Research article

Corelation of salivary calcium and vitamin D with dental caries - an *ex-vivo* study

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ABSTRACT

Introduction and Aim: Calcium and vitamin D3 play a pivotal role in many biological functions like hard tissue mineralization and tooth formation. Both are dietary elements with a known concentration in the saliva, therefore directly affecting the rate and progression of dental caries. The aim of the study was to compare the salivary vitamin D and calcium levels in caries active and caries free adult patients.

Materials and Methods: Tarson's saliva collection tubes were used to collect 5 ml of saliva samples from 110 patients. The supernatant obtained from the centrifuged saliva was stored at -4˚C. Analysis of calcium levels was done using the Arsenazo method. Commercially available vitamin D Elisa kit (Epitope diagnostics) was used for the analysis of vitamin D levels.

Results: The mean salivary vitamin D levels were higher in the caries free group (66.57mg/dl) when compared to the caries active group (56.43mg/dl). The mean salivary calcium level was elevated in the caries free group (27.09mg/dl) when compared to the caries active group (6.84 mg/dl).

Conclusion: The filtrates in saliva play a major role in the occurrence of dental caries through a demineralization and remineralization cycle. Vitamin D in saliva causes a decrease in bacterial aggregation and biofilm formation through the protective role of peptides, causing decreased demineralization of the tooth surface and increased level of free calcium ions in the saliva.

Keywords: Dental caries; saliva; calcium; vitamin D; oral health; demineralization.

INTRODUCTION

Dental caries is a microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth (1). In the caries development and progression cycle, calcium, phosphorus, and other inorganic ions present in the saliva influence the balance between demineralization and remineralization. Thus, calcium and phosphorus present in saliva form a barrier against dissolution of teeth (2).

Saliva is a perfect medium to be explored for caries surveillance as it represents all the ions usually present in body fluids. It serves as a true indicator of caries activity and the factors responsible for it. Saliva is a complex combination of water and organic and inorganic components and contains serum filtrates from blood (3). It plays a major role in delivering active metabolites directly to the tooth surface which influence the initiation and progression of dental caries. Therefore, it is important to study the various associations of these filtrates derived from blood in saliva to understand the multi factorial etiology of dental caries.

Vitamin D is an important element in our diets and has a role in calcium and phosphate homeostasis. It acts by regulating intestinal absorption, renal excretion and mobilization of calcium and phosphate ions from bone. Vitamin D and calcium play a crucial role in bone and tooth mineralization, and when levels are unregulated it can lead to the "rachitic tooth", which is a defective and hypo-mineralized organ highly susceptible to fracture and decay (4). Vitamin D deficiency is highly prevalent in South-East Asian countries, with India showing a prevalence of 67% (5). Several authors have studied the correlation of vitamin D and calcium levels to dental caries in the pediatric population (6,7). However, there is a scarcity of literature studying the effect of salivary vitamin D and calcium levels and its effect on dental caries in adults. Therefore, the objective of this present study was to compare the salivary vitamin D and calcium levels in caries active and caries free adult patients.

MATERIALS AND METHODS

An institutional ethical clearance was obtained. Subjects in the age group of 18-55 years reporting to the Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, India were screened for the selection (caries active and caries free groups) based on Decayed, Missing, and Filled Teeth (DMFT) scores. Smokers, patients with missing teeth, pregnant

and lactating women, patients with a known history of systemic diseases and patients on medications or supplements affecting salivary secretions and calcium levels were excluded from this study.

Collection of salivary sample

The study was explained to the subjects and an informed consent was obtained. Subjects were asked to refrain from any oral hygiene interventions like brushing, use of mouthwash and sugar free chewing gums. Patients were advised to not eat or drink for 2 hours prior to the sample collection. Patients were made to sit in an upright position and 5ml un-stimulated saliva samples were collected in the Tarson's saliva collection tube as per the Navazesh Protocol (8). Saliva collected from the patient was centrifuged, and the supernatant obtained was stored at -4°C.

Estimation of inorganic salivary calcium and vitamin D

The salivary samples were analyzed further for calcium levels by ARSENAZO method. Calcium ions (Ca^{2+}) react with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenear-sonic acid) to form an intense purple colored complex. Absorbance of the Ca-Arsenazo III complex was measured bio-chromatically at 660/700 nm. Commercially available vitamin D ELISA kit (Epitope diagnostics) was used to analyze the salivary vitamin D levels.

Statistical analysis

Vitamin D and calcium levels between caries active and caries free groups were compared using the Mann Whitney U test. The levels of salivary calcium and vitamin D levels in the caries active group were analyzed using the Chi square test. The value for statistical significance was set to $p<0.05$.

RESULTS

The salivary vitamin D and calcium levels were studied in study groups. The mean salivary vitamin D level was higher in the caries free group (66.57mg/dl) when compared to the caries active group (56.43mg/dl) and the difference was statistically significant (P< 0.02) (Table 1). The mean salivary calcium level was higher in the caries free group (27.09mg/dl) when compared to the caries active group (6.84mg/dl) and significant (Table 2).

Table 1: Comparison of vitamin D levels between study groups

Group	Mean (SD)	Range	Median (Q1-Q3)	Mann-Whitney U test	
				U Statistic	p-value
Caries Active (n=80)	56.43 (22.06)	0.25-113.82	58.25 (43.96-75.1)	669.00	0.02*
Caries Free (n=30)	66.57 (17.30)	10.15-88.40	69.48 (64.40-76.8)		

Table 2: Comparison of calcium levels between caries active and caries free group

Group	Mean (SD)	Range	Median (Q1-Q3)	Mann-Whitney U Test	
				U Statistic	p- value
Caries Active (n=80)	6.84 (2.75)	3.09-15.47	5.83 (5.01-8.10)	443.50	<0.001*
Caries Free (n=30)	27.09 (114.61)	3.03-589.0	4.62 (3.78-5.46)		

Table 3: Comparison of vitamin D levels with the severity of caries

Caries Active Group	N	Mean (SD)	Range	Median (Q1- Q3)	Kruskal Wallis Test	
					Chi-square value	p-value
Group 1(1 -3)	30	59.90 (20.30)	16.46- 113.82	59.14 (43.45-75.28)	5.68	0.13(NS)
Group 2(4- 10)	30	54.79 (23.02)	0.25-86.37	52.67 (40.90-75.74)		
Group 3(>10)	20	53.12 (24.29)	1.11- 75.06	62.20 (51.78-66.51)		
Group 4 (No caries)	30	66.57 (17.30)	10.15- 88.40	69.48 (64.40-76.85)		

Table 4: Comparison of calcium levels with severity of caries

Caries Active Group	N	Mean (SD)	Range	Median (Q1- Q3)	Kruskal Wallis Test	
					Chi-square value	p-value
Group 1(1 -3)	26	7.16 (2.69)	3.94- 14.56	6.51 (5.29- 8.23)	18.05	<0.001*
Group 2(4- 10)	41	6.51 (2.49)	4.12- 14.10	5.48 (4.87- 7.63)		
Group 3(>10)	7	7.59 (4.35)	3.09- 15.47	5.35 (4.67- 10.72)		
Group 4: No caries	26	27.09 (114.61)	3.03- 589.0	4.62 (3.78- 5.46)		

Furthermore, the salivary vitamin D and calcium levels were compared based on the caries activity. The mean vitamin D values were seen to be more (59.90 mg/dl) in the group 1, comprising 1-3 carious teeth only (Table 3). Similarly, the salivary calcium levels were seen to be higher (7.59mg/dl) in group 3, comprising less than 10 carious teeth (Table 4). As the severity of caries increased the vitamin D levels decreased.

DISCUSSION

It is proven that the cause for dental caries is multifactorial. Apart from oral hygiene practices and diet, the general nutritional status of an individual can reflect on his oral health. Micro-nutrients like vitamins and minerals are although required by the body in very limited amounts; their deficiencies can be detrimental to health. These biologically active molecules play a major role in tooth structure formation and maintenance of oral health (4). The levels of serum micronutrients in blood and their influences on dental caries have been studied extensively in the pediatric age group (6,7). Therefore, the present study was designed to compare salivary calcium and vitamin D levels in adult patients having dental caries and control groups.

Saliva plays a major role in delivering active metabolites directly to the tooth surface which influence the initiation and progression of dental caries. Therefore, it is important to study the various associations of these filtrates derived from blood in saliva to understand the multifactorial etiology of dental caries.

The current study revealed an increase in vitamin D and calcium levels in the caries free group, in comparison to the caries active group. Therefore, a direct relationship between salivary calcium and vitamin D levels can be established.

Vitamin D regulates calcium absorption during tooth formation and is essential for the development and maintenance of good oral health (9). It provides a cariostatic effect by modulating the antimicrobial peptides like cathecdellins through helper T cell pathways (10). Therefore, the decrease in demineralization on the tooth surface can be attributed to the antimicrobial role of vitamin D, thereby decreasing bacterial colonization.

The groups with high caries activity showed an increase in salivary calcium and vitamin D levels. This is in accordance with the other studies conducted in the adult population (11,12). The remineralization potential of enamel is enhanced by the presence of calcium in saliva, which exerts a protective effect by decreasing the adherence of caries-associated bacteria to the enamel thereby providing a local protective effect. When the tooth surface is super saturated with ions, there is a decrease in the bonding of calcium ions to the tooth surface. Therefore, there is an increase in the levels of free moving calcium ions in the saliva. The role of vitamin D in the reduction of dental caries has been studied (12, 13). The antimicrobial peptides that are produced through vitamin D LL-37 pathway reduces the risk of dental caries due to its antimicrobial properties (14). Antimicrobial peptides are essential components of the innate immunity and act against several bacteria, fungi, and viruses (13). Thus, salivary vitamin D levels influence defense mechanisms present in the oral cavity and promote dental health.

CONCLUSION

The changing outlook on management of dental caries has led research to broaden its focus on essential components of saliva. Hence the filtrates in saliva are vital for maintaining the equilibrium of the remineralization and demineralization cycle. These filtrates that we analyzed will add value to the already existing data, thereby throwing a light on factors other than the components of the classical key's triad. The limitations of this study would be a restricted sample size and a closed age group. The prospects of this study would include further research on polymorphism of specific genes which are responsible for the activity of these essential filtrates in saliva.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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