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Comparative Evaluation of Salivary Total Protein in Cleft Lip Palate Children to Their Mother and Between Healthy Children to Their Mother.

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ABSTRACT

Cleft lip and palate is a common developmental defect which disfigures the happy smile of a new born. The commonly used laboratory diagnostic procedure involves the analysis of cellular and chemical constituents of blood, which is an invasive. This study focuses on a non-invasive method to compare the levels of total proteins in saliva of children with cleft lip and palate to their mothers and healthy children to their mothers to aid as a bio-marker for further studies. The procedure involves the analysis and comparison of salivary total protein levels in cleft lip and palate children to their mothers and healthy children to their mothers. The saliva was allowed to drool into the funnel held to the lower lip. Diluted saliva sample were 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). Physical properties of saliva in healthy and in cleft lip and palate and showed a marked difference. The salivary total protein levels demonstrated higher levels of total proteins in patients with the cleft lip and palate defect than healthy children between mothers of cleft lip and palate to mothers of healthy children. Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes.

Keywords: Total Proteins, Saliva, Mother, Cleft Lip and Palate

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INTRODUCTION

Saliva is a unique fluid and has the potential to become the choice of diagnostic sample because of the advancements in detection technologies and a combination of clinically relevant bio molecules. Saliva is one of the most important factors in regulating oral health, with flow rate and composition changing throughout development and during disease [1]. Human saliva contains a plethora of compounds that can be informative in monitoring overall health and well-being. Components of saliva, therefore, may serve as biomarkers because the composition of oral fluid is responsive to behavioural, mechanical, genetic or ontogenetic stimuli [1, 2]. Saliva reflects the body's state of well-being; but its use as a diagnostic aid has been negatively affected because of our lack of understanding of the salivary bio molecules and their relevance to disease etiology combined with lack of highly sensitive detection systems. 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. The protein composition of saliva reflects cellular signal processing that results from day-to-day environmental influences as well as from acute or chronic stress [3]. This study aims at using saliva as a diagnostic tool and finding, if any, correlation exists in major salivary components of a mother and her child.

A study was conducted to see the methylenetetrahydrofolate reductase (MTHFR) is thought to be involved in the development of nonsyndromic cleft lip with or without cleft palate (NSCL/P). However, conflicting results have been obtained when evaluating the association between maternal MTHFR C677T and A1298C polymorphisms and the risk of NSCL/P [4]. 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 [5].

METHOD

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 [6].

RESULT

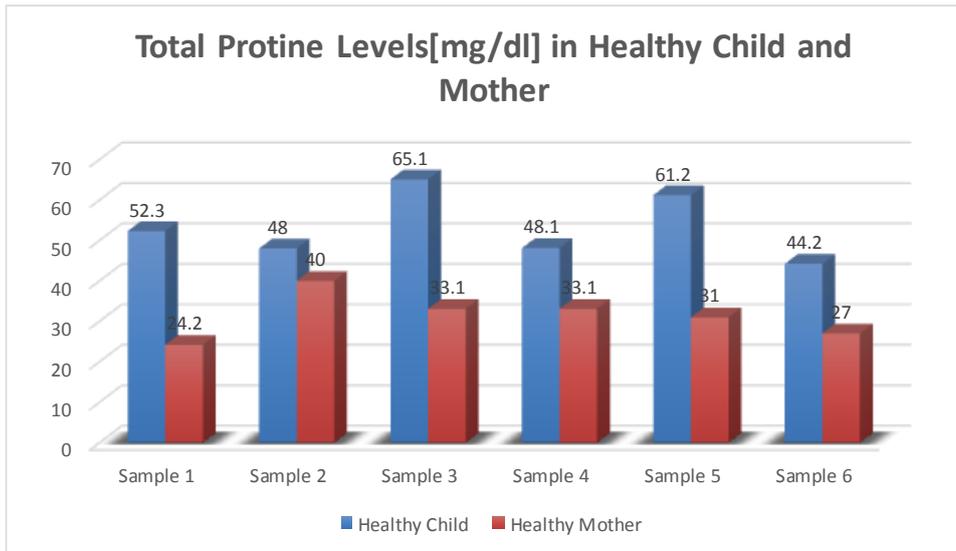
Most of the organic compounds in saliva are produced locally in the salivary glands, but some molecules pass into saliva from blood. Several pathways both intracellular and extracellular enable molecules to be transported from blood to saliva. Saliva is a good medium because its collection is non-invasive and the donation process is relatively stress-free, so that multiple collections can be performed without imposing too much discomfort on the donor [6]. The salivary total proteins after analysis in the laboratory using Light Chromatography coupled with Mass Spectrometry showed the following correlation between healthy children and their mothers and cleft lip/palate defect to their mothers.

Table 1 is showing comparison of total protein levels in healthy children and their mothers.

Table 2 is showing comparison of total protein levels of 6 cleft lip and palate children and their mothers.

Table 1: comparison of total protein levels in healthy children and their mothers

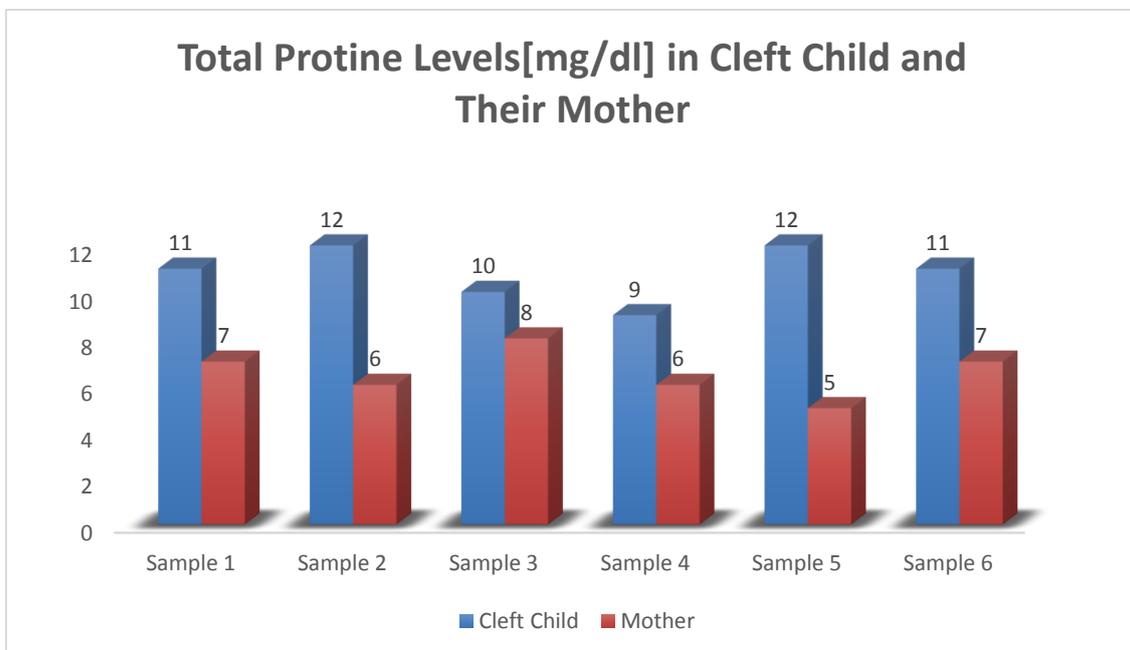
| Serial no. | Total Proteins Levels In Healthy children (mg/dl) | Total Proteins Levels In Healthy children's mothers (mg/dl) |
|------------|---|---|
| 1 | 52.3 | 24.2 |
| 2 | 48 | 40 |
| 3 | 65.1 | 33.1 |
| 4 | 48.1 | 33.1 |
| 5 | 61.2 | 31 |
| 6 | 44.2 | 27 |



Graph 1: Total protein levels in healthy children and their mothers

Table 2: comparison of total protein levels of 6 cleft lip and palate children and their mothers.

| Serial no. | Total Proteins Levels In cleft children (mg/dl) | Total Proteins Levels In cleft children’s mothers (mg/dl) |
|------------|---|---|
| 1 | 11 | 7 |
| 2 | 12 | 6 |
| 3 | 10 | 8 |
| 4 | 9 | 6 |
| 5 | 12 | 5 |
| 6 | 11 | 7 |



Graph 2: Total protein levels of 6 cleft lip and palate children and their mothers.

DISCUSSION

Saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders. Whole saliva can be collected in non-invasive manner by individuals with modest Congenital defects disfigurement causes not only psychological trauma for the family of the newborn but also affects .

Genomic constitution is unpredictable in a newborn. We can only see the manifestation of the genes in a body through the levels of proteins. Thus by drawing a correlation between the total proteins in patients with congenital defect and healthy individuals, through a non-invasive biomarker like saliva, would help us to further unfold the mysteries behind the genomic mutations. It is observed that the salivary total protein levels in patients with cleft lip and palate defect show a linear increase when compared with total protein levels in healthy individuals [7].

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 [8]. In this study Additional and more detailed biochemical analysis on a larger group of patients is needed. Findings from further studies on larger groups may justify the use saliva as a prognostic indicator in such patients. The concentrations of total proteins in saliva are co related with the development of the major salivary glands. The few of the abundantly seen amino acids in saliva are glycine, histidine, arginine, tryptophan, phenylalanine etc. Arginine is a non- essential amino acid which plays an important role in wound healing, cell division, immune function by increasing the size and function of thymus and hormonal secretion[9].

The salivary pH is dependent on various factors like the type of food intake, oral micro flora , the time of food intake and collection of saliva, etc. The salivary pH of children in our study was acidic (3-4); some studies done previously suggest that child's salivary pH is in the range of 6.9 to 7.5. This variation can be attributed to the fact that above factors were not considered in our study. 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. The protein composition of saliva reflects cellular signal processing that results from day-to-day environmental influences as well as from acute or chronic stress[10] This study aims at using saliva as a diagnostic tool and finding, if any, correlation exists in major salivary components of a mother and her child.

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