International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2013 Vol. 3 (1) January-April, pp.170-175/Javed B Mulla
Research Article

A STUDY IN BETWEEN DENTAL CAVITYAND DIABETES MELLITUS WITH REFERENCE TO MACRO- MINERALS ELEMENTS IN INDIAN ADULTS

***Javed B. Mulla**
[*]*Department of Medical Biochemistry, Bharati Vidyapeeth University, Sangli*
**Author for Correspondence*

ABSTRACT
This study was carried out to evaluate the possible protective role of macro-minerals elements like adequate level of calcium, phosphate and fluoride & pH in dental cavity of diabetes Mellitus. Total of 100 subjects of either sex, aged 20-60 were selected. Decayed, missed and filled teeth (DMFT) were used as indices for scoring the dental cavity and were distributed into 4 groups on the basis of DMFT indices as 30-40 (Group I), 41-50 (Group II), 51-60 (Group III) and more than 60 (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 cavity 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: *Dental Cavity, Serum Calcium, Phosphate, Fluoride, Lactic Acid*

INTRODUCTION
Dental caries, also known as tooth decay or a cavity, is an infection, bacterial in origin, that causes demineralization and destruction of the hard tissues (enamel, dentin and cementum), usually by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface. (Thaweboon, 2008) If demineralization exceeds saliva and other remineralization factors such as from calcium and fluoridated toothpastes, these hard tissues progressively break down, producing dental caries (cavities, holes in the teeth**)** (Hicks, 2003). The bacteria most responsible for dental cavities are the mutans streptococci, most prominently Streptococcus mutans and Streptococcus sobrinus, and lactobacilli. If left untreated, the disease can lead to pain, tooth loss and infection. Today, caries remain one of the most common diseases throughout the world. The systemic effects of periodontal diseases have been of increasing interest during the past two decades. Periodontitis is an inflammatory response to a bacterial challenge and represents a portal of entry for periodontal pathogens, bacterial endotoxins and proinflammatory cytokines (Dahlen *et al.,* 2010). Thus, the local oral inflammatory disease, periodontitis, may induce and perpetuate a systemic inflammation that may aggravate systemic diseases such as cardiovascular disease, pulmonary disease, rheumatoid arthritis and diabetes mellitus (Lalla**,** 2011**).**
Heterogeneous epidemiological data about the prevalence of periodontal diseases are available in the dental literature. Due to the advanced age of the subjects examined in the present study, the following epidemiological data predominantly focus on the prevalence of chronic periodontitis in adults and older subjects (Ximenez-Fyvie *et al.,* 2000).
Cariology is the study of dental caries. Dental cavity is a multifactorial disease, which has affected people throughout the ages. Many constituent of serum and saliva, both organic and inorganic have potentially protective role. These include calcium, phosphate, fluoride ions and bicarbonate buffer systems (Shahrabi, 2008). Epidemiological studies have supported the view that raised level of calcium, phosphate, and Fluoride in plaque might inhibit dental caries (Hicks, 2000). 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. In theory, continuous saturation of plaque fluid with mineral ions should completely overcome the harmful effect of plaque pH depressions and thus should be more effective than fluoride therapy (Rosin-Garget, 2001). 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 .The present study was done to estimate serum calcium, phosphate, and fluoride in the patients of dental caries in diabetes and to see and compare their level with the severity of disease and control (Jawed, 2011).

MATERIALS AND METHODS

A total of 100 subjects of either sex aged >30 years were selected from the department of Medical Biochemistry, Dental college, sangli. 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. Decayed, missed and filled teeth (DMFT) were used as index for scoring the dental caries. All subjects were distributed into 5 groups (Table-1) each having twenty individuals. Like group 1 with DMFT index 30-40, group 2 with DMFT index 41-50, group 3 with DMFT index 51-60 and group 4 with DMFT index more than 60, while the control subjects have the DMFT index equal or less than 3.

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 novel 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 hours for the estimation of lactic acid.

The blood in the syringe was covered, labeled 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^{o}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 colorimetric method using kit, cat # KC 120 supplied by Clonital Italy. Serum fluoride was also measured by colorimetric method using alazerine and zirconium dye. The fluoride was analyzed by the Magregian, Haier method cited by Farber 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 serum calcium, phosphate and fluoride among the control and diseased groups.

RESULTS AND DISCUSSION

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

Group	DMFT index	Distribution of subjects	Sex	
			Male	Female
Control	<30	20	13	7
Group - I	30-40	20	11	9
Group – II	41-50	20	11	9
Group – III	51-60	20	10	10
Group – IV	> 60	20	10	10

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

Parameters	Control (n=20)	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group 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)	+0.154	+0.128	+0.127	+0.139	+0.115
Phosphate (mg/dl)	4.22	4.03	3.59**	3.005**	2.295**
	+0.117	+0.099	+0.047	+0.032	+0.059
Fluoride	4.4	2.295**	1.615**	0.76**	0.58
(mg/dl)	+0.393	+0.317	+0.713	+0.044	+0.069
Lactic acid (mg/dl)	7.45	11.765**	15.32**	18.14**	22.875**
	+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 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 4: Inter group comparison of serum pH, calcium, phosphate, fluoride and lactic acid.

Parameters	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group IV (n=20)
PH	7.7407 +0.006	7.417 +0.005	7.419 +0.004	7.418 +0.005
Calcium (mg/dl)	9.72 +0.128	9.1** +0.127	8.6**† +0.139	7.955**††ÅÅ +0.115
Phosphate (mg/dl)	4.03 +0.09	3.59** +0.047	3.005**††† +0.032	2.295**††ÅÅ +0.059
Fluoride (mg/dl)	2.295 +0.317	1.615 +0.713	0.76** +0.044	0.58**Å +0.069
Lactic acid (mg/dl)	11.765 +0.809	15.32** +0.69	18.14**† +0.794	22.875**††ÅÅ +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. Å $P < 0.02$, ÅÅ $P < 0.001$ as compared group III vs. IV.*

Both dental cavity (periodontitis) and diabetes mellitus are frequent chronic diseases and generate enormous costs for the public health care system. Numerous studies, review articles and meta-analyses indicated a mutual influence between periodontitis and diabetes mellitus (Gurav, 2011). The mechanisms, whereby diabetes may negatively influence periodontal health, are primarily based on the impaired local immune defense and a reduced renewal of the periodontal tissues (Chávarry, 2009).

Moreover, higher levels of advanced glycation end products (AGE) can be found in the dental cavity of diabetics compared to non -diabetic subjects. The interaction between AGEs and collagen generates highly stable collagen macromolecules, that are resistant to physiologic enzymatic degradation.Hence, the renewal of all periodontal tissues is effectively compromised in diabetic subjects, especially when glycemic control is poor (Teeuw, 2010). These phenomena explain in part why diabetic patients are three times more likely to develop periodontitis than non-diabetic subjects.

The role of serum calcium, phosphate and fluoride and pH 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 regarding the estimation of salivary pH had showed that the pH of saliva remained below the critical level of 5.5 in dental caries of diabetic patients, than the caries free people. Another study carried out by Abelson and Mandel 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 (Rockenbach, 2006). 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 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 intracellularly. 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 our study as levels of serum calcium; phosphate and fluoride are significantly low in dental caries patient in comparison to the control (Larsen, 1999).

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 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 (Sinor, 2009) The study carried out by Murray 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 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. (Flores, 2007)

It is concluded that calcium, phosphate and fluoride deposited in plaque greatly reduces the development of experimental caries in the adjacent enamel because it tends to maintain the saturation of plaque fluid with respect to enamel mineral at low pH. (Hintao, 2007) 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 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 carcinogenicity of plaque. Rather, the degree of under saturation of plaque fluid with respect to enamel mineral is the principal factor to be considered.

ACKNOWLEDGEMENT

Authors are grateful to Dental College and Hospital, Sangli for providing facility to conduct this study.

REFERENCES

Chávarry NG, Vettore MV and Sansone C (2009). The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis. *Oral Health and Preventive Dentistry* **7** 107-127.

Dahlen G (2010). A microbiological study in relation to the presence of caries and calculus. *Acta Odontol Scand* **68** 199-206.

Flores MT and Andersson L (2007). The International Association of Dental Traumatology Guidelines for the management of traumatic dental injuries. I. Fractures and luxations of permanent teeth. *Dental Traumatology* **23**(2) 66-71.

Gurav A and Jadhav V (2011). Periodontitis and risk of diabetes mellitus. *Journal of Diabetes* **3** 21-28.

Hicks J and Garcia-Godoy F(2003). Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1). *Journal of Clinical Pediatric Dentistry* **28**(1) 47-52.

Hicks MJ and Flaitz CM (2000). Enamel caries formation and lesion progression with a fluoride dentifrice and a calcium-phosphate containing fluoride dentifrice: a polarized light microscopic study. *Journal of Dentistry for Children* **67**(1) 21-28.
Hintao J and Teanpaisan R (2007). The microbiological profiles of saliva, supragingival and subgingival plaque & dental caries in adults with and without type diabetes mellitus. *Oral Microbiology and Immunology* **22** 175-181.
Jawed M and Shahid SM (2011). Dental caries in diabetes mellitus: role of salivary flow rate and minerals. *Journal of Diabetes and Its Complications* **25**(3)183-186.
Lalla E and Papapanou PN (2011). Diabetes mellitus and periodontitis: A tale of two common interrelated diseases. *Nature Reviews Endocrinology* **7**(12) 738-748.
Larsen MJ and Jensen AF (1999). Individual variations of pH, buffer capacity and concentration of calcium and phosphate in unstimulated whole saliva. *Archives of Oral Biology* **44**(2) 111-117.
Rockenbach MI and Marinho SA (2006). Salivary flow rate, pH, and concentrations of calcium, phosphate, and sIgA in Brazilian pregnant and non-pregnant women. *Head and Face Medicine* **2** 44.
Rosin-Garget K and Lincir I (2001). Current concept on anticaries fluoride mechanism of action. *Collegium Antropologicum* **25**(2) 703-712.
Shahrabi M, Nikfarjam J and Alikhani A (2008). A comparison of salivary calcium, phosphate, and alkaline phosphatase in children with severe, moderate caries and caries free in Tehran's kindergartens. *Journal of Indian Society of Pedodontics and Preventive Dentistry* **26** 74-77.
Sinor Z, Yusoff A, Ismail AR and Rahman NA (2009). Salivary parameters and its effect on the occurrence of dental caries. *Internal Medicine Journal* **16** 47-52.
Teeuw WJ, Gerdes VE and Loos BG (2010). Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and metaanalysis. *Diabetes Care* **33** 421-427.
Thaweboon S and Thaweboon B (2008). Salivary secretary IgA, pH, flow rates, mutans streptococci and *Candida* in children with rampant caries. *Southeast Asian Journal of Tropical Medicine and Public Health* **39** 893-899.
Ximenez-Fyvie LA and Haffajee AD (2000). Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. *Journal of Clinical Periodontology* **27** 722-732.