International Journal of Community Medicine and Public Health
Raj G et al. Int J Community Med Public Health. 2022 Jun;9(6):2574-2578
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20221537

Original Research Article

Salivary urea and uric acid levels as biomarkers in dental caries: *in vivo* study

Gagan Raj*, Dhananjay Kumar, Suma B. S., Garima Mangal

Department of Public Health Dentistry, Buddha Institute of Dental Sciences and Hospital, Patna, India

Received: 11 April 2022
Accepted: 29 April 2022

***Correspondence:**
Dr. Gagan Raj,
E-mail: dr.graj2017@gmail.com

ABSTRACT

Background: A new approach in caries research is focused on the fact that salivary Substrates, like urea and uric acid, may play an important role in biofilm pH, homeostasis and in inhibiting dental caries and thus act as biomarker.

Methods: A clinical in vivo study was conducted in Dental college of Patna among simple random sample of 30 dental students (10 caries active and 20 caries free based on DMFT index) and of age group 18-25 years after satisfying the inclusion and exclusion criteria. Unstimulated saliva samples of dental students were collected at Coachman position using draining technique and salivary urea and uric acid estimation done at private pathology laboratory, Patna.

Results: The mean for urea level was higher among Caries free Group (61.15±18.85) as compared to Caries group (9.91±3.64) while the mean uric acid level was higher among Caries group (10.19±2.16) as compared to caries free group (5.25±2.78). The caries experience when assessed by correlating the DMFT Score with salivary urea and salivary uric acid concentration, it was found that the DMFT Score is in negative correlation with urea level (r=-0.790) and positive correlation with uric acid level (r=0.751) suggesting when salivary urea level increases there was decrease in caries rate and vice versa.

Conclusions: Thus value of salivary urea and uric acid in adolescence can serve as a parameter for determining the risk of caries, thus acting as biomarkers in dental caries and this, in turn, can be used in the planning and implementation of appropriate caries preventive measures.

Keywords: Draining technique, Coachman position, Salivary urea, Uric acid

INTRODUCTION

The oral cavity is an intricate environment composed of multiple structures and tissues types working in concert.[1] While each structure performs a unique function, all are colonized by bacteria and immersed in salivary fluids. Saliva principally consists of 99.5% water, 0.3% proteins and 0.2% trace, small organic compound like urea and uric acid, and inorganic substances.[2] Saliva is one of the innate defence systems of the human body that protects teeth by several mechanisms, such as improving tooth enamel by remineralization, neutralizing low plaque pH, rinsing food debris, microorganisms and sugar aggregation, and by its antibacterial and bacterial properties.[3] Saliva has been proved to be a credible diagnostic aid in detecting different Biomarkers for Dental caries.[4] A new approach in caries research is focused on the fact that alkali generation from salivary Substrates, like urea and uric acid, may play an important role in biofilm pH, homeostasis and in inhibiting dental caries and hence can act as biomarker of dental caries. Urea is present in saliva and crevicular fluids at 3-10mm in healthy individuals and it is hydrolysed by ureases to generate 2 molecules of ammonia and one molecule of CO2.[5] Uric acid is end product of purine metabolism, also

reported to act as an antioxidant. This information will facilitate the rationale design of strategies that rely on alkali production of caries risk assessment and interventions also formulating probiotic applications to enhance oral ureolysis and development of carious lesion and role of uric acid as an antioxidant in saliva.[6] Discovering, validating, and understanding saliva-based biomarkers will have a considerable role in establishing oral fluids as a credible diagnostic biofluid.[7] Hence the present study was done with the aim to assess the role of salivary urea and uric acid levels as biomarkers in dental caries.

Objectives

To estimate the level of urea and uric acid in salivary sample of dental students of Buddha Institute Of Dental Sciences and Hospital, Patna, Bihar. To find out association (if any) between salivary urea and dental caries. To find out association (if any) between salivary uric acid and dental caries.

METHODS

A clinical in vivo study was conducted among dental student from January 2020 to February 2020 in Patna. Ethical approval for the study was obtained from the Institutional Review Board, Patna and Informed Consent was obtained from the participants.

Based on DMFT Index, 30 (20 caries free and 10 caries active) Dental students of Buddha Institute of Dental Sciences and Hospital, Patna in the age group of 18-25 years were selected for the study by simple random sampling method and saliva sample was collected from the study subject after satisfying the inclusion and exclusion criteria. Dental students free of systemic or local condition which affects salivary secretion and Patients willing to consent to be a part of the study were included in the study. Subject who are undergoing orthodontic treatment, who have any adverse habits, subjects under restricted diet and subject who were medically compromised were excluded from the study.

Unstimulated saliva samples were collected from the study subject in Coachman position between 10.00 am – 12.00 pm by using draining technique and were sent for laboratory analysis for estimation of salivary urea and uric acid level.

The salivary urea level was estimated by using Bertholet method 8 and the salivary uric acid level was estimated by Colorimetric method 9 (by using Salimetrics uric acid enzymatic assay kit). The data obtained by analyzing different salivary samples for urea and uric acid concentration was systematically tabulated and subjected to statistical analysis. All the results were expressed in terms of percentage proportion. For the comparison of proportions, chi-square test and Independent sample T test, was used with continuity correction whenever appropriate. Correlation between the groups was done by Karl Pearson's correlation test. P<0.05 was taken to be statistically significant for the purpose of analysis.

RESULTS

In the present study based on DMFT Index, 20 caries free and 10 caries active students were selected for the study.

Table 1: Cross tabulation between DMFT (caries active/caries free) and gender.

Gender	Caries	No caries	Total	Chi-square value	P
	N (%)	N (%)	N (%)		
Male	6 (60.0)	9 (45.0)	15 (50.0)	0.600	0.439 NS
Female	4 (40.0)	11 (55.0)	15 (50.0)		
Total	10 (100.0)	20 (100.0)	30 (100.0)		

Statistical analysis: pearson's ch-square test. Statistically significant if p<0.05.

Table 2: Mean comparison of urea level (mg/dl) and uric acid level (mg/dl) between caries active and caries free group.

Clinical variables		Sample size	Mean	SD	Mean difference	P value
Urea level (mg/dl)	Caries active	10	9.91	3.64	51.24	<0.001 S
	Caries free	20	61.15	18.85		
Uric acid level (mg/dl)	Caries active	10	10.19	2.16	4.94	<0.001 S
	Caries free	20	5.25	2.78		

Statistical analysis: independent sample t test. statistically significant if p<0.05

Table 1 shows association between DMFT Score of the study subject and Gender in which it was found that 15 (50.0%) of the study subject were male among which 6 (60.0%) were caries active and rest 9 (45.0%) were caries free subject while rest 12 (50.0%) subject were female among which 4 (40.0%) were caries active and 11 (50.0%) were caries free subject thus caries experience was more among male and this result was found to be statistically insignificant (p=0.439).

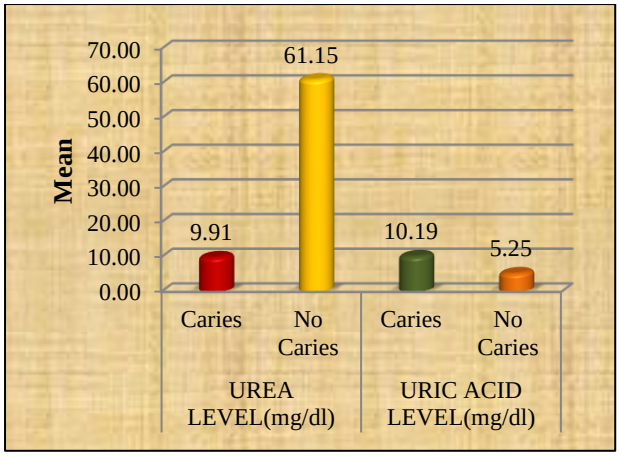

Figure 1: Mean comparison of urea level (mg/dl) and uric acid level (mg/dl) between caries active and caries free group.

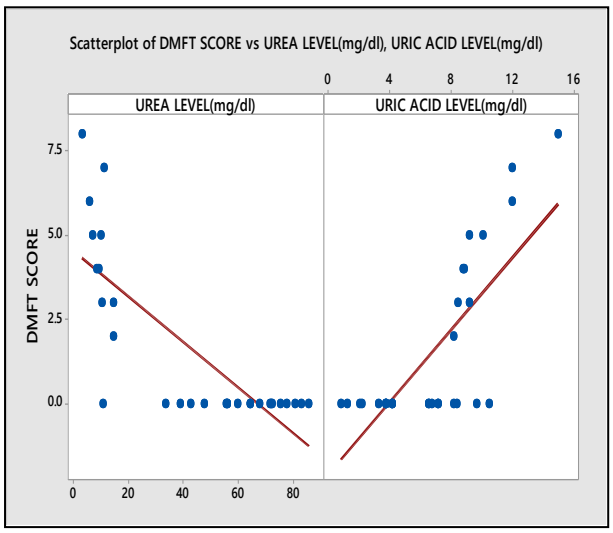

Figure 2: Correlation analysis.

Table 2 shows mean comparison of urea level (mg/dl) and uric acid level (mg/dl) between caries active and caries free group and it was found that the mean for urea level was higher among Caries free Group (61.15±18.85) as compared to caries active group (9.91±3.64) and this result was found to be statistically significant (p<0.001).Alternatively the mean uric acid level was higher among caries group (10.19±2.16) as compared to caries free group (5.25±2.78) and this result was found to be statistically significant (p<0.001) (Figure 1)

In the present study caries experience was assessed by correlating the DMFT Score with salivary urea and salivary uric acid concentration and it was found that the DMFT Score is in negative correlation with urea level (r=-0.790) and positive correlation with uric acid level (r=0.751) and this result was found to be statistically significant with p<0.001. This means when salivary urea level increases there was decrease in caries rate and vice versa. Alternatively, when salivary uric acid level increases there was increase in caries rate and vice versa (Table 3, Figure 2).

Table 3: Correlation analysis.

Correlation between	Correlation coefficient (r)	P value	Results
DMFT score and urea level (mg/dl)	-0.790	<0.001	Significant
DMFT score and uric acid level (mg/dl)	0.751	<0.001	Significant

DISCUSSION

In the present study, the saliva sample was collected from 30 Dental Students (20 caries free and 10 caries active) in the age group of 18-25 years from Buddha Institute of Dental Sciences and Hospital, Patna and sent for laboratory analysis for estimation of urea and uric acid. The data obtained by analyzing different salivary samples for urea and uric acid concentration show variation and there was statistically significant result between the mean salivary urea and uric acid concentration and dental caries of the study subjects.

As per the review of literature the normal salivary urea level ranges between 12-70 mg/dl and salivary uric acid level ranges between 0.25-8.6mg/dl.[10]

Our present study shows significantly higher levels of urea in caries free individual (61.15±18.85) as compared to caries active individual (9.91±3.64) which is in accordance with the study done by Nireeksha et al where the mean of the caries free individual was 7.223 and caries active individual was 4.663.[11] This may be attributed to generation of ammonia which favours the equilibrium between mineralization and demineralization, playing a key role in plaque pH homeostasis, preventing the emergence of a cariogenic micro biota, which could be a major impediment to the development of dental caries.[12]

The association between caries-free subjects and a higher ammonia generation as obtained in our study, in saliva may inhibit the development of a pathogenic microbiota, in an acidic oral environment.[13] Alkali generation is important in the physiology, ecology and pathogenicity of dental biofilms. A substantial body of evidence from microbiological, genetic, and biochemical analyses and clinical studies has suggested that the alkalinogenic potential of dental biofilms may be used as a strategy for caries control.[14]

On the other hand our study shows significantly higher levels of uric acid in caries active individual (10.19±2.16) as compared to caries free individual (5.25±2.78) which

is in contrast with the study done by Nireeksha et al where there was no significant change in uric acid levels in comparison between the groups. This elevation of uric acid can be related to more oxidative stress due to caries in caries active group than caries free group as one of the important defense mechanism of saliva is presence of antioxidants system which comprises of various enzymes (peroxidase, catalase, super oxide dismutase, glutathione peroxidase) and small molecules (uric acid, vitamin E, C).[15]

The present study also showed significant negative correlation between salivary urea level and DMFT Score (r=-0.790) which is in agreement with the study done by Xia et al who stated negative correlation between salivary urea and dental caries (r=-0.435).[16] This may be attributed to the fact that Urea is a part of the buffer system in saliva, which participates in neutralizing the acids in the oral environment and thus helps in preventing caries.[17]

However, limitations of this study are that only certain salivary component were evaluated, other salivary proteins and enzymes like histatins, and statherinsin saliva can also be evaluated. Despite the limitations, the findings of this study provide further insight in the role of various salivary components proving as potential biomarker of dental caries.[18]

CONCLUSION

The results of this study have demonstrated a variation in the role of salivary urea and uric acid levels as biomarkers in dental caries. From this study it is clear that the saliva with its constituents plays an important role in maintaining oral health of the individual. The urea contributes to maintaining the acidobasic balance of saliva, and thus affects the incidence of caries. The positive effect of urea was confirmed by the values found in this study: the respondents with a lower DMFT index presented a higher concentration of urea. Regulating salivary acidity, urea performs the role of a buffer, reducing the possibility of the occurrence of dental caries. On the other hand salivary uric acid acts as antioxidant system in maintaining oral and general health of the individual. Also the study depicts positive correlation between salivary uric acid and dental caries and this information may be helpful in caries prevention and progression.20 Thus value of salivary urea and uric acid in adolescence can serve as a parameter for determining the risk of caries, thus acting as biomarkers in dental caries and this, in turn, can be used in the planning and implementation of appropriate caries preventive measures.

Recommendations

Following recommendations are proposed: apart from urea and uric acid there are various salivary components like salivary protein such as IgG, IgA, C – Reactive protein and enzymes that are thought to play a defensive role in oral cavity and can act as biomarkers in dental caries thus further studies, with larger sample size and better molecular epidemiology can be done to prove salivary components as biomarkers in dental caries. Along with salivary urea and uric acid, blood urea and uric acid can also be estimated to find any association between oral health and systemic health of the individual. Salivary sample from different age group can be taken for better understanding the role of salivary biomarkers in initiation and progression of dental caries. In this study saliva samples of study subjects were collected based DMFT index, further studies can be done by selecting the study subject based on DMFS Index that would help in evaluating better relevance between salivary urea, uric acid and extent of dental caries. There is a need to expand knowledge and awareness regarding salivary biomarker among undergraduate and post graduates students so it should be included in the curriculum of all Dental institutions.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Silva PVD, Troiano JA, Nakamune ACMS, Pessan JP, Antoniali. Increased activity of the antioxidants systems modulate the oxidative stress in saliva of toddlers with early childhood caries. Arch Oral Biol. 2016;70:62-6.
2. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wonga DTW. Salivary Biomarkers: Toward Future Clinical and Diagnostic Utilities; Clinical Microbiology Reviews. 2013;48(3):781-91.
3. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. International Journal of Oral Science. 2017;1-8.
4. Nireeksha, Hegde MN, Kumari S, Ullal H, Kedilaya V. Salivary proteins as biomarkers in dental caries: In vivo study. Dent Oral Craniofac Res. 2017;3(2):1-7.
5. Lorenzo-Pouso AI, Pérez-Sayáns M, Bravo SB, López-Jornet P, García-Vence M, Alonso-Sampedro M et al. Protein-Based Salivary Profiles as Novel Biomarkers for Oral Diseases. Hindawi Disease Markers. 2018;6141845-22.
6. Wang A, Wang CP, Tu M, Wong DTW. Oral Biofluid Biomarker Research: Current Status and Emerging Frontiers. Diagnostics. 2016;6:45.
7. Nireeksha, Hegde MN, Kumari SN. Salivary Urea and Uric Acid Levels as Biomarkers in Dental Caries. Oral Health and Dentistry. 2018;3(1):528-31.
8. Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. FEMS Microbiology Letters. 2000;193:1-6.

9. Coulombe JJ, Favreau L. A new simple semi micro method for colorimetric determination of urea. Clin Chem. 1963;9:102-8.
10. Gao X, Jiang S, Koh D. Salivary biomarkers for dental caries. Periodontol. 2000;(2016):70.
11. Nascimento MM, Gordan VV, Garvan CW, Browngardt CM, Burne RA. Correlations of oral bacterial arginine and urea catabolism with caries experience. Oral Microbiol Immunol. 2009;24(2):89-95.
12. Morou-Bermudez E, Elias-Boneta A, Billings RJ, Burne RA, Garcia-Rivas V, Brignoni-Nazario, Suarez-Perez E. Urease activity in dental plaque and saliva of children during a three-year study period and its relationship with other caries risk factors. Arch Oral Biol. 2011;56(11):1282-9.
13. Toro E, Nascimento M, Suarez-Perez E, Burne R, Elias-Boneta A, Morou-Bermudez E. The Effect of Sucrose on Plaque and Saliva Urease Levels in vivo. Arch Oral Biol. 2010;55(3):249-54.
14. Bilbilova E, Ivkovska A, Ambarkova V. correlation between salivary urea level and dental caries. Biol Med Sci. 2012;213(1):289-302.
15. Ling Liu Y, Nascimento M, Burne RA. Progress toward understanding the contribution of alkali generation in dental biofilms to inhibition of dental caries. International Journal of Oral Science. 2012;4:135-40.
16. Ywan M. Chen YM, Weaver CA, Burne RA. Dual Functions of Streptococcus salivarius Urease. J Bacteriol. 2000;4667-9.
17. Farsi N. Dental Caries in Relation to Salivary Factors in Saudi Population Groups. J Contemp Dent Pract. 2008;(9)3:016-23.
18. Khozeimeh F, Torabinia N, Shahnaseri S, Shafaee H, Mousavi SA. Determination of salivary urea and uric acid of patients with halitosis. Dent Res J. 2017;14:241-5.
19. Hendi SS, Goodarzi MT, Moghimbeigi A, Ahmadi-Motamayel F. Evaluation of the status of salivary antioxidants in dental caries. Infect Disord Drug Targets. 2019;30.
20. Motamayel FA, Goodarzi MT, Hendi SS, Kasraei S, Moghimbeigi A. Total antioxidant capacity of saliva and dental caries. Med Oral Patol Oral Cir Bucal. 2013;18 (4):e553-6.

Cite this article as: Raj G, Kumar D, Suma BS, Mangal G. Salivary urea and uric acid levels as biomarkers in dental caries: in vivo study. Int J Community Med Public Health 2022;9:2574-8.