Levels of Salivary Proteins of Children with Proteinuria

Nihal Majeed[1], Amitha M Hegde[2], Sudarshan Shetty[3]

[1]Department of Dentistry, Middle East Specialised Medical Centre, Abu Dhabi, UAE. [2]Department of Pedodontics and Preventive Children Dentistry, A.B Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, India. [3]Department of Pediatrics, KS Hegde Medical Academy, Mangalore, India

Abstract

Aim: To determine the levels of total protein in saliva of children with proteinuria and their healthy siblings.

Method: A cross sectional study was conducted involving 65 children with significant proteinuria between 3-12 years of age. The data collected was tabulated and subjected for statistical analysis.

Results: The mean value of salivary proteins in the healthy siblings was 1117.14 µg/ml which is higher than that of the children with proteinuria. One-way ANOVA analysis, showed highly significant difference between the groups. ($p<0.001$) The levels of salivary proteins drop consistently as the grades of urinary protein loss increase.

Conclusion: Levels of salivary proteins in children with proteinuria was significantly lower than their siblings.

Key Words: Saliva, Proteins, Proteinuria

Introduction

Saliva plays a vital role in maintaining the health and integrity of the oral tissues. Among the various components of saliva, the proteins protect the oral cavity against pathogens and diseases like dental caries and mucositis [1-3]. Similar to salivary proteins, plasma proteins are essential in maintaining the various functions of the body. The kidneys play a major role in the retention of these proteins [4,5].

Proteinuria is a condition where plasma proteins are lost from the body via urine. Proteinuria is common in children and occurs as a result of a variety of factors. Urinary loss of plasma proteins results in hypoproteinemia [6].

This study aims at understanding the implications of hypoproteinemic state due to proteinuria on the levels of salivary proteins.

Material and Methods

A cross- sectional study was conducted based on data collected from K.S Hegde Charitable Hospital and A.B. Shetty Memorial institute of Dental Sciences, Mangalore, India. The study was approved by the Ethical Committee of Nitte University (ABSM/EC/101/2010).

The study included 95 children, divided into two groups. The study group comprised of 65 children (29 males and 36 females) between 3- 14 years of age with significant proteinuria. Diagnosis of significant proteinuria was made using dipstick method on spot using first morning urine samples of children. The dipstick test detected proteins in urine and was used for the quantification of the urinary proteins. (Significant proteinuria was defined as dip stick reading of 2+ or more.) [7]. Subjects who were unco-operative, subjects without consent form and subjects with other health complications were excluded from the study.

The control group comprised of 30 healthy siblings (18 males and 12 females) between 3- 14 years of age. (Dipstick test showing absence of proteins in urine.)

The control group was Group 1.The study group was again divided into 3 group based on the urine dipstick readings. Group 2 contained 31 children with 2+ reading, Group 3 contained 25 children with 3+ readings and group 4 contained 9 children with 4+ readings.

Consent was taken from parents and guardians. All the children who participated in the study were Indians. The unstimulated whole salivary samples were collected two hours after any oral or visual exposure to food stuffs. The salivary samples were collected in sterile vials between 10-11 am in order to prevent any bias in the concentration of the saliva due to the circadian rhythm. Children had to pool the saliva in the floor of their oral cavity and spit into a sterile vial intermittently.

After collection, these samples were frozen immediately at 2-8°C. It was then centrifuged and analysed for levels of total proteins using Lowry protein assay.

Lowry's assay for total protein is one of the most common colorimetric assays performed for protein detection. This procedure is particularly sensitive because it employs two color forming reactions. It uses the Biuret reaction in which Cu2+ (in the presence of base) reacts with the peptide bond to give a deep blue color. In addition Folin-Ciocalteu chemistry, in which a complex mixture of inorganic salts react with tyrosine and tryptophan residues to give an intense blue-green color, is also used. The combination of the two reactions makes this assay sensitive down to about 10 µg/ml [8].

The data collected were tabulated and subjected for statistical analyses. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS 17.00) for Windows. The levels of salivary protein among the four groups was compared by the one-way ANOVA test. The significance level was set at p-value <0.001.

Results

The study included 95 children, divided into two groups. The study group comprised of 65 children (29 males and 36 females) between 3- 14 years of age with significant proteinuria. The control group comprised of 30 healthy siblings(18 males and 12 females) between 3- 14 years of age. (Dipstick test showing absence of proteins in urine).

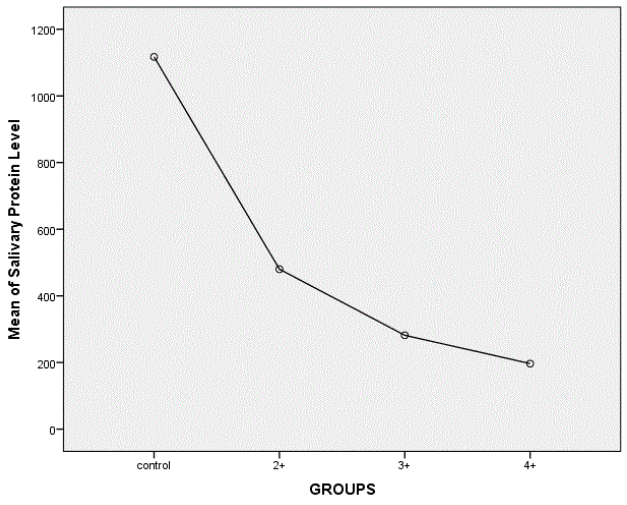
Corresponding author: Dr Nihal Majeed, BDS, MDS, MFDS RCSEd, Department of Dentistry, Middle East Specialised Medical Centre, PO Box: 3781, Abu Dhabi, UAE; e-mail: nihalmajeed@gmail.com

The control group was Group 1. The study group was again divided into 3 group based on the urine dipstick readings. Group 2 contained 31 children with 2+ reading, Group 3 contained 25 children with 3+ readings and group 4 contained 9 children with 4+ readings.

The mean value of salivary proteins in the Group 1 was 1117.14 µg/ml which is higher than all other groups *(Table 1)*. The mean value of group 2 is 480 µg/ml, group 3 is 281.72 µg/ml and group 4 is 196.56 µg/ml. One-way ANOVA analysis, showed highly significant difference between the groups. ($p<0.001$) The levels of salivary proteins drop consistently as the grades of urinary protein loss increase *(Graph 1)*.

Table 1. *Comparison of salivary protein levels : One way anova.*

		N	Mean	Std. Deviation	df	Mean Square	F	Sig.
Salivary Protein Level	Control	30	1117.1	179	3	4066116	259.6	<0.001
	2+	31	480	120.7				
	3+	25	281.7	53.8				
	4+	9	196.6	28				



Graph 1. *Comparison of levels of total proteins in saliva.*

Discussion

Among the vital organs of our body, the kidneys are responsible for a multitude of bodily functions. One of the most important roles of these organs involves the regulation of intravascular volume and concentration of fluids in the body by producing urine. Kidneys thus play a principal role in maintenance of the internal environment. The kidneys play a major role in the retention of plasma proteins, using renal tubules to reabsorb them as the proteins pass through the glomerular filtration barrier. There are normally more than 2 million functioning glomeruli that regulate total body water and solute concentrations. In addition, kidneys are involved in regulation of blood pressure, detoxification of harmful substances, secretion of hormones, control of acid/base balance and concentration of several electrolytes [9].

Normal urine protein excretion especially albumin is <4 mg/m^2. Therefore, the detection of abnormal quantities or types of protein in the urine is considered an early sign of significant renal or systemic disease.

Proteinuria means the presence of an excess ofserumproteinsin theurine. The presence of protein in urine is a common laboratory finding in children. Proteinuria is present at routine urine testing in up to 10 percent of school-aged children, although this decreases to 0.1 percent at repeated testing. Although proteinuria is usually benign, the condition can be a marker for a serious underlying renal disease or systemic disorder. Proteinuria may occur secondary to glomerular or tubular dysfunction [6].

This study was aimed at determining the levels of salivary proteins in children with and without significant loss of proteins in urine. Urinary dip stick test was used to classify the subjects into study group and controls.

The urine dipstick test uses the tetrabromophenol blue colorimetric method, which is the most widely used screening method. The intensity of color changes from yellow to blue and correlates with the amount of protein in the urine: trace (10 mg per dL), 1+ (30 mg per dL), 2+ (100 mg per dL), 3+ (300 mg per dL), and 4+ (1,000 mg per dL or greater) [10].

The control subjects were those whose dipstick test showed absence of urinary proteins. The control group was Group 1.

In this study the population was classified on the basis of dipstick test. The study population contained subjects showing a reading of 2+ or more for proteinuria. The study group was again divided into 3 group based on the urine dipstick readings. Group 2 contained 31 children with 2+ reading, Group 3 contained 25 children with 3+ readings and group 4 contained 9 children with 4+ readings.

Whole saliva is clinically more significant since it is the secretion that bathes the mouth. It comprises of not only pure secretions from the major and minor salivary glands but also the gingival exudates, micro organisms and their products, epithelial cells, food remnants, and also to some extent nasal exudates [11]. Standardised collection of saliva was carried out to avoid any circadian rhythmic changes or carry over stimulation of saliva after meals [12].

It has also been previously concluded by Doddset al. that the unstimulated state is the predominant condition in terms of the salivary gland activity, and the unstimulated saliva flow is the critical determinant of salivary clearance [13].Thereby unstimulated whole saliva of all the subjects was collected for analysis in this study.

It was observed that children with proteinuria had significantly lower levels of salivary proteins. A direct co-relation between the levels of total proteins in saliva and the levels of proteins in urine was noted. This is in accordance the studies of Johanson et al and other studies conducted on children with protein energy malnutrition [14]. The decrease in protein pool in saliva could be due to the fact that many of the salivary protein components are serum derived. When

plasma proteins are lost in urine, the circulating plasma proteins also decreases.

Conclusion

Levels of salivary proteins in children with proteinuria were significantly lower than their healthy siblings.

Acknowledgement

I would like to acknowledge Dr. Abdul Jaleel and Mr Shan Mohammed for all the help rendered.

References

1. Whelton H. The anatomy and physiology of salivary glands. In: Edgar WM, O'Mullane DM, editors. Saliva and oral health, 2 nd ed. British Dental Association; 1996. p. 2.

2. Roa NS, Chaves M, Gómez M, Jaramillo LM. Association of salivary proteins with dental caries in a Colombian population. *Acta Odontológica Latinoamericana*. 2008; **21**: 69-75.

3. Aps JK What does saliva represent to the dentist? *Revue Belge de Médecine Dentaire*. 2006; **61**: 133-40.

4. Morris G, Murray TG. Acid-base and fluid homeostasis in CRF. *Medical Clinics of North America*. 1981; **65**: 429.

5. Cotran RS, Kumar V, Robbins SL. The Kidney: Pathological basis of disease. 4th ed. Philadephia; PA. Saunders. 1989. pg 1011-1082.

6. Leung AK, Wong AH. Proteinuria in children. *American Family Physician*. 2010. 15; **82**: 645-51.

7. eMedicine. [Internet].[Updated: Apr 21, 2008]; available from http://emedicine.medscape.com/article/984289-overview

8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*. 1951; **193**: 265.

9. Raja K, Coletti DP. Management of the dental patient with renal disease. *Dental Clinics of North America*. 2006; **50**: 529-545.

10. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review [published correction appears in Am Fam Physician. 2006;74(7):1096]. *American Family Physician*. 2005; **71**: 1153-1162.

11. Rudney JD, KL Hickey, Z Ji. Commulative correlations of lysozyme, cactoferrin, peroxidase, S-IgA, Amylase, and total protein concentrations with Adherence of oral vividans streptococci to microplates coated with human saliva. *Journal of Dental Research*. 1999; **78**: 759-768.

12. Dawes, C.: Saliva for Human Rhythmometry, Proceedings Second International Symposium on Chronobiology, Florence, Italy, 1970.

13. Dodds MW, Johnson DA, Mobley CC, Hattaway KM. Parotid saliva protein profiles in caries-free and caries-active adults. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*. 1997; **83**: 244-51.

14. Johanson. I., Lenander. M., SaellstromA-K., Saliva composition in Indian chsildren with chronic Protein Energy malnutrition. *Journal of Dental Research*. 1994; **73**: 11-19.