ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Evaluation of Salivary Electrolytes in Cleft Lip and Palate and Healthy Children.

Rahul R Deshpande[1, 2], Samrat Sabhlok[1], Vishwas Patil[1], Shubham Singh[1]*, Rajdeep Singh Chhabra[1], Mayuri Mutha[1], Shreya Dasgupta[1], and Kumar Ankit[1].

[1]Dr. D. Y. Patil Vidyapeeth, Dr. D.Y. Patil Dental College and Hospital, Pimpri Pune-18, Maharashtra, India.
[2]Deenanath Mangeshkar Hospital, Pune-4 Maharashtra, India.

ABSTRACT

Saliva, a modern age biomarker for defects and pathological diseases, was used in our study to compare the levels of electrolytes in healthy individuals and the individuals having congenital defect. Saliva being a non-invasive method for determining the status of health can be the most widely used tool for the diagnosis of such cases. Materials & Method: Group A - Mixed dentition children free from any congenital defects. Group B - Mixed dentition children with cleft lip and palate. Method of saliva collection- The saliva was allowed to drool into the funnel held to the lower lip. Methods of laboratory analysis- Diluted saliva sample were subjected to inductively coupled plasma emission spectroscopy. The electrolytes were detected by liquid chromatography coupled with mass spectrometry (LC-MS) which were sodium, potassium, calcium and chlorine. Result and conclusion - It was observed that the level of potassium showed a linear increase in patients with cleft lip and palate. Thus this study helps us correlate the salivary electrolytic values between cleft lip and palate and healthy children in mixed dentition age group and showcases the importance of utilizing saliva as a biomarker.

Keywords: cleft lip and palate, salivary electrolytes, congenital defects, saliva, mixed dentition.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Saliva is a dynamic fluid that has varying spectrum of proteins, polypeptides, nucleic acids, electrolytes, and hormones. It is an exocrine secretion of the salivary glands which is hypotonic in nature with a pH of 7.2–7.4[1].

It consists of a complex mixture deriving from the secretion of salivary glands, gingival fold and oral mucosa transudate, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products [2]. It is a clear, slightly acidic muco-serous exocrine secretion, composed of a variety of electrolytes, small organic substances, proteins, peptides and polynucleotides [3].

Components of saliva, therefore, may serve as biomarkers because the composition of oral fluid is responsive to behavioral, mechanical, genetic or ontogenetic stimuli [4, 5]. Saliva is mainly composed of water (95–99.4%) and various minerals, electrolytes, hormones, enzymes, immunoglobulin, cytokines, and other components whose abundance is dependent upon the gland from which it is secreted. Whole saliva is not a homogeneous fluid, but is made up of secretions from a number of sources, predominantly the extrinsic glands, but also fluids from the intrinsic glands, epithelial cell secretions, and the gingival crevicular fluid [6, 7].

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 [8].

Congenital defects are a curse in disguise as we are merely puppets to unforeseen forces and laws of nature. Cleft lip and palate is one such defect which not only distorts the happy smiling face of a newborn but also brings grief into the lives of the parents.

Cleft lip with or without cleft palate (cleft lip and palate) is the most common facial malformation, affecting approximately one in 700 live births worldwide. Structures anterior to the incisive foramen, including the lip and alveolar ridge, constitute the primary palate. The secondary palate is made of palatal structures posterior to the incisive foramen. A cleft of any element of the primary palate, with or without cleft secondary palate, is considered cleft lip and palate. It results from failure of one or both of the medial nasal prominences to fuse and merge with the maxillary prominences during weeks 4-6 of gestation; the secondary palate fuses between weeks 8 and 12 of gestation. Cleft secondary palate alone is etiologically different from cleft lip and palate and occurs in only one in 2,500 live births [9]. Children in India who suffer from cleft lip and palate present complex problems. Although national epidemiological data of cleft lip and palate is not available, many studies from different parts of India have shown a wide variation in its incidence, from 0.25 to 2.29 per 1000 live births [10-14].

The following study aims at deriving a correlation between the salivary electrolytes in children with cleft lip and palate and healthy children. Using saliva as a biomarker we can determine the possible parameters which may be useful adjunct to diagnosis of congenital defects later on. The salivary electrolytes were analyzed by spectrophotometric methods to obtain various salivary electrolytes namely – calcium, sodium, potassium and chlorine which are thus compared.

Although a new revolutionary era of saliva as a diagnostic tool has been arrived but studies and researches done on it are scarce. Hence, the reason for this study is to show the potential ability of saliva to be used as a non-invasive mean of diagnosing diseases which will lead to a whole new edge to diagnosis of congenital defects such as cleft lip and palate.

ISSN: 0975-8585

MATERIALS AND METHODS

Diagnostic instruments

- Sterile mouth mirrors
- Sterile probe
- Sterile explorers
- Sterile tweezers
- Sterile kidney trays
- Sterile cotton
- Disposable gloves, mouth mask, head caps
- Disinfecting solutions
- Instruments for saliva collection
- Disposable plastic funnel
- Sterile glass vials
- Saliva collection tubes (Tarsen tubes)
- Ice box for storing saliva during transportation to laboratory

Equipments for salivary analysis

- pH strips
- Measurement of salivary total protein done in laboratory using Light
- Chromatography coupled with Mass Spectrometry- Shimadzu LC 2010-CHT
- Measurement of salivary trace elements done by inductively coupled plasma emission spectroscopy- Lab Pro Nich

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 cryo-precipitation vial with a funnel inserted into it, in a calm atmosphere to simulate unstimulated conditions. The saliva was allowed to drool 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 1ppm for each element.

RESULTS AND DISCUSSION

Saliva is a biological fluid, with an important role in the oral physiology. It is a plays a major role in the process of oral and general health maintenance. Being a non-invasive and tearless method of sample collection it can be used in children. Cleft lip with or without palate being one of the common congenital defects hence the following study correlates electrolytic levels between cleft lip and palate and healthy children of mixed dentition age group.

Unstimulated whole saliva samples were collected in morning session 1 hour after breakfast. The collection was continued until 2 ml of saliva per subject was obtained. The electrolytes were detected by liquid chromatography coupled with mass spectrometry (LC-MS) that are sodium, potassium, calcium and chlorine.

ISSN: 0975-8585

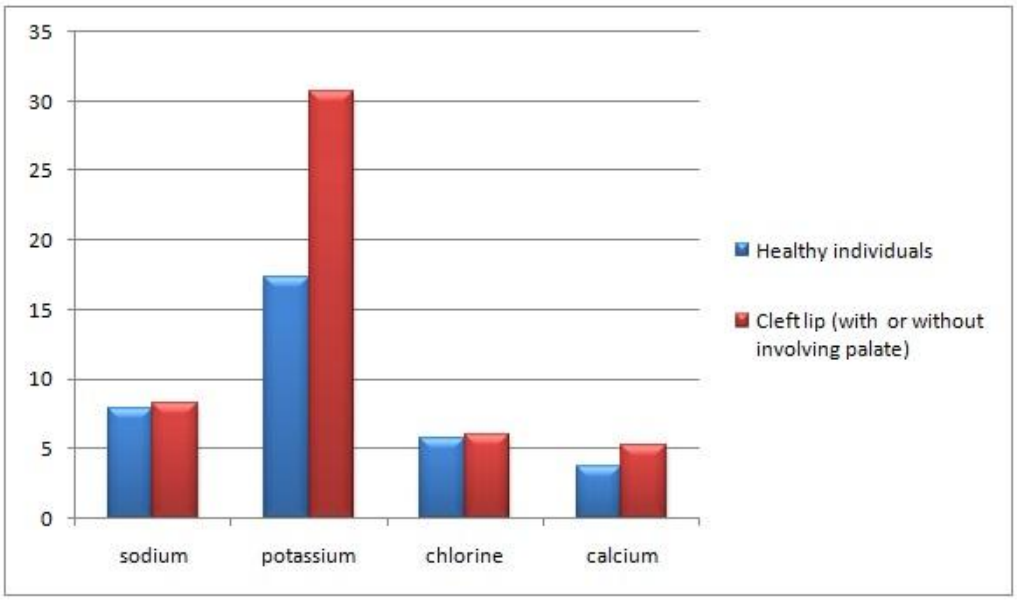
Table 1: Salivary electrolytic values in healthy children in mEq/L:-

sample	Sodium	Potassium	Chlorine	Calcium
1	7.9	18.22	5.72	4.84
2	6.6	16.86	4.80	0.40
3	7.3	17.20	5.4	4.55
4	9.10	16.90	6.8	4.12
5	8.7	17.2	6.14	4.7
Total	39.6	86.38	28.86	18.61
Mean	7.92	17.23	5.77	3.72

Table 2: Salivary electrolytic values in cleft lip and palate children in mEq/L:-

Sample	Sodium	Potassium	Chlorine	Calcium
1	9.7	31.97	7.08	5.74
2	6.1	28.43	4.42	4.49
3	8.7	30.97	6.08	4.74
4	10.7	32.97	8.08	6.74
5	6.4	28.83	4.62	4.59
Total	41.6	153.17	30.28	26.30
Mean	8.32	30.63	6.06	5.26

Graph 1: Comparison in electrolytic values of cleft lip and palate with healthy children:



In earlier studies conducted, where salivary electrolytes comparison of children diagnosed with leukemia and healthy children showed no changes in potassium level. The results showed a marked increase of calcium levels in cancer patient when compared with healthy children which can also be used as an indicator in diagnosis with the help of saliva [15].

The salivary calcium levels did not show a marked difference between mother and child salivary samples. The salivary potassium levels in the study were higher in children in accordance to the previous

studies which also suggest higher potassium levels in mixed dentition subjects (children) than permanent dentition (adults). The sodium levels were high in mother's saliva [16].

Accordingly, the results revealed that there was a linear increment seen in salivary electrolytic level of potassium in cleft lip and palate patients as compared to healthy individuals by the spectrophotometric analysis. It also gave us knowledge that other electrolytes in comparison showed no significant changes in cleft lip and palate children.

CONCLUSION

In cleft lip and palate children potassium levels were high when compared with healthy children (mean value for cleft lip and palate – 17.23mEq/L and for healthy children – 30.63mEq/L) which showed a linear increase.

As we can conclude that there was an increased level of electrolyte potassium in cleft lip and palate 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.

ACKNOWLEDGEMENTS

Agharkar Research Institute, Pune, India. Deshpande's Oral Health Clinic, Pune.

REFERENCES

[1] L Chicharro, A Luc'ıa,M. P'erez, AF Vaquero, and R Urena. Sports Med 1998;26(1):17-27.
[2] Humphrey SP, Williamson RT. J Prosthet Dent 2001; 85: 162-9.
[3] Edgar WM. Br Dent J 1992; 172:305-12.
[4] Katie P. Wu, et al Salivary flow and compositions in children.
[5] Thiruvanamalai Sivakumar et al. J Oral Sci 2009; 51(4):573-580.
[6] Marieb, E.N. and Hoehn, K. 2009, Human Anatomy and Physiology. 8th ed. Benjamin Cummings.
[7] Martini FH and Nath JL. 2009, Fundamentals of Anatomy and Physiology. 8th ed. Benjamin Cummings.
[8] Rahul R Deshpande et al. Res J Pharm Biol Chem Sci 2011; 2(4): 343-350.
[9] TW. Langman's medical embryology. Philadelphia, PA: Lippincott, 2000:366-375.
[10] Kannappan J. G. Cleft Palate Cleft lip and Oro-tacacial anomalies – A multi-disciplinary approach, 1st edition, Shanti Anand Printers and Publishers, Madras, India, 1988; 3-17.
[11] Sharma B. et al. Ind J Pediat 1972; 39: 286- 292.
[12] Tibrewala N. S. and Pal P. M. Ind Pediat 1974; 11: 403-411.
[13] Saifullah S. et al. Ind Pediat 1967; 4: 251-261.
[14] Goravalingappa JP and Nashi HK. Ind J Med Res 1979; 69: 140-146.
[15] R Deshpande et al. Res J Pharm Biol Chem Sci 2014; 5(1): 126-130.
[16] Rahul R Deshpande et al. Res J Pharm Biol Chem Sci 2014; 5(1): 219-224.