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Imbalance of some Salivary Components in children with Dental Caries and Fluorosis.

[1]Dr. Mujtaba Ali S, Senior Resident, Government Dental College and Hospital, Hyderabad, Telangana

[2]Dr. E R Reddy, Professor, Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, Telangana

[3]Dr. Khan Z A, Assistant Professor, Buddha Institute of Dental Sciences and Hospital, Patna, Bihar

[4]Dr. M Kiranmayi, Professor, Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, Telangana

Corresponding Author: Dr. Mujtaba Ali S, Senior Resident, Government Dental College and Hospital, Hyderabad, Telangana

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Abstract

Background: This study compares the Salivary Calcium, Phosphorus and Alkaline Phosphatase levels in children with Dental Caries and Fluorosis.

Study design: 80 children both males and females aged between 12 to 18 years, from the Nalgonda region were included in the study. Children were divided into 4 groups: Caries group, Fluorosis group, Fluorosis affected by Caries group and Control group. The Salivary Calcium, Phosphorus and Alkaline Phosphatase levels in the sample were assessed using Auto-analyzer.

Results: The mean Salivary Calcium and Phosphorus levels were lower in caries group ($p>0.05$) whereas Alkaline Phosphatase levels was higher in the caries group when compared to control group which was statistically significant ($p<0.05$). The mean Salivary Calcium ($p>0.05$), Phosphorus and Alkaline Phosphatase levels were lower in fluorosis group when compared to control group which was statistically significant ($p<0.05$). The mean Salivary Calcium ($p<0.05$) and Phosphorus ($p>0.05$) levels were lower in Fluorosis affected by Caries group and Alkaline Phosphatase ($p>0.05$) levels were higher when compared to control group.

Conclusion: There is strong relation between the levels of salivary calcium and alkaline phosphatase with the disease process of dental caries and fluorosis.

Keywords: Calcium, Phosphorus, Alkaline Phosphatase, Fluorosis, Caries.

Introduction

Saliva has many functions in protecting the integrity of the oral mucosa: it participates in the clearing of the oral cavity of food residues, debris and bacteria; it buffers the deleterious effects of strong acids and bases and provides the ions needed to remineralize the teeth like calcium ,phosphate[1]. The delicate balance between

demineralization and remineralization, to which dental hard tissues are subjected to, dwells on the Salivary Calcium, Phosphate and Salivary Alkaline Phosphatase (ALP) levels[2]. Saliva should be saturated to affect the bioavailability of the calcium and phosphate in amounts adequate for remineralization[3].

The concentration of inorganic calcium (Ca) and phosphorus (P) in saliva shows considerable variation depending on a number of factors. Previous studies, such as the one reported by Karshan et al[3], stated that the calcium and phosphorus content of saliva is low in caries-active persons. But, most investigators have been unable to confirm this finding. The levels and state of calcium and phosphate in saliva may be related to the susceptibility to dental caries.

Saliva is also rich in enzymes. Some of these enzymes, such as alkaline phosphatase, may play an important part in the dental caries process. The role of alkaline phosphatase in the mineralization process has been established unequivocally.

In many of the previous studies the salivary levels of Ca, P and ALP has been determined in patients with caries. But in a study by Gavriliuc et al[4] the author determined these salivary components in patients with fluorosis. The level of ALP is more sensitive and easier to measure than the actual concentration of Fluoride in saliva. Because of this it is considered reasonable to use ALP as an indicator for early diagnosis of fluorosis[5]. Hence, the present study was envisaged to study the possible relationship of salivary calcium, phosphorous and alkaline phosphatase activity with the incidence of dental caries and dental fluorosis in children.

Materials and Methods

The present study was conducted in the department of Pediatric and Preventive Dentistry, Kamineni Institute of Dental Sciences. Children and Young Adolescents of an age group of 12 to 18 years were included in the present study through a random selection method. Both sexes were included, precaution was taken to exclude cases having acute oral afflictions, systemic diseases (liver disorders, renal and thyroid disorders), chronic debilitating diseases, metabolic bone disorders (e.g., Osteitis deformans) to avoid interference in salivary calcium, phosphorus and alkaline phosphatase activity estimation. Special Children (Mental Retardation) and Children with Physical Disabilities were also excluded.

All the children were examined adequately using a mouth mirror and an explorer. The ICDAS II index was calculated for each patient and fluorosis scores were given according to Tooth Surface Index of Fluorosis (TSIF).

The study comprised of 80 children, divided into the following groups:

Group I: Consisted of 20 children with no caries and fluorosis (Control group)

Group II: Consisted of 20 children with Caries (ICDAS II Criteria 2-4)

Group III: Consisted of 20 children with dental fluorosis (Tooth Surface Index of Fluorosis score 2-4)

Group IV: Consisted of 20 children with fluorosis affected by caries.

The samples of saliva were collected one week after performing oral prophylaxis. The patients were asked to perform mouth rinsing after breakfast (1 h 30 min before saliva collection)[6, 7] and during this period children were not permitted to eat or drink. Unstimulated, directly expectorated, whole saliva samples (5ml) were collected by asking children to spit in the tubes once a minute for 10 min in clean, dry, sterilized plastic containers on dental chairs and under resting condition as described by Scully[8]. All the samples were collected between 10.30 and 11.30 AM. After saliva collection, the container's

caps were closed and stored in an ice box and within 5 min transported to immunochemistry laboratory for biochemical analysis.

Saliva samples were centrifuged under centrifugal force: 1000 g [6] to remove bacteria and other extraneous material. The supernatant was used for immunochemical assays for estimation of calcium, phosphorus and alkaline phosphatase levels using an Auto-analyzer.

- The Salivary Calcium levels were assessed using reagent which contains Arsenazo III (Lifechem LTD, Hyderabad, India).
- The Salivary Phosphorus levels were assessed using reagent which contains Sulfuric acid and Ammonium Molybdate (Lifechem LTD, Hyderabad, India). Inorganic phosphorus reacts with ammonium molybdate in presence of sulfuric acid to form reduced phosphomolybdate complex, which is measured as an end point reaction at 340 nm.
- The Salivary Alkaline Phosphatase levels were assessed using Alkaline Phosphatase kit (Lifechem LTD, Hyderabad, India). Alkaline phosphatase was measured by kinetic method. Alkaline phosphatase effects on 4-nitrophenyl phosphate and converts it to yellow 4-nitrophenol which could absorb the light. The absorption of light is proportional with alkaline phosphatase enzyme activity.

The data thus obtained was statistically analyzed by SPSS-20 software. One Way ANOVA test was used where applicable; $P < 0.05$ was considered as statistically significant.

Results

The mean salivary calcium content in Group I (4.07 mg %) was found to be slightly higher when compared to the Group II (3.55 mg %), which is statistically not significant ($P > 0.05$) and also with Group III (3.14 mg %) as well as the Group IV (3.03 mg%) which are statistically significant ($P < 0.05$).

The mean phosphorus content in the Group I (14.56 mg %) is found to be only slightly higher as compared with Group II (13.42 mg %), Group III (14.10 mg %) and Group IV (13.94 mg %) which are statistically not significant ($P > 0.05$)

This enzyme activity for the Group II is much higher (27.20 K.A.) than that for the Group I (17.85 K.A), which is statistically significant ($P < 0.05$). The enzyme activity for the Group III is much lower (7.20 K.A.) than that for the Group I (17.85 K.A), which is statistically significant ($P < 0.05$). The enzyme activity for Group IV is slightly higher (17.90 K.A.) than that for the Group I (17.85 K.A), which is statistically not significant ($P > 0.05$).

Table 1: Comparison of Salivary Calcium, Phosphorus and Alkaline Phosphatase levels between group I and group II.

Variables	Group I (Control Group)	Group II (Caries Group)	P value
Calcium	4.07 mg%	3.55 mg%,	P > 0.05
Phosphorus	14.56 mg%	13.42 mg%	P > 0.05
ALP	17.85 K.A	27.20 K.A	P < 0.05

Table 2: Comparison of Salivary Calcium, Phosphorus and Alkaline Phosphatase levels between group I and group III.

Variables	Group I (Control Group)	Group III (Fluorosis Group)	P value
Calcium	4.07 mg%	3.14 mg%,	P < 0.05
Phosphorus	14.56 mg%	14.10 mg%	P > 0.05
ALP	17.85 K.A	7.20 K.A	P < 0.05

Table 3: Comparison of Salivary Calcium, Phosphorus and Alkaline Phosphatase levels between group I and group IV.

Variables	Group I (Control Group)	Group IV (Caries with Fluorosis Group)	P value
Calcium	4.07 mg%	3.03 mg%,	P < 0.05
Phosphorus	14.56 mg%	13.94 mg%	P > 0.05
ALP	17.85 K.A	17.90 K.A	P > 0.05

Discussion

Saliva has protecting effects on oral tissues. It acts as a lubricant which facilitates oral function including talking, chewing and so on. Recent studies showed that saliva has different effects on dental caries regarding to its organic components, such as alkaline phosphatase, and its inorganic components, such as calcium, phosphate and other ions [9, 10].

Skeletal and Dental Fluorosis, caused by long-term intake of high levels of systemic fluoride, is characterized by clinical manifestations in bones and teeth [11, 12]. Though young teeth affected with dental fluorosis are resistant to dental caries, fluorosis at moderate and severe levels is associated with a higher prevalence of dental caries[13, 14]. This is due to brittle enamel and chipping, fracture of enamel in severe cases exposing dentin. Fluorosis is a serious public health problem in developing nations of the world, where drinking water contains more than 1 ppm of fluoride[15].

Alkaline phosphatase, an enzyme present in saliva, is active at pH 9-10 and is important for the process of remineralization. A variation in the level of alkaline phosphatase affects the ionic concentration of phosphate and calcium, which in turn can alter the equilibrium of demineralization and remineralization of enamel. The interplay of the various components of saliva and their protective role against dental caries has been an area of research in the recent years (last few decades)[16]. Examination of alkaline phosphatase levels in adolescents with fluorosis is significant, because the activity of this enzyme may be changed before the clinical manifestations of the disease and resultant decrease of inorganic phosphate concentration in saliva [4].

In the present study the calcium concentration of control group was slightly higher than the caries group but there was no significant difference. This was in accordance with the study done by Karshan et al[3] and various other studies [17, 18]. Contradictory results were seen in the studies conducted by Turtola et al[19], Elizarova and Petrovich et al[20] in which calcium concentration was more in caries group. The calcium concentration of control group in the present study was significantly higher compared to fluorosis group and caries with fluorosis group. This was in accordance with the study done by Gavriliuc et al[4].

The mean phosphorus content in the Group I was found to be only slightly higher as compared with Group II which was statistically not significant. This was in accordance with the studies conducted by Shahrabi et al[21] and others [22, 23]. But the studies conducted by Karshan et al[3] and others[17,24] has shown decreased mean phosphorus content in caries free group when compared to caries group. Group III as well as the Group IV has also shown decreased phosphorus content compared to Group I which was statistically not significant.

The importance of calcium and phosphate precipitation on the tooth surfaces in remineralization process has been already known[25, 26], but the concentration of these ions in saliva could not be a predictor of caries susceptibility. Organic molecules, such as statherin and proline-rich protein have the ability to inhibit

precipitation of calcium and phosphate on teeth and although saliva is supersaturated with respect to calcium and phosphate ions, spontaneous precipitation from saliva to tooth enamel does not normally occur[26].

The ALP enzyme activity for the Group II was much higher than that for the Group I which was statistically significant. This was in accordance with the studies done by various authors[2,18,27]. This could be due to maintenance of equilibrium between demineralization and remineralization depending on the ionic concentration of calcium and phosphate in saliva, which in turn is influenced by levels of alkaline phosphatase. Variation in ALP activity causes changes in phosphate levels which lead to initiation and progression of caries lesion[2]. Mandel et al in his study stated that there is no significant difference in ALP activity in caries free and caries group[28]. Decreased ALP content was seen in caries group compared to caries free group in a study conducted by L Shaw et al[17]. The enzyme activity for the Group III in the present study was much lower than that for the Group I which was statistically significant. This could be due to inhibitory effect of Fluorine ions on Alkaline Phosphatase enzyme[29]. Our results were in accordance with the study conducted by Gavriliuc et al[4]. The enzyme activity for the Group IV was slightly higher than that for the Group I, which was statistically not significant.

It would be difficult to establish an association between the salivary components and their role in Fluorosis and Dental Caries because of the different sampling techniques, age groups of children and different laboratory tests between the studies.[30]

Conclusion

Salivary calcium levels were significantly lower in Fluorosis group and also in Caries with fluorosis group. There was no significant difference in salivary phosphorus levels in all the groups. Alkaline phosphatase levels were higher in caries group and lower in fluorosis group which was statistically significant.

The results of this study indicate that there is strong relation between the levels of salivary calcium and alkaline phosphatase with the disease process of dental caries and fluorosis. Further studies with a larger sample are needed to establish a proper relationship.

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