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Comparative Evaluation of Total Salivary Proteins in Leukemic and Healthy Children of Mixed Dentition Age Group.

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ABSTRACT

Saliva has been used as a diagnostic aid in a number of systemic diseases. Leukemia is a fatal disease. The oral manifestations of the leukemias occur early in the course of the disease and these oral features can at times act as a diagnostic indicator. So the aim of our study was to correlate total salivary protein and electrolytes in saliva of healthy and leukemic children of mixed dentition age group. Materials & Method: Group A - Mixed dentition children free from any systemic or local diseases which affect salivary secretions and totally caries free with dmft/DMFT score of 0. Group B - Mixed dentition children with leukaemia. Method of saliva collection- The saliva was allowed to drull into the funnel held to the lower lip. Methods of laboratory analysis- Diluted saliva sample was subjected to inductively coupled plasma emission spectroscopy. The total proteins and free amino acids were detected by liquid chromatography coupled with mass spectrometry (LC-MS). Sodium, potassium and calcium analysis performed using shimadzu atomic absorption spectroscopy. Result& Conclusion – Physical properties of saliva in healthy and systemic diseased patients were different.

Keywords: Saliva, Total Protiens, Lukemia, Mixed dentition age group.

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INTRODUCTION

At present, saliva represents an increasingly useful auxiliary means of diagnosis [1]. Many researchers have made use of sialometry and sialochemistry to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health [2].

Leukemia is a uniformly fatal disease of unknown etiology that is characterized by excessive and abnormal proliferation of primitive white blood cells and their precursors, with infiltration of the various tissues of the body, especially bone marrow, spleen, and lymph nodes [3].

The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Other biologic fluids are utilized for the diagnosis of disease, and 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 older 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.

So in this study we have compared the salivary total proteins and electrolytes of healthy and leukemia children of mixed dentition age group. Diluted saliva sample was subjected to inductively coupled plasma emission spectroscopy. The total proteins and free amino acids were detected by liquid chromatography coupled with mass spectrometry (LC-MS). Sodium, potassium and calcium analysis performed using Shimadzu atomic absorption spectroscopy.

MATERIALS AND METHODS

Criteria for patient selection

In the present study, 9 normal healthy male children ranging from 6 to 15 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. Exclusion criteria included patients who were physical or mentally compromised, having developmental delay, auditory or visual dysfunction, known neurological diseases, history of drug intake for group A as a control group. Informed consent forms were obtained from the custodial parent or guardian of the subject after explaining the procedure to the parent or guardian. For group B patients with acute lymphocytic leukemia, whose chemotherapy of age 6 to 15 were taken under consideration.

Method of saliva collection

Unstimulated whole saliva samples were collected in morning session 1 hour after breakfast. 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 5ml cryoprecipitation vial with a funnel inserted into it, in a calm atmosphere to simulate unstimulated conditions. The saliva was allowed to drull 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.

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. The instrument used was Varian Vista Pro with detection limits of 1 ppm for each element.

Amino acid analysis

LCMS: Shimadzu 2010 with single quadrupole and electro spray ionization source.

Mode of ionization: sample scanned in dual mode both positive and negative.

Drying gas used is: Nitrogen.

HPLC: Shimadzu 2010 with quaternary gradient, autosampler and PDA detector.

Sodium, potassium and calcium analysis: Performed using shimadzu atomic absorption spectroscopy. For atomic absorption the samples were first lyophilised and then subjected to analysis to minimise interference from water. Sample reconstitution is done in methanol.

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.

RESULT AND DISCUSSION

Most of the organic compounds in saliva are produced locally in the salivary glands, but some molecules pass into saliva from blood. Several pathways both intracellular and extracellular enable molecules to be transported from blood to saliva. Saliva is a good medium because its collection is non-invasive and the donation process is relatively stress-free, so that multiple collections can be performed without imposing too much discomfort on the donor.

Scientists who studied various microelement concentrations in the saliva in other diseases, observed oral pathologies too. Błoniarz et al. found higher concentrations of calcium, magnesium and sodium in the saliva of patients with oral carcinoma planoepitheliale spinocellulare[4].

Table no. 1 is showing sodium, potassium, calcium levels of 9 healthy children and also glycine, Phenyl alanine, Tryptophan levels. Table no. 2 explains the total protein levels i.e. arginine, histidine, tryptophan, glutamic acid and also sodium, potassium, calcium levels in leukemic children. Our results showed a markable increase of calcium levels in cancer patient when compared with a healthy child which can also be used as a indicator in diagnosis with the help of saliva. Only tryptophan is common amino acid which we found in both leukemia and healthy children.

Table 1: healthy saliva samples of group A

SR. NO.	pH	Cations in ppm			Amino acids		
		Sodium	Potassium	Calcium	Glycine %	Phenyl alanine %	Trptophan %
1	3-4	5	5	10	10	9	11
2	3-4	3	6	6	11	8	12
3	3-4	4	4	4	12	9	10
4	3-4	2	5	5	10	10	9
5	3-4	3	6	4	15	11	12
6	3-4	4	7	3	12	10	11
7	3-4	5	4	4	13	9	10
8	3-4	5	5	5	14	11	11
9	3-4	4	7	6	12	10	10
Mean		3.77	5.44	5.22	12.11	10.77	9.55

Table 2: leukemia saliva samples of group B

SR. NO.	pH	Cations in ppm			Amino acids			
		Sodium	Potassium	Calcium	Arginine %	Histidine %	trptophan %	Glutamic acid %
1	2-3	3	4	12	5	3	8	6
2	2-3	4	6	15	6	4	9	7
3	2-3	6	5	16	5	5	5	8
4	2-3	5	7	12	8	8	6	5
5	2-3	6	4	15	6	4	8	6
6	2-3	7	5	13	5	2	5	8
7	2-3	6	6	14	9	5	2	9
8	2-3	7	7	12	5	8	4	7
9	2-3	6	5	15	8	8	6	5
Mean		5.55	5.44	13.77	5.77	5.22	5.88	6.77

Salivary amylase and total protein content in the saliva of leukemia patients are found to be elevated and therefore can be of diagnostic value [5]. Rahematulla et al. analyzed the salivary constituents in AML and acute lymphoblastic leukemia (ALL) patients and reported that the mean values for amylase activity and total protein concentrations were significantly higher in these subjects. They assessed several salivary components in stimulated whole saliva from patients with acute leukemia who were undergoing chemotherapy. Saliva samples were collected at the time of diagnosis and longitudinally during the treatment period. Data analysis showed that patients with leukemia had significantly higher peroxidase and amylase activity and elevated concentration of salivary total protein at the time of diagnosis [5].

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and can be especially valuable in large-scale screening and epidemiologic studies. Saliva is being used as a diagnostic aid in an increasing number of clinical situations in systemic diseases that can affect salivary gland function and the composition of saliva [6].

Ashok L et al in 2010 concluded in his that the saliva of leukemia patients demonstrated obvious changes in composition. A rise in salivary amylase and total protein levels was evident, with the increase in amylase levels being statistically significant[7].

Additional and more detailed biochemical analysis on a larger group of patients is needed. In this study it was difficult to have the same number of age-matched patients in each group due to the nature of disease. We included all patients with newly diagnosed leukemia and the number of patients in each group could not be controlled. Findings from further studies on larger groups may justify the use salvia as a prognostic indicator in such patients.

CONCLUSION

In lukemic children calcium levels are significantly high when compared with healthy children (mean value for leukemia – 13.77% and for healthy children – 5.22%) which statistically significant. We found tryptophan a common amino acid prominently than any other amino acid in both groups. As we can conclude that there is increase level of total salivary proteins in leukemic children than healthy children. But we need to have more number of samples to define accurate difference in both groups and to use saliva as a diagnostic tool.

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