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Serum Calcium, Phosphate, Fluoride and Lactic Acid in Dental Caries

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This study was carried out to evaluate the possible protective role of some serum factors like pH and adequate level of calcium, phosphate and fluoride in dental caries. A total of 100 subjects of either sex, aged 10-40 were selected. Decayed, Missed and Filled Teeth (DMFT) were used as indices for scoring the dental caries and were distributed 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. Serum was collected and pH, calcium, phosphate, fluoride and lactic acid were analyzed. Patients of dental caries showed significantly decreased levels of calcium, phosphate, fluoride ($p<0.001$) and significantly increased level of lactic acid ($p<0.001$) were observed in Groups I, II, III and IV as compared to controls. Among groups prominent significant changes were observed in Group IV. This study did not show any significant change in serum pH with the progression of disease. From the findings of present study, it can be concluded that the adequate level of calcium, phosphate and fluoride is responsible for the significant deposition of these minerals in plaque which greatly reduces the developmental caries in the adjacent enamel.

Key words: Serum calcium, phosphate, fluoride, lactic acid, dental caries

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INTRODUCTION

Dental caries is a multifactorial disease, which has affected people throughout the ages (Kedjarun *et al*., 1997a, b). Many constituent of serum and saliva, both organic and inorganic have potentially protective role. These include calcium, phosphate, fluoride ions and bicarbonate buffer systems (Speirs, 1984; Rapisarda and Longo, 1981; Robinson *et al*., 2000). Epidemiological studies have supported the view that raised level of calcium, phosphate and Fluoride in plaque might inhibit dental caries (Wen *et al*., 1995; Schamschula *et al*., 1985; Ashley and Wilson, 1977; Shaw *et al*., 1983). It is commonly thought that the organic acid produced in dental plaque is responsible for caries, but this is only partly true because it is a complex effect of pH, calcium, phosphate and fluoride, which brought about minerals dissolution (Pearce, 1998). In theory, continuous saturation of plaque fluid with mineral ions should completely over come the harmful effect of plaque pH depressions and thus should be more effective than fluoride therapy. In low concentration, fluoride alone only partially inhibits the net dissolution of enamel and the production of acid by plaque organisms, while demineralization requires the presence of calcium and phosphate (Fejerskov *et al*., 1981; Duckworth, 1993; Gaffar *et al*., 1999).

The present study was done to estimate 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 of either sex aged 10-40 were selected from the department of dentistry, Jinnah Postgraduate Medical Centre and from the Out Patient Department of Fatima Jinnah Dental Hospital Karachi, Pakistan. All the subjects were free 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 missed tooth 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 at Fatima Jinnah Dental Hospital out patient department. Decayed Missed and Filled Teeth (DMFT) were used as index for scoring the dental caries (Shah, 1986). All subjects were distributed into 5 groups (Table 1) each having twenty individuals. Like Group I with DMFT index 4-8, Group II with DMFT index 9-16, Group III with DMFT index 17-24 and Group IV with DMFT index more than 25, while the control subjects have the DMFT index equal or less than 3.

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

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

A 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. 0.5 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 h 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 min at 3000 rpm. The hemolyzed samples were discarded. The supernatant layer of serum was then separated and poured in labelled glass bottles and stored in deep freezer at -20°C.

The serum pH was 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 serum sample and pH was noted from the screen of digital pH meter.

The serum calcium was estimated calorimetrically by using kit (Ref # 995936) supplied by Quimica Clinical Aplicade SA Aposta Spain. Serum inorganic phosphorus was measured by calorimetric method using kit, cat # KC 120 supplied by Clonital Italy. Serum fluoride was also measured by calorimetric method using alazerine and zirconium dye. The fluoride was analyzed by the Magregian, Haier method cited by Farber (1960) 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. All assays were done at department of Biochemistry, Fatima Jinnah Dental College and Hospital and Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi.

The student's t-test was used to compare the serum 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 distribution of sex is approximately equal in all groups. The base line comparison of mean values of age, DMFT, index and number of 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.

Table 3 shows the comparison of the mean values of serum pH, calcium, phosphate, fluoride and lactic acid between control and all groups. In Group I there is a significantly decreased level of serum, calcium and fluoride and significantly increased level of lactic acid when compared to control subjects ($p<0.001$). In Group II, III and IV serum, calcium, phosphate and fluoride observed decreased significantly and a significant increased in serum lactic acid when compared to control subjects ($p<0.001$). No significant change is observed in serum pH of all groups when compared to control group.

Table 4 shows the intergroup comparison of mean values of serum pH, calcium, phosphate, fluoride and lactic acid. A significantly decreased serum calcium and phosphate and increased lactic acid were observed in Group II, III and IV when compared to Group I whereas fluoride was significantly decreased in Group II and IV when compared to Group I. When Group III and IV were compared with Group II, the decreased serum calcium, phosphate and increased lactic acid were observed. In contrary when Group IV compared with Group III, significantly decreased level of calcium, phosphate, fluoride and increased lactic acid were observed. In Group II serum calcium and phosphate were significantly decreased while lactic acid was significantly increased when compared to Group I ($p<0.001$). In Group III and IV serum calcium, phosphate and fluoride were decreased significantly while lactic acid was increased significantly when compared to Group I ($p<0.001$). In Group III serum calcium and phosphate were significantly decreased and lactic acid is significantly raised when compared to Group II ($p<0.05$).

Table 2: Baseline comparison of personal data of the control and patients

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

Values are expressed as mean±SEM, * $p<0.001$ as compared to control

Table 3: Comparison of serum pH, calcium, phosphate, fluoride and lactic acid between control and groups

		Groups			
Parameters	Control (n = 20)	I (n = 20)	II (n = 20)	III (n = 20)	IV (n = 20)
pH	7.412	7.407	7.417	7.419	7.418
	±0.005	±0.006	±0.005	±0.004	±0.005
Calcium	10.275	9.72**	9.1**	8.6**	7.955**
(mg dL^{-1})	±0.154	±0.128	±0.127	±0.139	±0.115
Phosphate	4.22	4.03	3.59**	3.005**	2.295**
(mg dL^{-1})	±0.117	±0.099	±0.047	±0.032	±0.059
Fluoride	4.4	2.295**	1.615**	0.76**	0.58
(µg dL^{-1})	±0.393	±0.317	±0.713	±0.044	±0.069
Lactic acid	7.45	11.765**	15.32**	18.14**	22.875**
(mg dL^{-1})	±0.413	±0.809	±0.695	±0.794	±0.956

Values are expressed as mean±SEM, ** $p<0.001$ as compared to control

Table 4: Inter group comparison of serum pH, calcium, phosphate, fluoride and lactic acid

	Groups			
Parameters	I (n = 20)	II (n = 20)	III (n = 20)	IV (n = 20)
pH	7.7407	7.417	7.419	7.418
	±0.006	±0.005	±0.004	±0.005
Calcium	9.72	9.1**	8.6**†	7.955**††⊕⊕
(mg dL^{-1})	±0.128	±0.127	±0.139	±0.115
Phosphate	4.03	3.59**	3.005**††	2.295**††⊕⊕
(mg dL^{-1})	±0.09	±0.047	±0.032	±0.059
Fluoride	2.295	1.615	0.76**	0.58**⊕
(µg dL^{-1})	±0.317	±0.713	±0.044	±0.069
Lactic acid	11.765	15.32**	18.14**†	22.875**††⊕⊕
(mg dL^{-1})	±0.809	±0.69	±0.794	±0.956

Values are expressed as mean±SEM, * $p<0.05$, ** $p<0.001$ as compared Group I vs all groups, † $p<0.005$, †† $p<0.001$ as compared Group II vs III and IV, ⊕$p<0.02$, ⊕⊕ $p<0.001$ as compared Group III vs IV

DISCUSSION

The role of 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 (1944), 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 the caries free people. Another study carried out by Ahelson and Mandel (Larsen *et al.*, 1999; Ahelson and Mandel, 1981) demonstrated that 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 caries susceptible subjects. However this study did not show any significant change in the blood pH with the progression of disease.

The study carried out by previous workers (Keith and Sophia, 1998) revealed that 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 intra cellularly. 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 "reservoir" of calcium, phosphate and fluoride in dental plaque and also maintains their saturation. These observations are quite identical with our 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 fall in serum calcium, phosphate and fluoride as the disease process advances. This observation is in complete agreement with the study carried out by Pearce (1998) 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 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 study carried out by Murray *et al.* (1991) on fluoride in caries prevention observed that the crystals formed in the presence of fluoride dissolved more slowly in acid as they have lower intrinsic rate of dissolution, particularly of F are taken up during remineralization and the crystals formed in the presence of F are large, dense and more perfect (Rosin-Garget and Lincir, 2001). Another observation made in this study was that, the rate of remineralization was raised in the presence of F in early carious lesion at those time when the pH has risen so that remineralization is the dominant process and he also demonstrated the antibacterial property of F 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 (Mg^{++}) which can be inhibited up to 100% by F with the level of 95 ppm in the solution.

It is concluded that calcium, phosphate and fluoride deposited in plaque greatly reduces the development of experimental caries in the adjacent enamel (Pearce *et al.*, 1983; Ten Cate, 1997) 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 previous workers (Murray *et al.*, 1991; Geddes, 1972) 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.

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