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Age-Related Changes of the Human Salivary Secretory Total Protein Complex and Trace Elements in Children between the Age Group of 3-16 Years

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Abstract:
Human saliva plays a vital role in maintaining the integrity of oral tissues and its composition changes during childhood due to maturation of the salivary glands thus indicating the need of age-matched controls for the clinical use of saliva as a diagnostic tool for diseases. Thus this study aims at analyzing physiologic variability of naturally occurring total protein concentration and trace elements in unstimulated whole saliva of children as a function of age. For this study unstimulated whole saliva specimens were collected from 30 healthy male children equally divided into: Primary (3 - 5 years); Mixed (6 - 11); Permanent (12 - 15years).The samples were studied for estimation of trace elements by inductively coupled plasma emission spectrometer and total protein content by light chromatography coupled with mass spectrometry. The trace elements detected in this study were calcium, potassium, magnesium, silica with calcium, magnesium and silica showing linear increase with age from deciduous to mixed to permanent dentition with potassium concentration highest in the mixed dentition group. The total protein content showed a linear increase with age. Thus this study establishes a correlation between age and salivary composition. Hence constructing a comprehensive catalogue which is physiologic for salivary electrolytes and proteins with newer biochemical aids is necessary for saliva to serve as a diagnostic aid.
Keywords: *age, saliva, trace elements, total proteins.*

Background:

Father Ricardo Rezend once said 'What we see is the tip of an iceberg'. But the actual size of the iceberg is not what we see above the water level but is the large unexplored mass which is present below the water level. Historically the demise of the Titanic was because it could not gauge the power and the strength of an iceberg.

The idea behind this concept of an icerberg is that saliva is like and iceberg with the role of saliva in maintainence of oral health being only the tip of the iceberg. The role of saliva is much broader and it can also serve as a diagnostic tool for monitoring health and disease status of an individual[1]. This aspect of saliva is a late bloomer and is now coming to the forefront. But for this approach to succeed we must understand the basic concept of salivary composition and the role of its constituents.

Mouth is a unique , highly complex multifactorial interface between the body and its external environment. It has greater structural and biologic complexity as compared to the other body orifices. It contains mineralized tissues (teeth) which are continuously exposed to environmental changes. Thus saliva , an oral biofluid is important in maintaining homeostasis in the oral cavity and its presence is vital to the maintainence of healthy oral tissues [2].

Saliva is composed of organic, inorganic contents and macromolecules. Salivary composition changes during childhood due to maturation of salivary glands. Thus for saliva to serve as a diagnostic aid there is a need for age-matched controls with physiologic levels of salivary electrolytes and proteins established for particular age groups[3].

There are only few studies on salivary composition of healthy children are available[4]. Thus this study aims at analyzing physiologic variability of naturally occurring total protein concentration and trace elements in unstimulated whole saliva of children as a function of age.

Material & methods:

Criteria for patient selection:

In the present study, 30 normal healthy male children ranging from 3 to 16 years were selected from housing societies in and around Pimpri- Chinchwad area of Pune district who were free from any systemic or local diseases which affect salivary secretions and totally caries free with dmft/DMFT score of 0 [5] in November 2010. After assessing and confirming their caries status these children were stratified equally into three dentition groups : Primary (10 children ,ranging from 3-5 years) , Mixed (10 children ,ranging from 6-11years) and Permanent(10 children ,ranging from 12-16 years). Exclusion criteria included patients who were physical or mentally compromised, having developmental delay, auditory or visual dysfunction, known neurological diseases, history of drug intake and patients with arrested carious lesions[6].Informed consent forms were obtained from the custodial parent or guardian of the subject after explaining the procedure to the parent or guardian.

Method of saliva collection:

To minimize the effect of circadian rhythms, all whole saliva samples were collected one hour after lunch for the unstimulated condition[4]. The child was seated in a well-ventilated and well-lit room. The head was kept at 45 degrees flexion with one hand holding onto a 4ml cryoprecipitation vial with a funnel inserted into it, in a calm atmosphere to simulate unstimulated conditions. The saliva was allowed to drip into the funnel held to the lower lip. For each trial, the collection continued for 2 minutes but if the saliva sample was insufficient within 2 minutes, the collection was continued until 2 ml of saliva per subject was obtained[6].

Methods of laboratory analysis:

For detection of trace elements in saliva, the saliva samples obtained from each subject were diluted with distilled water in a proportion of 1:4.This diluted saliva sample was then subjected to inductively coupled plasma emission spectroscopy. The basic aim of analytical atomic spectroscopy is to identify elements and quantify their concentrations in various media[7]. The machine used was Varian Vista Pro with detection limits of 1 ppm for each element.

For detection of total proteins, light chromatography coupled with mass spectrometry (LCMS) was used. Mass spectrometry (MS) is an analytical technique used for determining masses of particles, for determining the elemental composition of a sample or molecule and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds.

Results:

On subjecting the salivary samples to laboratory diagnosis the following results were obtained calcium, potassium, magnesium and silica were detected in salivary samples of all three age groups as their concentration levels were more than 1ppm.

Comparing between the primary, mixed and permanent dentition groups, calcium, magnesium and silica levels showed a linear increase in concentration from primary to mixed to permanent dentition age groups. Salivary potassium concentration showed a significant increase in the mixed dentition age group. The potassium concentration was lower in the permanent dentition age group than deciduous dentition age group. There was no statistically significant difference in the trace elements detected in

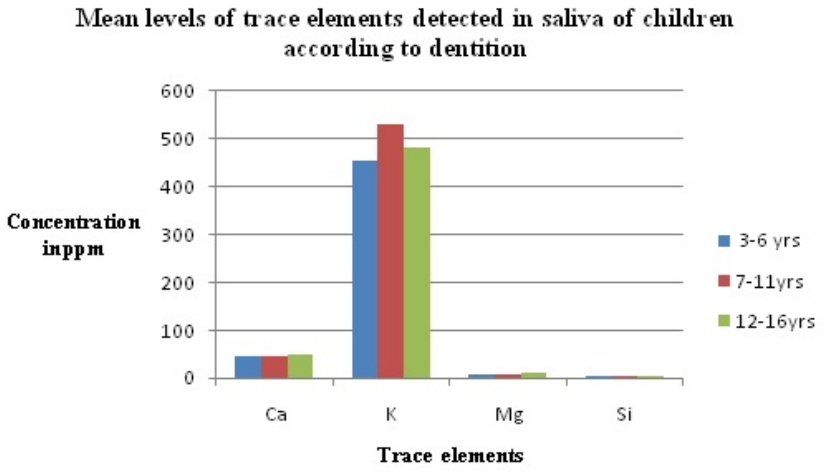

Figure 1: Potassium concentration is the highest among the trace elements detected with calcium, magnesium and silica showing a linear increase with age. Potassium concentration is highest in the 7-11 years group**.**

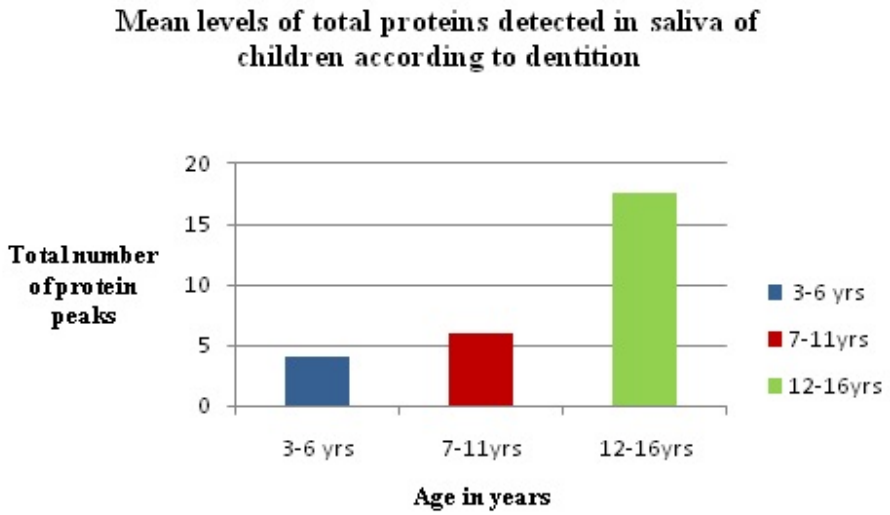

Figure 2: The total number of protein peaks observed is highest in 12-16 years group with linear increase in total protein peaks

unstimulated whole saliva of children in all three dentition age groups. (Figure 1)
For analyses of total proteins as per results obtained from LCMS ,the number of peaks of high molecular weight proteins were analysed as per lab specifications molecular weight more than 700 Kda were taken into consideration. The number of peaks observed in the unstimulated whole saliva of children in primary, mixed and permanent dentition did not show any statistically significant difference, though the average number of peaks in permanent dentition was higher than the primary and mixed dentition age groups (Figure 2).

Discussion:

The organic and inorganic contents of whole saliva were analysed in this study. A number of physiological factors influence the composition of whole

saliva. These are, the source of saliva, the method of collection and the degree of stimulation. Because it is difficult to use a collecting device with children unstimulated whole saliva was collected in this research. The time of saliva collection is also important. In this study saliva was collected during acrophase as salivary flow rate peaks during afternoon time [4].

The elements detected in this study were, calcium, potassium, magnesium and silica as their concentration in whole saliva of children of all three age groups was more than 1 ppm.

An overview of values showed a linear increase in calcium concentration from primary to mixed to permanent dentition age groups but did not show statistical significance. It has been proved that an inverse relationship exists between salivary calcium concentration and dental caries. The results of this study highlight one aspect of increased caries susceptibility in deciduous dentition.

Kavanagh and Svelha (1998) postulated that a key salivary parameter to consider in terms of remineralization is the extent of variations in calcium concentration .While phosphate levels in resting saliva do not vary markedly, large fluctuations in calcium concentrations occur in one individual. A lower calcium concentration results in a lower thermodynamic driving force for hydroxyapatite precipitation at normal oral pH, a higher driving force for hydroxyapatite dissolution at low pH, and a higher critical pH than normal value of 5-5.3[8]. Salivary calcium concentrations are lower in children than adults. The critical pH is significantly higher for children than for adults in saliva. Therefore, when compared to adults, children have a greater thermodynamic driving force for demineralization at low oral pH, and a lower force for remineralization at normal oral pH[9]. This is one contributor to the increased risk of demineralization in children. Thus the results of this study postulate that the remineralization potential of saliva increases from deciduous dentition to mixed to permanent dentition age groups.

The potassium concentrations in this study did not show a linear increase from one dentition to the next. But the potassium concentration in the mixed dentition group was recorded the highest. The reason for this still needs to be postulated. The studies show the salivary potassium levels do not have any relation to caries[4]. The salivary sodium - potassium concentration can be used for detecting functioning of aldosterone hormone in the body alongwith detection of kidney disorders [10].

In this study magnesium concentration rose linearly with age from deciduous to mixed to permanent dentition age groups. This finding does not match the findings of Ben Aryeh et al, in which they found that salivary magnesium concentration does not differ significantly between young and old [11]. There is a controversy that exists regarding the concentration of salivary magnesium and its relationship to caries. Few studies show that there was no relation between caries and magnesium[4]. However, others have found magnesium to decrease with increasing caries surfaces. Most surveys have found a negative correlation between magnesium in drinking water and caries prevalence and high magnesium to be usually present with high calcium. The literature reports very low levels of cariogenic streptococci in the presence of magnesium. Thus high magnesium levels have a caries preventive effect. Thus results of this study postulate that the higher the

magnesium levels in saliva lesser the colonization of streptococcus mutans and hence decreased caries risk[12]. Thus the caries risk decreases from deciduous to mixed to permanent dentition groups. To completely understand magnesium function, it is necessary to explore magnesium's relationship with calcium. Magnesium plays a major role in multiple body functions. Calcium and magnesium are known to be necessary for the normal function of various systems in animal and human organisms. There are many diseases caused by abnormal concentration of electrolytes. The mechanisms of homeostasis indicate only the ionized forms of these elements. Therefore, it is very important to define levels of total and ionized forms of calcium and magnesium in blood and saliva. According to Iryna N. Andrusishina , taking into account that the ratios of calcium and magnesium in serum and saliva are identical, determination of the ionized forms of these elements in saliva can be acceptable[13].

Silica concentration showed a linear increase with age from deciduous to mixed to permanent dentition. It has been proven that silica plays a major role in mineral absorption. Silica is essential in maintaining the calcium magnesium balance in mineralized structures. Silica also plays a major role in collagen formation. Thus maintenance of silica concentration in the body plays a important role in maintainence of vitality of hard and soft connective tissues. Thus this study postulates that the permanent dentition has improved property of maintainence of mineralization of hard tissues.

Total proteins in saliva may have both protective and detrimental properties[14] .Thus salivary proteins can be known as "double-edged swords". Function of total proteins may depend on molecule's location or site of action. Some proteins such as antimicrobial and pH modulating proteins play a protective role in the oral cavity, while adhesions and agglutinins play a detrimental role by increasing the colonization of micro-organisms. Thus quantitative and qualitative identification of salivary proteins is a necessary first step in identifying potential protein biomarkers of disease[15]. In this study the total protein concentration showed a linear increase with age, but qualitative analysis of these proteins is essential to prove its role in health and disease.

Conclusion:

From this study we can conclude that saliva has a great potential for clinical disease diagnostics. It has long been recognized that saliva serves as a mirror of body's health as it contains proteins, hormones, antibodies, electrolytes and other molecules that are frequently measured in standard blood tests to monitor health and disease[16]. Thus there is a necessity for constructing a comprehensive catalogue which is physiologic for salivary electrolytes and salivary total proteins alongwith qualitative analysis of individual amino acids, their linkages and formations with newer biochemical approaches. Thus this study lays a foothold and may serve as a reference value for growing interest in saliva as a diagnostic tool.

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References:

[1] David T. Wong, Journal of American Dental Association, 2006,137, 313-21

[2] Donald I Hay, William H Bowen.,in:The Functions of Salivary Proteins, S. (Eds.), Edgar's Saliva and oral health, British Dental Association, London 1996, p.p 105-119

[3] H. Ben-Aryeh, M. Fisher, R. Szargel and D. Laufer**,** Archives of Oral Biology, 1990, 35(11),929-931

[4] Betul Kargul, Aysen Yarat, Ilknur Tanboga, The Saudi Dental Journal,1998, 10(3) ,100-106

[5] A.R. Vieira, M.L. Marazita, T. Goldstein-McHenry, Journal of Dental Research, 2008, 87(5), 435-439

[6] Katie P. Wu, Chang Gung Med J, 2008, 31, 281-6

[7] Thomas J. Manning, The Chemical Educator, 1997,2(1),1-19

[8] Kavanagh DA, Svehla G, Archives Oral Biology. 1998,43(12),1023-7.

[9] Anderson P, Hector MP, Ramprasad MA, International Journal of Paediatric Dentistry, 2001, 11(4),266-73.

[10] David P. Lauler, Roger B. Hickle, and George W. Thorn, N Engl J Med 1962, 267, 1136-1137

[11] Aryeh Ben, Fisher M,Szargel R et al, Archives of Oral Biology, 1990, 35(11), 929 - 931

[12] Curzon MEJ, Cutress, TW,in: Trace elements and dental disease postgraduate dental handbook. London: John Wright, PSG Inc., 1983, pp.222 -223

[13] Iryna N. Andrusishina, J. Elementol. 2010, 15(3), 425–433

[14] Lawrence A. Tabak**,** Pediatric Dentistry, 2006 ,28(2),110-116

[15] Hongwei Xie, Nelson L. Rhodus, Robert J. Griffin, John V. Carlis, Timothy J. Griffin, Molecular & Cellular Proteomics, 4.11, 2005,1826-1830

[16] Preethi BP, Anand Pyati, Reshma Dodawad, Biomedical Research 2010, 21 (3), 289-294.