dev.		
pH	6.99±.36	6.79±0.42	2.316	0.023*
Flow rate	1.33±.32	1.09±0.29	3.456	0.001*
Buffering capacity	5.05±.81	4.69±0.86	1.929	0.057
Total protein	0.09±.06	0.13±0.09	-2.011	0.048*
Total antioxidant	0.68±.25	0.79±0.28	-1.880	0.064

*** p<0.05, Significant**



Graph 1: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries free and caries active individuals

On observation, in case of caries free individuals the mean pH before was 6.99±.36 while that of caries active was 6.79±0.42 and it was statistically significant {p<0.05}.

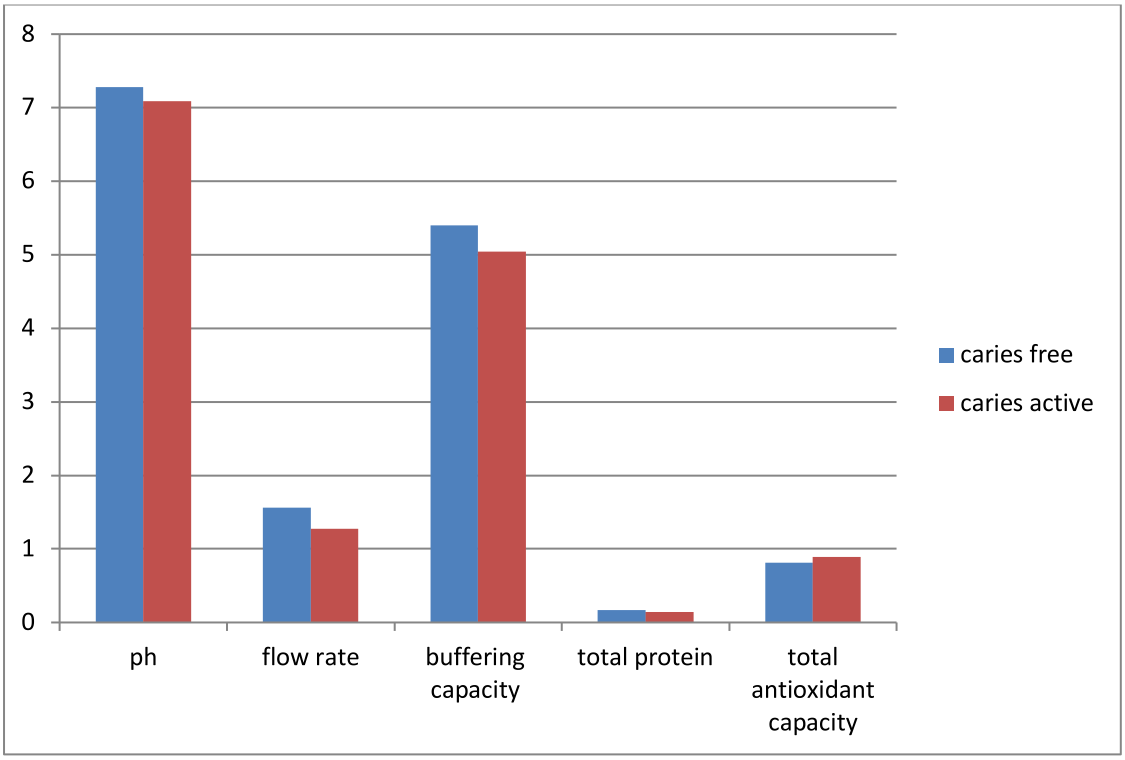
The mean flow rate for caries free individuals before the application of fluoride was 1.33±.32 and in caries active, it was 1.09±0.29 and was statistically significant {p<0.05}. Mean buffering capacity in caries free individuals before the application of fluoride was 5.05±.81 and in caries active, it was 4.69±0.86 and was not statistically significant {p>0.05}. Mean total protein in caries free individuals before the application of fluoride was 0.09±.06 and in caries active, it was 0.13±0.09 which was found statistically significant {p<0.05}.

Mean total antioxidant capacity in caries free individuals before the application of fluoride was 0.68±.25 while in caries active, it was 0.79±0.28 and the difference was not statistically significant {p>0.05}.

Table 2: Physicochemical Properties of Saliva for Caries Free and caries Active Group after intervention with fluoride agents.

Variables	Caries free (n=40)	Caries active (n=40)	t-value	p-value
	Mean ± Std. Dev.	**Mean ± Std. Dev.**		
pH	7.28±0.41	7.09±0.42	2.09	0.04*
Flow rate	1.56±0.37	1.27±0.34	3.56	0.001*
Buffering capacity	5.40±0.92	5.04±0.87	1.79	0.08
Total protein	0.17±0.10	0.14±0.07	-1.53	0.13
Total antioxidant	0.81±0.33	0.89±0.35	-0.88	0.32

*** p<0.05, Significant**



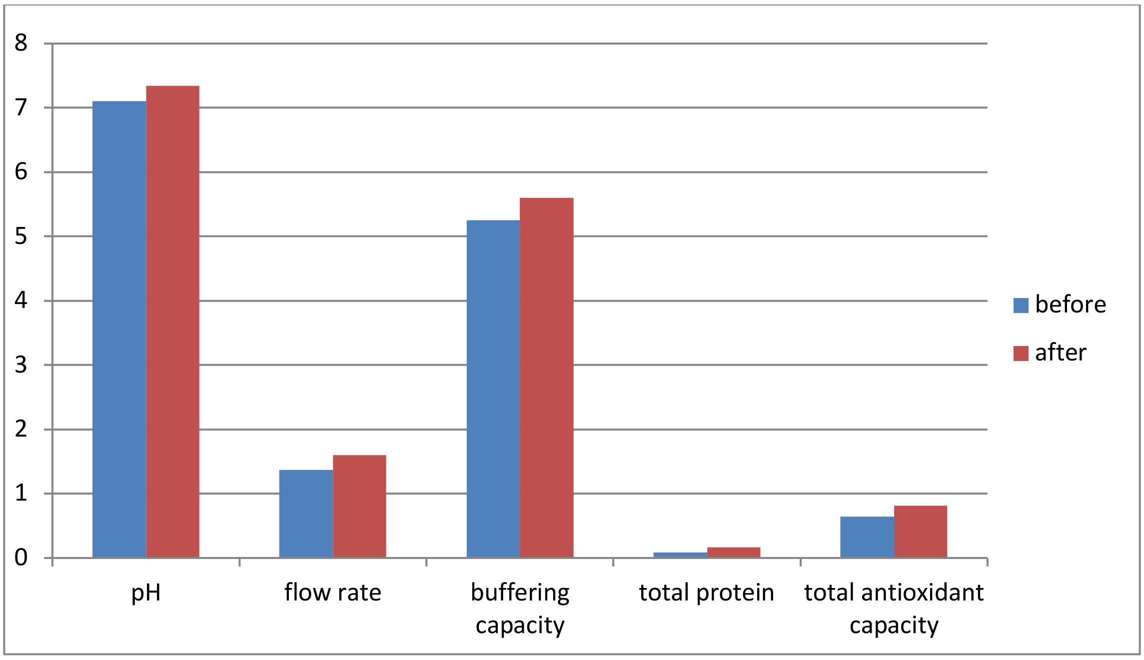
Graph 2: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries free and caries active individuals after the intervention of topical fluoride agents.

On observation, after the intervention with fluoride agents, in case of caries free individuals the mean pH was 7.28±0.41while that of caries active was 7.09±0.42 and the difference was statistically significant {p<0.05}. The mean flow rate for caries free individuals after the application of fluoride was 1.56±0.37 and in caries active, it was 1.27±0.34 and the difference was statistically significant {p<0.05}. Mean buffering capacity in caries free individuals after the application of fluoride was 5.40±0.92 and in caries active, it was 5.04±0.87 and was not statistically significant {p>0.05}. Mean total protein in caries free individuals after the application of fluoride was 0.17±0.10 and in caries active, it was 0.14±0.07 and was statistically not significant {p>0.05}. Mean total antioxidant capacity in caries free individuals after the application of fluoride was 0.81±0.33 and in caries active, it was 0.89±0.35 and the difference was not statistically significant {p>0.05}.

Table 3: Comparison of different variables in caries free group before and after the administration of fluoride gel.

			N	Mean ± Std. Deviation	t-statistic	P value
CARIES FREE	pH	Before	20	7.1±0.30		.0001*
		After	20	7.34±0.39	-6.019	
	Flow rate	Before	20	1.37±0.37		
		After	20	1.60±0.42	-4.305	.001*
	Buffering capacity	Before	20	5.25±0.67		
		After	20	5.60±0.72	-4.449	.001*
	Total protein	Before	20	0.09±0.05		.016*
		After	20	0.17±0.134	-2.653	
	Total antioxidant	Before	20	0.64±0.28		.010*
		After	20	0.81±0.36	-2.839	

*** p<0.05, Significant**



Graph 3: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries free group before and after the administration of fluoride gel.

On observation, in caries free individuals the mean pH of saliva before the application of fluoride gel was 7.1±0.30 while after the application of fluoride gel, it was 7.34±0.39. The difference in the pH of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean flow rate of saliva before the application of fluoride gel in caries free individuals was 1.37±0.37 while after the application of fluoride gel, the mean flow rate was 1.60±0.42. The difference in the flow rate of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean buffering capacity of saliva before the application of fluoride gel in caries free individuals was 5.25±0.67 while after the application of fluoride gel it was 5.60±0.72. The difference in the buffering capacity of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

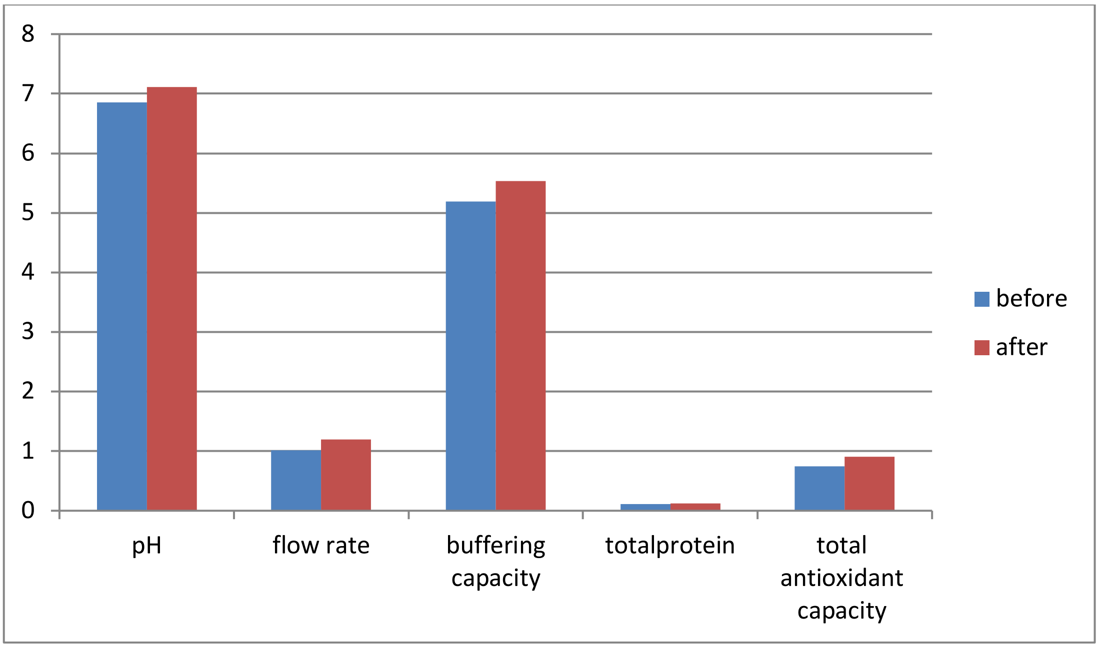
The mean total protein of saliva before the application of fluoride gel in caries free individuals was 0.09±0.05 while after the application of fluoride gel it was 0.17±0.134. The difference in the total protein of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean total antioxidant capacity of saliva before the application of fluoride gel in caries free individuals was 0.64±0.28 while after the application of fluoride gel it was 0.81±0.36. The difference in the total antioxidant capacity of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

Table 4: Comparison of Different Variables in Caries Active Group Before and After the Administration of Fluoride Gel.

CARIES ACTIVE			N	Mean ± Std. Deviation	t-statistic	P value
	pH	Before	20	6.85±0.47	-6.110	.0001*
		After	20	7.11±0.49		
	Flow rate	Before	20	1.01±0.06	-6.131	.001*
		After	20	1.20±0.30		
	Buffering capacity	Before	20	5.19±0.75	-10.802	.001*
		After	20	5.53±0.78		
	Total protein	Before	20	0.11±0.09	-.191	.851
		After	20	0.12±0.05		
	Total antioxidant	Before	20	0.75±0.27	-3.526	.002*
		After	20	0.91±0.34		

*** p<0.05, Significant**



Graph 4: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries active group before and after the administration of fluoride gel.

On observation, in caries active individuals the mean pH of saliva before the application of fluoride gel was 6.85±0.47 while after the application of fluoride gel, it was 7.11±0.49. The difference in the pH of saliva in caries active individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean flow rate of saliva before the application of fluoride gel in caries active individuals was 1.01±0.06 while after the application of fluoride gel it was 1.20±0.30. The difference in the flow rate of saliva in caries active individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean buffering capacity of saliva before the application of fluoride gel in caries active individuals was 5.19±0.75 while after the application of fluoride gel; the it was 5.53±0.78. The difference in the buffering capacity of saliva in caries active individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

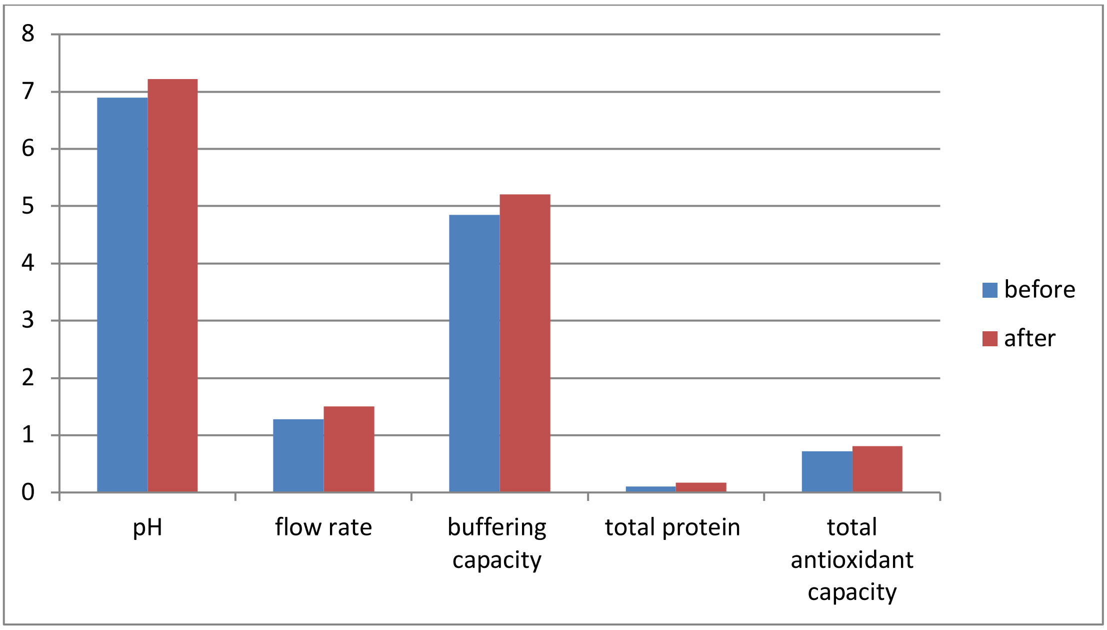
The mean total protein of saliva before the application of fluoride gel in caries active individuals was 0.11±0.09 while after the application of fluoride gel; the mean total protein was 0.12±0.05. The difference in the total protein of saliva in caries free individuals before and after the application of fluoride gel was not statistically significant {p>0.05}.

The mean total antioxidant capacity of saliva before the application of fluoride gel in caries active individuals was 0.75±0.27 while after the application of fluoride gel it was 0.91±0.34. The difference in the total antioxidant capacity of saliva in caries active individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

Table 5: Comparison of different variables in caries free group before and after the administration of fluoride varnish.

			N	Mean ± Std. Deviation	t-statistic	P value
CARIES FREE	pH	Before	20	6.89±0.40	-9.571	.0001*
		After	20	7.22±0.44		
	Flow rate	Before	20	1.28±0.26	-5.132	.001*
		After	20	1.50±0.31		
	Buffering capacity	Before	20	4.85±0.90	-3.266	.004*
		After	20	5.21±1.07		
	Total protein	Before	20	0.10±0.07	-4.394	.001*
		After	20	0.17±0.06		
	Total antioxidant	Before	20	0.72±0.22	-1.762	.094
		After	20	0.81±0.30		

*** p<0.05, Significant**



Graph 5: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries free group before and after the administration of fluoride varnish.

On observation, in caries free individuals the mean pH of saliva before the application of fluoride gel was 6.89±0.40 while after the application of fluoride gel, it was 7.22±0.44. The difference in the pH of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean flow rate of saliva before the application of fluoride gel in caries free individuals was 1.28±0.26 while after the application of fluoride gel it was 1.50±0.31. The difference in the flow rate of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

The mean buffering capacity of saliva before the application of fluoride gel in caries free individuals was 4.85±0.90 while after the application of fluoride gel it was 5.21±1.07. The difference in the buffering capacity of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p<0.05}.

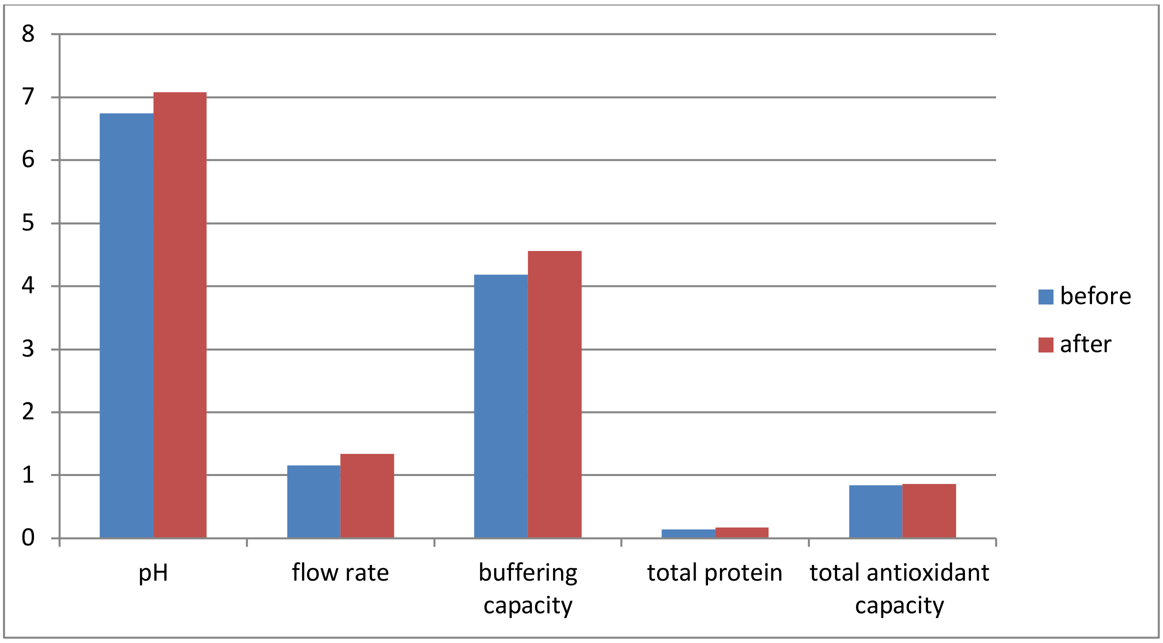
The mean total protein of saliva before the application of fluoride gel in caries free individuals was 0.10±0.07while after the application of fluoride gel it was 0.17±0.06. The difference in the total protein of saliva in caries free individuals before and after the application of fluoride gel was statistically significant {p>0.05}.

The mean total antioxidant capacity of saliva before the application of fluoride gel in caries free individuals was 0.72±0.22 while after the application of fluoride gel it was 0.81±0.30. The difference in the total antioxidant capacity of saliva in caries free individuals before and after the application of fluoride gel was not statistically significant {p<0.05}.

Table 6: Comparison of different variables in caries active group before and after the administration of fluoride varnish.

CARIES ACTIVE			N	Mean ± Std. Deviation	t-statistic	P value
	pH	Before	20	6.74±0.36	-6.449	.0001*
		After	20	7.08±0.35		
	Flow rate	Before	20	1.16±0.31	-4.547	.001*
		After	20	1.34±0.38		
	Buffering capacity	Before	20	4.18±0.63	-10.771	.001*
		After	20	4.56±0.69		
	Total protein	Before	20	0.14±0.09	-.869	.395*
		After	20	0.17±0.08		
	Total antioxidant	Before	20	0.84±0.29	-.459	.652*
		After	20	0.86±0.38		

*** p<0.05, Significant**



Graph 6: Comparison of mean pH, Flow Rate, Buffering Capacity, Total Protein and Total antioxidant capacity in caries active group before and after the administration of fluoride varnish.

On observation, in caries active individuals the mean pH of saliva before the application of fluoride varnish was 6.74±0.36 while after the application of fluoride varnish it was 7.08±0.35. The difference in the pH of saliva in caries active individuals before and after the application of fluoride varnish was statistically significant {$p<0.05$}.

The mean flow rate of saliva before the application of fluoride varnish in caries active individuals was 1.16±0.31 while after the application of fluoride varnish it was 1.34±0.38. The difference in the flow rate of saliva in caries active individuals before and after the application of fluoride varnish was statistically significant {$p<0.05$}.

The mean buffering capacity of saliva before the application of fluoride varnish in caries active individuals was 4.18±0.63 while after the application of fluoride varnish it was 4.56±0.69. The difference in the buffering capacity of saliva in caries active individuals before and after the application of fluoride varnish was statistically significant {$p<0.05$}.

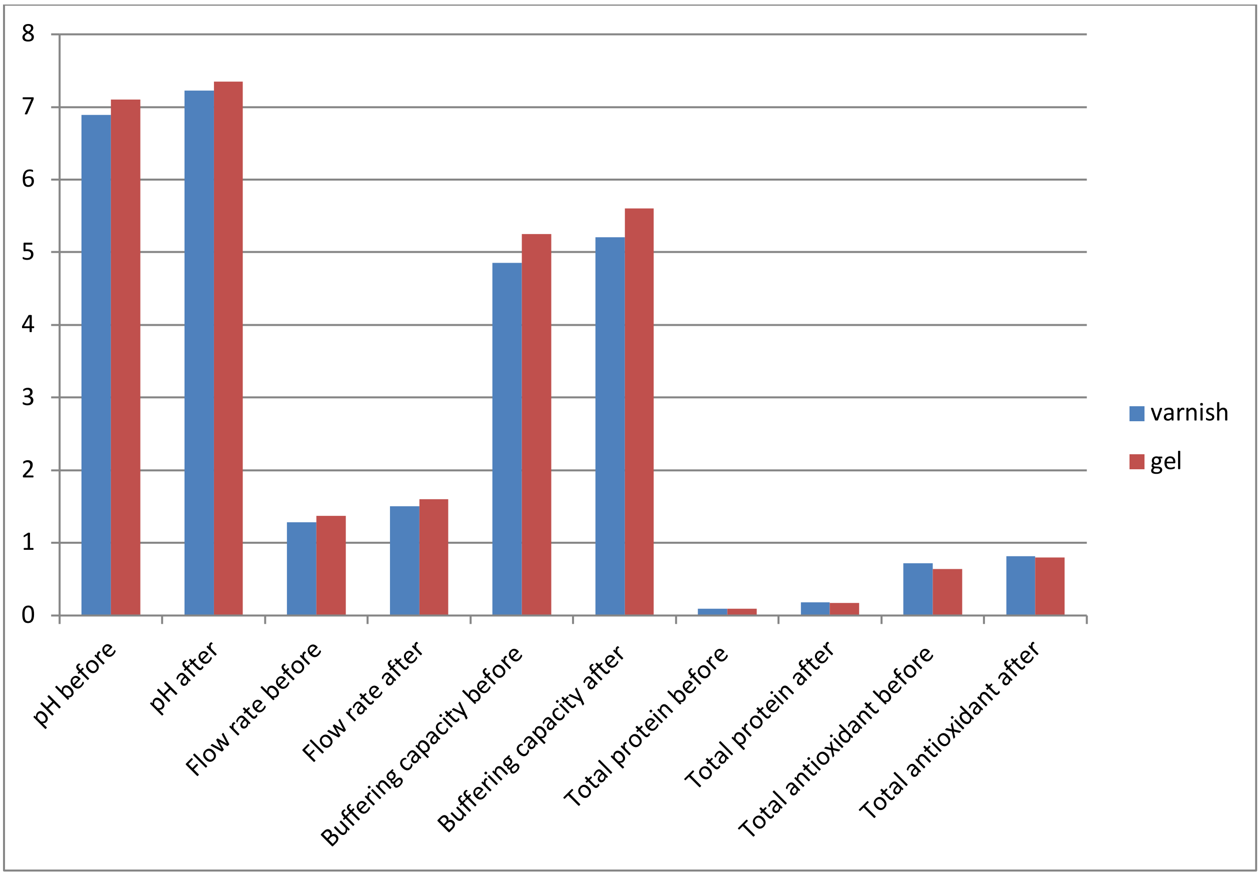
The mean total protein of saliva before the application of fluoride varnish in caries active individuals was 0.14±0.09 while after the application of fluoride varnish it was 0.17±0.08. The difference in the total protein of saliva in caries free individuals before and after the application of fluoride varnish was not statistically significant {$p>0.05$}.

The mean total antioxidant capacity of saliva before the application of fluoride varnish in caries active individuals was 0.84±0.29 while after the application of fluoride varnish it was 0.86±0.38. The difference in the total antioxidant capacity of saliva in caries active individuals before and after the application of fluoride varnish was not statistically significant {$p>0.05$}.

Table 7: Comparison of Varnish and Gel Materials with Different Variables before and After Treatment in Caries Free Group.

variables		Varnish (n=20)	Gel (n=20)		
		Mean ± Std. Deviation	Mean ± Std. Deviation	t-value	p-value
pH	Before	6.89±0.39	7.10±0.30	-1.885	0.067
	After	7.23±0.44	7.35±0.39	-.920	0.363
Flow rate	Before	1.28±0.26	1.37±0.37	-.856	0.397
	After	1.50±0.31	1.60±0.42	-.842	0.405
Buffering capacity	Before	4.85±0.90	5.25±0.67	-1.589	0.120
	After	5.21±1.08	5.60±0.72	-1.360	0.183
Total protein	Before	0.097±0.07	0.090±0.05	.360	0.721
	After	0.18±0.06	0.17±0.13	.287	0.776
Total antioxidant	Before	0.72±0.22	0.64±0.28	1.067	0.293
	After	0.82±0.31	0.80±0.36	.093	0.926

*** $p<0.05$, Significant**



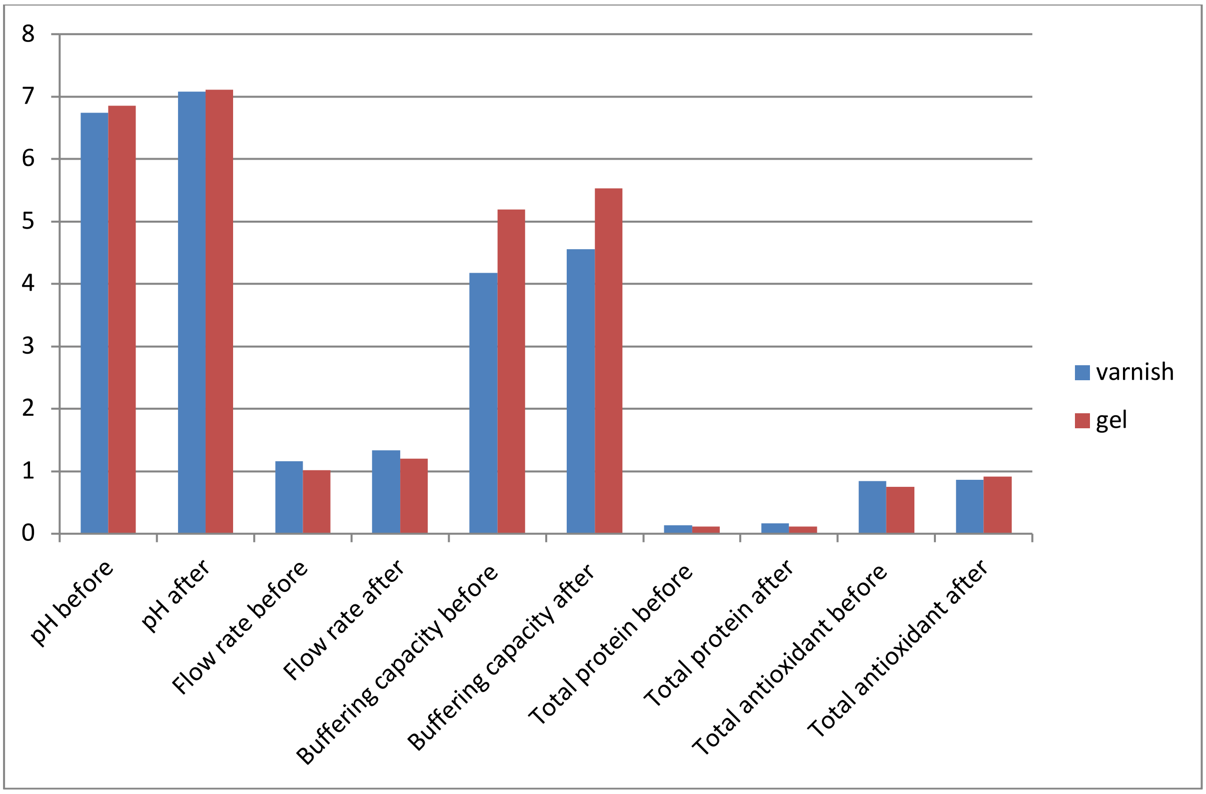
Graph 7: Comparison of varnish and gel materials with different variables before and after treatment in caries free group.

Comparison of varnish and gel materials with different variables before and after treatment in caries free group shows no significant correlation.

Table 8: Comparison of Varnish and Gel Materials with Different Variables before and After Treatment in Caries Active Group.

Variables		**Varnish (n=20)**	**Gel (n=20)**	
		Mean ± Std. Deviation	**Mean ± Std. Deviation**	**p-value**
pH	Before	6.74±0.36	6.85±0.47	0.433
	After	7.08±0.35	7.10±0.49	0.825
Flow rate	Before	1.16±0.31	1.02±0.26	0.131
	After	1.34±0.38	1.20±0.30	0.228
Buffering capacity	Before	4.18±0.63	5.19.±0.75	0.001*
	After	4.56±0.69	5.53±0.78	0.001*
Total protein	Before	0.14±0.09	0.12±0.09	0.389
	After	0.17±0.81	0.12±0.49	0.032
Total antioxidant	Before	0.83±0.29	0.75±0.27	0.343
	After	0.86±0.37	0.91±0.34	0.633

*** p<0.05, Significant**



Graph 8: comparison of varnish and gel materials with different variables before and after treatment in caries active group.

Comparison of varnish and gel materials with different variables before and after treatment in caries active group shows no significant correlation except for the buffering capacity and which showed a significant increase in fluoride gel group.

Discussion

Dental caries is an infectious bacterial biofilm disease which is expressed in a predominantly pathologic oral environment. Although acid generating bacteria are the etiologic agents, dental caries has been thought of as multi-factorial since it is influenced by dietary and host factors as well. Dental caries covers that caries is a continuum from the very first atomic level of demineralization i.e. loss of calcium and phosphate from the crystal surface involving the initial enamel or root lesion, through to eventual cavitation.[2] . The eventual outcome of dental caries is determined by the dynamic balance between pathological factors that lead to demineralization and protective factors that leads to remineralization.

Clinical dentistry, even today, generally considers that placing a restoration ''fixes'' dental caries. Unfortunately, placing the restoration only removes the offending bacteria from that cavity in that tooth. It does not deal with the disease in the rest of the mouth. Remineralization therapy produces mineral crystals that are much more resistant to acid challenge than the original mineral of the enamel and dentine. Therefore, remineralization alone is often sufficient to stop the progression of caries and to repair the subsurface lesions so that operative dentistry is not needed. The many clinical trials with fluoride have shown dramatic reductions in overall decay levels as measured by cavity formation. Application of fluoride results in the formation of fluorapatite or fluorhydroxyapatite that is more resistant to acid dissolution. It also influences the bacterial composition by affecting the carbohydrate metabolism of mutans streptococci and alters the plaque ecosystem which results in inhibition of cariogenic microorganism.

Apart from fluoride, the most effective and natural defence system is the saliva which decides the outcome of dental caries. It is one of the most important protective body fluids but remains the least understood [3]. Saliva is well adapted to protection against dental caries. Some of the functions of saliva include the mechanical action of cleaning, broad spectrum of antimicrobial proteins, buffer capacity and formation of enamel acquired pellicle, protection of mineralised tissues, participation in chewing, swallowing and speech [4]. These salivary functions are involved in the prevention of dental caries directly or indirectly and contribute to its essential role in the health of the teeth. Knowledge of the functional properties of saliva and of its separate components may permit a better assessment of dental caries susceptibility (Dowd, 1999). The cario-preventive action of fluoride due to its effects on the teeth, bacteria and plaque has been extensively studied however the effect of salivary physico-chemical properties (which is the main deciding factors for the progression of dental caries,) after the application of topical fluoride has not been reported in literature. So, the present study aims to evaluate some of the physico-chemical properties of saliva before and after the application of topical fluoride agents.

Inclusion and Exclusion criteria

The amount of saliva in the mouth is not constant and varies within a person over time and between individuals (Ship et al., 1991). It has been shown that the salivary flow rate, pH, sampling conditions, contamination and many other pathophysiological factors may influence the physico-chemical properties in saliva (Liu and Delgado, 1999). Medications may lead to alteration in salivary physicochemical property [5]. Thus in other to reduce bias and to standardize the study physically or medically compromised children, children on medication, children who had any systemic disease and who had undergone any type of preventive therapy to combat caries process were excluded from the study.

Fluoride Agent

Two fluoride agents have been evaluated in this study: - fluoride gel and fluoride varnish. Several reports show that different fluoride salts inhibit peroxidase activity, particularly under low pH conditions [6,7,8,9,10]. To avoid a possible influence of the low pH of acidified topical fluoride gels on peroxidise activity, a commercially available neutral NaF fluoride gel was used in this investigation. The concentration of fluoride in neutral 2% NaF gel is … ppm.

Commercially available 5% NaF Fluoritop-SR® varnish with a concentration of 22.6 mgF/ml was used for the study. It is easily available and cost effective, thus been used for this study [11].

Parameters Used In the Study:

Many studies have showed that salivary parameters such as salivary flow rate, pH and buffering capacity were lower in subjects with high dental caries [12]. The proteins in saliva have been found to have antibacterial properties as they include both non-immunoglobulins and secretory antibodies, especially secretory IgA, lysozyme, lactoferrin and peroxidise which helps in inhibition of cariogenic microorganism.

Imbalances in levels of free radicals, reactive oxygen species and antioxidants in saliva may play an important role in the onset and development of chronic degenerative diseases and dental caries [13]. Hence this study was undertaken to evaluate and compare the flow rate, pH, buffering capacity, total protein and total antioxidant levels among caries free and caries active children and also to evaluate the effect of fluoride on the properties of saliva in these children.

Collection of Saliva

The standardized collection of saliva is the most important step to obtain reliable results (Tenovuo and Lagerlöf, 1994). The timing of the sample collection is important, since the flow rate can vary with time. Variation in individual flow rates can be as high as 50% over a 24-hour period due to circadian rhythms (Ferguson and Botchway, 1979), and have been reported to exceed 50% in cross-sectional healthy population studies (Ship et al., 1991). In the present study the saliva was collected between 9 to 12 a.m. to prevent circadian variation and hence to standardize the collection procedure during the whole sampling period. It also helps to keep the secretion rate as constant as possible.

Saliva can be collected under both resting and stimulated conditions. However stimulated saliva sample was collected in the present study as it is more or less impossible to obtain true resting saliva as salivary flow is always influenced by some kind of stimulation.

Stimulated saliva was collected using spitting method in which the patient was asked to chew on sterile rubber band. The saliva collected in the mouth is asked to spit in a pre-weighed container [14] after asked chewing on sterile rubber bands [15].

Flow Rate:

The main factor affecting salivary composition is the flow index which varies in accordance with the type, intensity, and duration of the stimulus.

As the salivary flow increases the clearance become faster and lesser is the microbial attacks [16] along with that the concentrations of total protein, sodium, calcium, chloride, and bicarbonate as well as the pH increases to various levels, whereas the concentrations of inorganic phosphate and magnesium diminish [17]. Measurement of salivary flow rate is used as a screening method on a population level to identify people with low salivary flow which is often but not always related to caries susceptibility and activity [18].

Stimulated saliva is secreted in response to either masticatory or gustatory stimulation, or to other less common stimuli such as activation of the vomiting centre. A wide variation among individuals has been found (Ghezzi et al., 2000). Men have higher flow rates than women (Parvinen and Larmas, 1981). The factors affecting the flow of stimulated saliva are nature of stimulus, vomiting, smoking, gland size, gag reflex, olfaction, unilateral stimulation, and food intake (Dawes, 1996). Reduced salivary flow may cause a variety of mostly unspecific symptoms to the patient, so the establishment of salivary flow rates is of primary importance in oral medicine and dentistry (Ghezzi et al., 2000).

Widely accepted normal values for stimulated flow rates are 1.0 - 3.0 ml/min. Values below 0.7 ml/min are considered as hyposalivation, and values 0.7 – 1.0 ml/min low (Tenovuo and Lagerlöf, 1994).

In our present study, the mean salivary flow rate was 1.33 ±0.32 ml/min which is within the range as given by Tenovuo and Lagerlöf, 1994. Studies have shown that the flow rate of saliva decreases in caries active children compared to caries free children. In our present study the salivary flow rate was decreased in caries active children compared to caries free children and were statistically significant. This is in accordance with the studies conducted by Browen et al and Scully where they showed that dental caries is probably the most common consequence of hypo salivation.

Various authors [19,20,21,22] have reported similar finding in which they concluded that various components of saliva plays a major role in the development of caries.

In contrast to the above studies; Mandel 1987, Birkhed, Heintze 1989, and Russell et al 1990 reported that no linear relationship exists among salivary secretion rate, caries activity, and DMFS/DMFT values, except when the salivary flow rate was below threshold level [23].

From the present study, it was observed that the stimulated salivary flow rate in caries free as well as caries active children increases after the application of either fluoride gel or fluoride varnish. However there was no significant correlation in the flow rate of saliva between fluoride varnish and fluoride gel.

The increase in the stimulated salivary secretion may be due to the altered pH of products and to the mechanical influence, taste change, different distribution of slurry / foam/gel within the oral cavity and retention in niches.[24]

pH

Salivary pH is the acidity or alkalinity of the saliva. Oral cavity contains normal oral flora that are capable of metabolizing fermentable carbohydrates, leading to the production of a variety of acidic by-products. According to the international journal of drug testing (http://www.livestrong.com/article/192281-what-is-ph-of-saliva/), saliva has a pH of 6.3, with a range of 5.6 to 7.9, but could be modified by the oral health [25]. If salivary pH diminishes it can increase the enamel demineralization. There is no definite pH at which demineralization begins; it may be in the range of 5.5 to 5.0 which is the critical pH. The critical pH is the pH at which a solution is just saturated with respect to a particular mineral, such as tooth enamel. If the pH of the solution is above the critical pH, then the solution is supersaturated with respect to the mineral, and more mineral will tend to precipitate out. Conversely, if the pH of the solution is less than the critical pH, the solution is unsaturated, and the mineral will tend to dissolve until the solution becomes saturated [26]. Saliva and plaque fluid, for instance, are normally supersaturated with respect to tooth enamel because the pH is higher than the critical pH, so our teeth do not dissolve in our saliva or under plaque [27]

Studies have shown that patient with low or no caries activity had a resting salivary pH of around 7.0 and those with extreme caries activity had a resting pH below critical pH 5.5 [28].

In the present study, the mean pH of the saliva in caries free and caries active children were 6.99±.36 and 6.79±0.42 respectively. A reduction in the pH was seen in caries active children and the difference was found to be statistically significant.

The results obtained are in accordance with the study conducted by Animireddy D et al 2014[29,30,31,32]. However, in their study the salivary pH was only slightly reduced in caries-active children compared with caries-free children and were statistically not significant. In contrast to our study, the study carried out by Thaweboon et al. in 2008 [33,34,35,36] reported a weak or no relationship between the incidence of dental caries and the pH of saliva.

From the present study, it was observed that the pH of saliva in caries free group increases after the application of either fluoride varnish or fluoride gel and was statistically significant. In caries active group, pH increases after the application of fluoride agents and was also found to be statistically significant. However, there was no significant correlation in the pH changes of saliva between fluoride varnish and fluoride gel. Dąbrowska et al. reported a similar finding who observed a slight increase in salivary pH after a single application of each of the preparations [37]

Balunowska M et al analysed the effect of preparations used in professional fluoride prophylaxis on the chosen parameters of human saliva. They found that the pH value was elevated after fluoride application which is in accordance with our result.

The increase in the pH after fluoridation may be attributed to the increase in stimulate saliva secretion, resulting in elevated pH level [38].

Buffering Capacity

Saliva behaves as a buffer system to protect the mouth [39] as it prevents colonization by potentially pathogenic microorganisms by denying them optimization of environmental conditions and maintaining a pH level in saliva and plaque by neutralizing and cleaning the acids produced by acidogenic microorganisms, thus, preventing enamel demineralization. The buffer capacity of unstimulated and stimulated whole saliva involves three major buffer systems i.e. bicarbonate system, phosphate system and protein system. The most important buffering system, in saliva is the carbonic acid / bicarbonate system. Bicarbonates release weak carbonic acid. When acid is added, bicarbonate is rapidly decomposed to H_2O and CO_2 which leaves the solution resulting in complete removal of acids. The saliva bicarbonate increases the pH and buffer capacity of saliva, especially during stimulation [41]. The second buffering system is the phosphate system, which contributes to some extent to the buffer capacity at low flow rate. The mechanism for the buffering action of inorganic phosphate is due to the ability of the secondary phosphate ion, $HPO4^{2-}$, to bind a hydrogen ion and form an $H2PO4^{-}$ -ion.

The third buffering system is the protein system. In the low range of pH (very low pH 4-4.5)[42] .The buffering capacity of saliva is due to the macromolecules (proteins) containing H-binding sites (Tenovuo and Lagerlof, 1994).

Bicarbonate is the chief determinant of the buffer capacity, there is an interrelationship between pH, secretion rate and salivary buffering capacity (Birkhed and Heitze, 1989) and suggest that bicarbonate concentration is strongly dependent on secretion rate.

Ericcson (1959) developed a saliva buffer capacity test, which showed that the final pH is an acceptable measure of saliva buffering ability. The normal buffering capacity is within the range of 4-5 pH according to his method. He also showed that the bicarbonate levels show diurnal variation.

In the present study, the mean buffering capacity of the saliva in caries free and caries active children were 5.05±.81 and 4.69±0.86 respectively. A reduction in the buffering capacity was seen in caries active children but the difference in the buffering capacity were statistically not significant. From the present study, it was observed that the buffering capacity of saliva in caries free group increases after the application of either fluoride varnish or fluoride gel and was statistically significant. Similar result was seen in caries active group in which the buffering capacity increases after the application of fluoride agents and was also statistically significant. However there was no significant correlation in the buffering capacity of saliva between fluoride varnish and fluoride gel.

The increase in the buffering capacity after the application of topical fluoride may be due to the increased in the bicarbonate and total protein secretion which are the main component of buffer in saliva [43].

Proteins

Saliva contains a large number of proteins that participate in the protection of the oral tissues. On an average 300mg/100ml of total protein is present in human saliva. These saliva proteins can have a wide range of functional properties i.e. multifunctional (performing more than one function) redundant (performing similar function but to different extent) and amphifunctional (Acting both for and against the host) [44] Because of which there seems to be a considerable overlap in functionality.

Salivary proteins act directly and indirectly through various methods on plaque and bacteria modulating susceptibility of the tooth to dental caries. (Mandel ID. Electrophoretic Studies of Saliva. J Dent Res 1966; 45:634.). They can be related to the immune response and oral defence, like lysozyme, lactoferrin, lactoperoxydase, immunoglobulins, agglutinins, chitinases and mucins which in saliva participate in the protection of the oral tissues; other proteins possess bacteria-killing properties, like histatins and defensins. They also inhibit mineral precipitation and aids in remineralization [45]. The present study evaluate the total protein concentration of saliva in caries free and caries active children and the mean total protein of the saliva in caries free and caries active children were 0.09±.06 gm/dl and 0.13±0.09 g/dl respectively. An increased in the total protein concentration of the saliva was seen in caries active children and the difference was statistically significant.

Similar results were reported in the study conducted by Tulunoglu O.S. and Preethi BP where higher concentrations of total proteins in the saliva of children with greater cariogenic activity were reported [46,47].

In contrast to our study Leone and Oppenheim (2001) reported that fourteen studies which examined the correlation between caries and salivary proteins, found no correlation between them. Murray et al stated that concentration of salivary proteins was reduced in case of caries active children in developing countries who were subjected to mild starvation on natural diet.

Total antioxidant capacity:

Biological reduction of molecular oxygen (O_2) generates products collectively termed reactive oxygen species (ROS). By accepting a single electron, O_2 is transformed into the superoxide radical anion $\cdot O_2$ -, which plays a key role in biological systems. Superoxide radicals are generated under natural conditions during mitochondrial respiration, by UV-B radiation, and in phagocytosis of cells engaged in immune response [48].

ROS exhibit a wide spectrum of pathogenic properties. The uncontrolled production of ROS/RNS has been strongly implicated in several human diseases and ageing-related degenerative processes [49].

Living organisms possess several antioxidative species and mechanisms protecting them against the harmful action of ROS. 'Antioxidants' are substances that neutralize free radicals or their actions (Sies, 1996). These include the enzymes superoxide dismutase, catalase, glutathione peroxidises and many other peroxidises together with non-enzymatic antioxidants, like tripeptide glutathione, uric acid, ascorbate, selenium compounds, vitamins A, E, and C, and compounds containing thiol groups. Imbalance between ROS and antioxidants is referred to as oxidative stress [50]. Fluoride is also known to be an inhibitor/activator of numerous enzymes [51,52,53]. It is recognised to affect oxygen metabolism by inducing the production of ROS, and inhibiting the activity of several antioxidant enzymes in several tissues and cells such as brain, skeletal muscles, nervous system, erythrocytes and osteoblasts [54].

Saliva also presents an antioxidant action due its enzymatic (mostly peroxidise system) and non-enzymatic compounds (uric acid, glutathione, sialic acid, etc.) that were closely related to dental caries, odontogenic cyst, smoking, periodontitis, oral cavity cancer, temporomandibular disorders, etc[55,56]. Fluoride is known to be the main caries preventive agent available and is being used widely in different forms. However, there are no reports of the effect of topically applied fluoride on the total antioxidant capacity of saliva.

It is suggested that free radicals and antioxidant system appear to act in concert rather than alone. Investigations of individual antioxidant activity may be misleading and the measurement of any individual antioxidant may be less representative of the whole antioxidant status. Moreover, the number of different antioxidants makes it difficult and also expensive to measure each of them separately.

The present study evaluated the effect of topically applied fluoride on the total antioxidant capacity of saliva in caries free and caries active children. In the present study, the mean total antioxidant capacity of saliva in caries free and caries active children were 0.68±.25 and 0.79±0.28 respectively. An increase in the total antioxidant capacity was seen in caries active children and the difference in the total antioxidant capacity were statistically significant.

From the present study, it was observed that the total antioxidant capacity of saliva in caries free group increases after the application of either fluoride varnish or fluoride gel and was statistically significant. Similar result was seen in caries active group in which the total antioxidant capacity increases after the application of fluoride agents and was also statistically significant. However, there was no significant correlation in the total antioxidant capacity of saliva between fluoride varnish and fluoride gel.

Similar result was reported by Mariana Ferreira Leite et al 2012 in which they evaluated the effect of topical fluoride gel NaF 2% application on antioxidant parameters of whole saliva from children. They concluded that topical fluoride gel NaF 2% favourably stimulated the release of non-enzymatic antioxidant components of saliva, sustaining the reducing power of saliva and the natural defences of the oral cavity [57]. The fluoride topical application can stimulate the salivary flow rate which could explain the increase of non-enzymatic antioxidant parameters of saliva [58]. Increased in the flow rate results in increased concentration of protein in saliva as observed in our study. In a previous study [59] it has been suggested that saliva is also rich in antioxidant mainly uric acid with lesser but definite contributions from albumin, ascorbate and glutathione and all of these are proteins or have proteins in their structure. It has been reported that uric acid is the major antioxidant in saliva accounting for more than 85% of the TAC of both unstimulated and stimulated saliva. Thus, there is a linear association between antioxidant level and total protein level of saliva. Saliva is considered as one of the most important factor in caries process. The physicochemical properties of saliva like pH, buffering capacity, salivary flow rate, concentration of various components like proteins and antioxidant defence system play a major role in the development of caries. Applications of fluoride have shown dramatic reductions in overall decay levels as measured by cavity formation [60]. However, the effects of fluoride on the physicochemical properties of saliva have not been studied extensively.

The present study highlights the effects of fluoride intervention on both low and high caries risk groups. Thus, this study further reinforces the fluoride regime on high caries risk group. One of the possible limitations of this study could be limited follow up as it was a cross-sectional study done at 1 hour interval after intervention with fluoride. Also, we evaluated selected variables of saliva as analyzing all the possible variable were beyond the scope in limited tenure of time. Longitudinal/cohort studies with longer follow up with more variables of saliva should be done in future for strengthening the above evidence. Other alternatives agents of fluoride can be used for better correlation.

Conclusion

The following conclusions were drawn from the present study: -

- At baseline level the difference in pH, flow rate and total protein in saliva in cries free and caries active group were statistically significant. After intervention with fluoride the difference between the two groups in respect to total protein level became statistically insignificant.
- In caries free group the difference before and after the application of both fluoride gel and varnish in respect to all salivary parameters became statistically significant except, total antioxidant capacity in children with fluoride varnish.
- In caries active group the difference before and after the application of fluoride gel in respect to all salivary parameters became statistically significant, except total protein. Whereas in the same group, the difference before and after the application of fluoride varnish was statistically significant for all parameters, except total protein and total antioxidant capacity.
- It was also found that effect of both fluoride gel and varnish was almost equivocal in their efficacy on physiochemical properties of saliva in both the groups.

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