ORIGINAL ARTICLE

SERUM AND SALIVARY MINERALS IN DENTAL CARIES

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ABSTRACT

Background: Dental caries is a multifactor disease, affecting people of all ages. Inorganic mineral of serum and salva can also have protective role in dental caries. This study was carried out to evaluate and compare the possible role of salivary and serum factors like pH, adequate level of calcium, phosphate and fluoride in dental caries.

Methodology: A Total of 100 subjects aged 10-40 were selected. Decayed, missed and filled teeth (DMFT) were used as indices for scoring the dental caries and were distributed or divided into 4 groups on the basis of DMFT indices as 4-8 (Group I), 9-16 (Group II), 17-24 (Group III) and more than 25 (Group IV), while the control subjects had DMFT index equal to or less than 3. pH, calcium, phosphate, fluoride and lactic acid were analyzed in saliva and serum.

Results: Patients of dental caries showed significantly low levels of calcium, phosphate, fluoride ($P<0.001$) and significantly high level of lactic acid ($P<0.001$) in all the groups as compared to control subjects. Prominent significant changes were observed in different groups. The salivary and serum pH, calcium, fluoride, phosphate and lactic levels were found to be significantly changed among the patients having dental caries.

Conclusion: It can be concluded from the findings of present study that the adequate levels of calcium, phosphate and fluoride in saliva as well as serum are responsible for the significant deposition of these minerals in plaque which greatly reduces the developmental caries in the adjacent enamel.

Keywords: Serum, Saliva, Calcium, Phosphate, Fluoride, Lactic acid, Dental caries.

INTRODUCTION

Dental caries is a multifactorial disease, which has affected people throughout the ages.[1,2] Many constituent of serum and saliva, both organic and inorganic have potentially protective role. These include pH, calcium, phosphate, fluoride ions and bicarbonate buffer systems.[3-5] Epidemiological studies have supported the view that raised level of calcium, phosphate, and Fluoride in plaque might inhibit dental caries.[6-9] It is commonly thought that the organic acid produced in dental plaque is responsible for caries, but this is partly true because it is a complex effect of pH, calcium, phosphate and fluoride, which brought about minerals dissolution.[10] In low concentration, fluoride alone partially inhibits the net dissolution of enamel and the production of acid by plaque organisms, while demineralization

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requires the presence of calcium and phosphate.[11-13] The present study was done to estimate and compare the salivary and serum calcium, phosphate, and fluoride in the patients of dental caries and to see and compare their levels with the severity of disease and control.

MATERIALS AND METHODS

A total of 100 subjects aged 10-40 years were selected from the Department of Dentistry, Jinnah Postgraduate Medical Centre and from the Out Patient Department of Fatima Jinnah Dental Hospital Karachi, Pakistan. The subjects were not suffering from any systemic illness and were not taking any caries preventive regimen like fluoride toothpaste, fluoride rinses or NaF/calcium tablets. Subjects who gave improper history about missing teeth or suffering from any type of Xerostomia or having any oral inflammatory problems were not included in the study.

Dental examination was done with the assistance of dentist under natural light source. Decayed, missed and filled teeth (DMFT) were used as index for scoring the dental caries.[14] All subjects were distributed into 5 groups (Table 1) each having twenty individuals. Group 1 with DMFT index 4-8, group 2 with DMFT index 9-16, group 3 with DMFT index 17-24 and group 4 with DMFT index more than 25, while the control subjects have the DMFT index equal or less than 3.

About 10 mL of unstimulated mixed saliva was obtained from the individual 2 hours after the breakfast and 10 minutes after mouthwash with deionized water several times. Saliva was collected in 10 cc disposable plastic syringes after removing the piston and blocking the needle. Syringes were than labeled, covered and transferred in icebox to the laboratory. The saliva samples were centrifuged for 15 minutes at 3000 rpm. The clear supernatant of saliva was separated and labeled tubes were stored at -20^{0}C.

Approximately 10 mL of venous blood sample was drawn after applying a tourniquet, followed by proper aseptic precautions with a sterile disposable plastic syringe without any anticoagulant. A drop of blood was put on the electrode of pH meter from the nozel of syringe carefully for blood pH determination. Half mL of blood was immediately put into sterile bottle containing 0.5 mg of EDTA (Ethylene Diamine Tetra Acetic acid) powder, shaken gently and stoppered. This blood was used within 24 hours for the estimation of lactic acid.

The blood in the syringe was covered, labelled and transferred in an ice box to the laboratory. Blood sample was centrifuged for 15 minutes at 3000 rpm. The hemolyzed samples were discarded. The supernatant layer of serum was then separated and poured in labeled glass bottles and stored in deep freezer at -20^{0}C.

The salivary and serum pH were measured electrometrically with the glass electrode by digital pH meter HI 8014 (Hanna Instrument, USA). After calibration and temperature adjustment the bulb of glass electrode was immersed in a drop of sample and pH was noted from the screen of digital pH meter.

The calcium was estimated calorimetrically by using kit (Ref # 995936) supplied by Quimica Clinical Aplicade SA Aposta Spain. Inorganic phosphorate was measured by colorimetric method using kit, cat # KC 120 supplied by Clonital Italy. Fluoride was also measured by colorimetric method using alazerine and zirconium dye. The fluoride was analyzed by the Magregian, Haier method cited by Farber,[15] in which the fluoride reacts with dye lake, dissociating a portion of it into a colorless complex anion (ZrF_6) and the dye. As the amount of fluoride increased, the color produced becomes progressively lighter or different in hue depending on the reagent used. The student's "t-test" was used to compare the salivary and serum pH, calcium, phosphate and fluoride among the control and diseased groups.

RESULTS

One hundred individuals were divided into five groups according to their DMFT index (Table 1). The base line comparison of mean values of age, DMFT, index and number of tooth brushing per day (Table 2) shows a significant decrease in number of brushing and significant increase in DMFT index in all groups when compared to control.

The comparison between salivary and serum pH, calcium, fluoride, phosphate and lactic acid levels is given in Table 3. According to the findings of present study, the serum pH, calcium, fluoride and lactic acid levels were significantly high ($p<0.01$) where as serum phosphate levels were found significantly low ($p<0.01$) in all patients of dental caries as compared to saliva in same patients.

DISCUSSION

The role of salivary and serum pH, calcium, phosphate and fluoride in dental caries has been the point of interest since the mid of this century by many oral hygienist in the field of oral biochemistry. The early work of Stephan[16], regarding the estimation of salivary pH had showed that the pH of saliva remained below the critical level of 5.5 in caries patients, than those without dental caries. The saliva exert its major influence on caries initiation by means of plaque formation rather than by direct contact on the tooth surface, they showed that plaque pH fall was greater in dental caries susceptible subjects. However this study did not show any significant change in the blood pH with the progression of disease.[17, 18] The calcium ions are present normally in dental plaque bound to matrix and other proteins attracting phosphate and fluoride as counter ion, other phosphate and fluoride occurs intracellularly.[19] All three ions occur as an inorganic mineral in serum and are in continuous exchange phase with the saliva over the dental plaque. This is responsible for the "pool" or "reservior" of calcium, phosphate and fluoride in dental plaque and also maintains their saturation. These observations are quite identical with this study as levels of serum calcium, phosphate and fluoride are significantly low in dental caries patient in comparison to the control.

Our study quite clearly gives the information that there is significant difference in salivary and serum calcium, phosphate and fluoride as the disease process advances (Table 3). This observation is in complete agreement with the study carried out by Pearce [10] who explained that salt dissolution is governed by the concentration of calcium, phosphate and OH^- ions in the surrounding fluid. These results are also supported by the research study of previous investigators who explained the process of dental caries on the basis of ionic product and solubility product. They explained that these ions are the main constituent of the enamel apatite lattice. The crystals formed in the presence of fluoride dissolved more slowly in acid as they have lower intrinsic rate of dissolution [20] particularly of fluoride are taken up during remineralization and the crystals formed in the presence of fluoride are large, dense and more perfect. [21] Another observation made in this study was that, the rate of remineralization was raised in the presence of fluoride in early carious lesion at those time when the pH has risen so that remineralization is the dominant process. The investigations also demonstrated the antibacterial property of fluoride as it has a tendency to bind with the active metal of enzyme system e.g. in case of enolase, an enzyme that require magnesium which can be inhibited up to 100% by fluoride with the level of 95 ppm in the solution.

CONCLUSION

It is concluded that salivary and serum pH, calcium, phosphate, fluoride, and lactic acid variations in which are greatly influenced by the progression of dental caries. These minerals found in both the fluids and deposited in plaque greatly reduce the

development of experimental caries in the adjacent enamel [22, 23] because it tends to maintain the saturation of plaque fluid with respect to enamel mineral at low pH. This saturation is a combined result of reduced plaque pH depression due to the acid neutralizing properties of apatite, and the high concentrations of calcium, phosphate and fluoride leached into plaque fluid by acids. Secondly, these results support the findings of Geddes [20,24] that total plaque acid production does not correlate well with plaque pH following incubation with sugar, and thirdly, lead us to predict that pH measurement alone is inadequate to assess the potential cariogenicity of plaque. Rather, the degree of under saturation of plaque fluid with respect to enamel mineral is the principal factor to be considered. The levels of there influential factors in both the characteristic body fluids; saliva and serum have variations to be maintained during the treatment against the progression of the disease.

Table 1: Distribution of control and patients in groups. (According to the DMFT index)

Group	DMFT index	Distribution of subjects	Sex	
			Male	Female
Control (n=20)	= 3	20	13	7
Group – I (n=20)	4-8	20	11	9
Group – II (n=20)	9-16	20	11	9
Group – III (n=20)	17-24	20	10	10
Group – IV (n=20)	=25	20	10	10

Table 2 : Baseline comparisan personale data of the controlad patients.

Group	Age (years)	DMFT Index	Brushing (No. of times/day)
Control (n=20)	23.9 +1.623	1.35 +0.208	2.05 +0.05
Group – I (n=20)	27.75 +1.680	6.3* +0.291	1.6* +0.11
Group – II (n=20)	28.25 +1.769	12.15* +0.099	1.05* +0.135
Group – III (n=20)	31.7* +1.818	19.8* +0.47	0.5* +0.114
Group – IV (n=20)	31.95* +1.59	26.95* +0.364	0.15* +0.08

*** P < 0.01 as compared to control**

Table 3: Comparison of serum and salivary pH, calcium, fluoride, phosphate and lactic acid in groups

Group	pH		Calcium		Fluoride		Phosphate		Lactic acid	
	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum
I	6.948 ±0.217	7.407* ±0.006	7.425* ±0.363	9.72* ±0.128	0.036 ±0.014	2.295* ±0.317	19.70 ±0.529	4.03* ±0.099	6.03 ±0.315	11.765* ±0.809
II	6.39 ±0.088	7.417* ± 0.005	4.24* ±0.342	9.1* ±0.127	0.024 ±0.015	1.615* ±0.713	16.28 ±0.655	3.59* ±0.047	8.815 ±0.412	15.32* ±0.695
III	5.845 ±0.175	7.419* ±0.004	2.3* ±0.201	8.6* ±0.139	0.012 ±0.004	0.76* ±0.044	13.05 ±0.400	3.05* ±0.032	13.27 ±0.43	18.14* ±0.794
IV	5.05 ±0.734	7.418* ±0.005	2.38* ±0.177	7.95* ±0.115	0.013 ±0.004	0.58* ±0.069	10.44 ±0.328	2.295*±0 .059	17.27 ±0.444	22.875* ±0.956

*** P < 0.01 as compared to control**

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