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Salivary Investigations - The Art of Balance

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ABSTRACT

As we enter the era of genomic medicine, sialochemistry will play an increasingly important role in the early detection, the monitoring and progression of the systemic and oral diseases and serve as a tearless diagnostic tool. For saliva to play a role as a diagnostic aid it is necessary to examine the consequences of preanalytical physiologic variations with respect to age .Thus the aim of this study was to detect the physiologic levels of different electrolytes, total proteins and amino acids with the use of newer biochemical methods like atomic absorption spectroscopy and Light chromatography coupled with mass spectroscopy. 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 amino acids isolated from the salivary samples did not show any specific trend depending on age. Establishment of amino acid sequences and not only individual amino acids is necessary to establish the genetic component of saliva along with our quest to establish saliva as a diagnostic fluid. With new and highly sensitive techniques, however, the lower level of analytes in saliva is no longer a limitation. Almost anything one can measure in blood, one can measure in saliva.

Keywords: saliva, electrolytes, total proteins, amino acids, biochemical, balance

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INTRODUCTION

The arrival of the twenty-first century has suddenly forced on dentistry a new paradigm regarding the standards used in patient health care management. Traditional methods and procedures used in early times are being questioned in the light of new emerging methods in emerging information/technologies. [1]

New science and technologies are creating a wave in dentistry and have changed traditional approaches to diagnostics, risk assessment, prevention, and many procedures in clinical dentistry. These new science advances are primarily directed toward connective tissue biophysics/mechanics, tissue engineering, and the large areas of biotechnology (gene therapy, drug delivery, and transport dynamics), molecular engineering (macromolecular structure, protein structure, and molecular therapies) and basic sciences in the form of organic chemistry, microbiology and biotechnology.

In fact, the recent development of saliva as a diagnostic medium has placed dentistry at the forefront of monitoring systemic health and disease and has served saliva as a ' **Tearless'** diagnostic tool. The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Many diagnostic analytes and biomarkers present in the blood in the form of hormones, electrolytes, antibodies are also present in saliva. Saliva offers some distinctive advantages. Whole saliva can be collected non-invasively, and by individuals with limited training. No special equipment is needed for collection of the fluid. Diagnosis of disease via the analysis of saliva is potentially valuable for children and adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations [2].

As we enter the era of genomic medicine, sialochemistry will play an increasingly important role in the early detection, the monitoring and progression of the systemic and oral diseases. This analysis is possible only with the favorable unison of efforts between the basic sciences and clinical minds to establish a diagnostic tool which is present in quantums and still unexplored.

This togetherness of organic chemistry, microbiology and biotechnology and dentistry can help us to investigate the large unexplored side of saliva in diagnosis of systemic health and disease.

For the detection of these biomarkers there has to be an amalgamation of the knowledge obtained from newer techniques used in basic sciences and their clinical applications. Thus reciprocity of these newer technologies into clinical diagnostics is the need of the hour.

It is likely that the use of saliva in assays will continue to expand thus providing a new instrument of investigation for physiologic as well as pathophysiologic states.

Thus for saliva to play a role as a diagnostic aid it is necessary to examine the consequences of preanalytical physiologic variations with respect to age as the physiologic levels of different analytes in blood are established as a function of age before it can be used in detection of pathologies.

Thus the aim of this study was to detect the physiologic levels of different electrolytes, total proteins and amino acids with the use of biochemical methods

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 [3] 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[4].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[5]. 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 instrument used was Varian Vista Pro with detection limits of 1 ppm for each element.

The total proteins and free amino acids were detected by liquid chromatography coupled with mass spectrometry (LC-MS). Mass spectrometry (MS) is an analytical technique used for determining masses of particles of ions which gives the elemental composition of that ion for elucidating the molecular masses of molecules, as atomic mass units and hence their molecular structures.

RESULTS AND DISCUSSION

On subjecting the salivary samples to elemental analysis ,the presence of calcium, potassium, magnesium and silica were detected .The salivary samples of all three age groups exhibited elemental concentration levels of 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 unstimulated whole saliva of children in all three dentition age groups.

For analyses of total proteins as per results obtained from LCMS ,the number of peaks of high molecular weight proteins were analyzed .

The number of major 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.

The amino acids detected from the salivary samples did not show any specific trend depending on age. The most commonly detected amino acids from all the samples were proline, glycine and glutamic acid and their aggregates. The other amino acids isolated from saliva were valine, serine, arginine, histidine, phenylalanine, tyrosine, hydroxyproline and glutamine. The amino acids detected did not show any specific pattern in three age groups. (Table 1, Table 2, Table 3)

Human saliva has great potential for clinical disease diagnostics. Constructing a comprehensive catalogue of saliva analytes using proteomic approaches is a necessary first step to identifying potential biomarkers of disease [8]. However, because of the challenge presented in cataloguing saliva with widely varying abundance, new approaches are needed.

Table 1: Amino acids detected in saliva samples from deciduous dentition age group

SAMPLE ID	Molecular weight	Molecular weight	Molecular weight	Molecular weight
D1	117.85 Valine	238 Serine dimer	310 Arginine dimer	563 Proline dimer
D2	152.95 Glycine dimer	238 Serine dimer	318 Valine +Serine	619 proline +Threonine+Glycine
D3	155.9 Arginine	235 Valine dimer	372 Proline +Glutamine	660 Histidine +Valine +Glutamine
D4	118 Valine	240 Serine dimer	318 Valine +Serine	563 (Proline dimer)
D5	116 Glycine	238 Serine dimer	372 Proline +Glutamine	480 Proline dimer

TABLE 2: Amino acids detected in saliva samples from mixed dentition age group

Sample ID	Molecular weight	Molecular weight	Molecular weight	Molecular weight
M1	117.95 Valine	288 Serine +Glutamic acid	371 Proline +Hydroxyproline	587 Proline +Tyrosine + Valine
M2	166 Tyrosine	210.85 Glycine +Glutamine	354.75 2Glycine +Valine	474.65 Serine +2 Glycine + Serine
M3		218 Glycine + Serine	434 2 Serine + 2 Glycine	584 3 Serine + Valine
M4	152 2 Glycine	288 Glycine + Hydroxyproline	338 2 Glutamic acid 445 Proline +threonine	551 Proline + Serine + Glutamic acid
M5	178 Arginine	218 Glycine +Serine	315 Proline +Glycine 451 Proline +Glycine +Glutamine	520 Proline + Proline

TABLE 3: Amino acids detected in saliva samples from permanent dentition age groups

Sample ID	Molecular weight	Molecular weight	Molecular weight	Molecular weight
P1	171 Hydroxyproline	222 Threonine	394 Lysine	496 Proline +Lysine
P2	178 Arginine	238 Serine Dimer	295 Arginine +Valine	469.5 Arginine+ Valine +Lysine
P3		227 Phenylalanine	365 Histidine	527 2 Proline
P4	193 Glycine + Valine	287 Glutamic acid + Valine	363 Glycine + Glutamic acid + Valine	515 Glycine +Valine + Glutamic acid + Hydroxyproline
P5	171 Hydroxyproline	288 Glycine + Hydroxyproline	434 2 Serine + 2 Glycine	555 Proline dimer

The impetus for development of these diagnostic aids comes from the need to strike a balance between the evolving new technologies in the fields of biochemistry, biotechnology and clinical sciences .One must identify specific salivary biomarkers of disease or health state, and develop technologies that detect biomarkers of interest.

This study thus aimed at analyzing the levels of different electrolytes, total proteins and the amino acids detected from the saliva samples and establishing a physiologic profile for particular age groups in collaboration with biochemical and modern techniques.

The trace elements detected by inductively coupled plasma emission spectroscopy indicated the presence of calcium, potassium, magnesium and silica as their concentration in whole saliva of children of all three age groups was more than 1 ppm. The role of salivary calcium, magnesium, potassium and silica along with total proteins in caries dynamics and oral homeostasis has been established. [9]The levels of calcium, magnesium, silica show a linear increase with age except potassium which does not show a linear increase.

But calcium and magnesium play an important function in the body metabolism. 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[10].Thus ratios in saliva can be used in detecting osteoporosis, heart disease, central nervous system defects, neuromuscular conductivity and immunity related disorders. (Trakhtenberg 2006, Kudrin 2006). Therefore, it is very important to define levels of total and ionized forms of calcium and magnesium.

The salivary sodium - potassium concentration can be used for detecting functioning of aldosterone hormone in the body along with detection of kidney disorders and drug overdoses eg: Digitalis toxicity [11] It has been proven that silica plays a major role in mineral absorption and maintaining the calcium magnesium balance in mineralized structures and collagen formation. Thus for saliva to serve as a biomarker it is necessary to correlate the values of calcium, magnesium, potassium and silica in serum and saliva.

Total proteins in saliva may have both protective and detrimental properties. From analysis of the total proteins, the proline-rich proteins (PRPs) constitute about 70% of human salivary proteins and are unusual in their amino acid compositions, with proline comprising 25%-45% of the amino acid residues and proline, glycine, and glutamic acid/glutamine constituting 70%-85% of the amino acid residues[12]. Similarly the amino acids detected in this study give the picture of dominance of proline rich proteins. Large numbers of amino acids other than these three amino acids were also detected from this saliva sample which did not show a specific pattern. The differences in the amino acids isolated from these samples can be attributed to the differences in the diet intake of these subjects as the diet intake was not standardized as well the oral flora and the body components are also variable.

Large number of DNAs, RNAs and proteins are present in saliva which provides genetic information of hosting human bodies, oral microbes present in the mouth, infecting DNA viruses. Salivary m-RNAs provide information of transcription rates of host genes and micro biota and RNA virus infections. Salivary proteins represent genetic information by establishing amino acid sequences which will help us to understand the translational regulation of the host body and oral microbiota [13]. Quantitative and qualitative identification of salivary proteins is a necessary first step in identifying potential protein biomarkers of disease Thus establishment of amino acid sequences and not only individual amino acids is necessary to establish the genetic component of saliva along with our quest to establish saliva as a diagnostic fluid. Finally, the possibility of physiological variations in any component whose estimation is proposed as a diagnostic aid should be investigated before it is used. This is possible only with the integration of ideas emerging in new research technologies in the form of biochemistry, biotechnology, and genomics.

CONCLUSION

The post genomic era provides opportunities for high-throughput approaches to genomics and proteomics. With the advancement in these technologies, researchers have been able to prove that, the components of saliva act as a “mirror of the body’s health” [14]. These discoveries and technological advances in conjunction with the ability to diagnose disease through the use of a biofluid obtained noninvasively would offer a revolutionary change in medicine [15]. A major drawback for using saliva as a diagnostic fluid has been the notion that informative analytes generally are present in lower amounts in saliva than in serum. With new and highly sensitive techniques, however, the lower level of analytes in saliva is no longer a limitation. Almost anything one can measure in blood, one can measure in saliva.

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