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Demonstration of Urease Activity in Subgingival Plaque Sample of Periodontitis Patients at a Tertiary Care Centre of Central Nepal

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ABSTRACT

Introduction: The urease activity is produced by many oral and gastric microorganisms, which have demonstrated systemic implications as well. Urea can be detected both from saliva and gingival crevicular fluid, which could suggest that the oral cavity can act as an extragastric reservoir for many microbes leading to serious systemic diseases.

Objective: The main objective of this current study was to find out the urease activity in human dental plaque.

Methods: An analytical cross-sectional study was conducted from September to November 2023 in patients visiting the Department of Periodontology and Oral Implantology. The urease activity was detected using a rapid urease test (RUT) kit from a hundred cases diagnosed with periodontitis. All systemically healthy patients excluding patients on ongoing proton-pump inhibitor therapy were selected for the presence/absence of periodontitis as per the 2017 World Workshop classification. Data were collected and entered into Microsoft Excel, and further analysis was done using SPSS v.20.

Results: Out of 100 patients, urease activity was found positive in 85 (85%) patients. Regarding gender and age, the urease activity was not much different and was not statistically significant (P value= 0.163 and 0.382 respectively).

Conclusions: The results of this study suggest there is a high urease activity in dental plaque samples whose removal is essential to prevent our body from systemic threats like bacterial endocarditis, gastric carcinoma, etc. caused by urease-producing microorganisms.

Keywords: Dental plaque; nepal; periodontitis; urease activity.

INTRODUCTION

Urease is an enzyme that hydrolyses urea and is produced by several bacterial species including oral microorganisms like *Streptococcus salivarius, Actinomyces naselundii, Haemophilus parainfluenzae, Staphylococcus epidermidis*, etc.[1,2] Urea is delivered in the gingival crevicular fluid and salivary secretions even in normal healthy individuals but was found in greater concentration in the presence of gingival inflammation.[3,4] Dental plaque is a complex structure with different microbial colonies protected by a resistant sheath. So, dental plaque can act as a suitable platform for the survival of many microorganisms. Interestingly, oral microorganisms commonly found in dental plaque and generating the urease activity have been linked with systemic diseases like bacterial endocarditis (*H. parainfluenzae, S. epidermidis*).[5-7] Thus, it is paramount to understand the link between the urease activity which could indicate the presence of potential bacteria leading to perio-systemic diseases. Rapid urease test is a simple, cost-effective way to evaluate the urease activity in a given sample. The current study is hence believed to reveal the presence of urease activity in oral cavity that might relate it to the causation of systemic disease as mouth is a known doorway for contamination.

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METHODS

The study was conducted in the Department of Periodontology and Oral Implantology, Kathmandu University School of Medical Sciences (KUSMS), Dhulikhel, Kavreplanchok, Nepal after obtaining the ethical clearance letter from the Institutional Review Committee of KUSMS (KUSMS-IRC Ref. 148/23). It was three-month (September- November 2023), analytical cross-sectional study conducted on a patient fulfilling the definitive set of criteria. The inclusion criteria were patients above 20 years of age, systemically healthy patients with periodontitis diagnosed as per World Workshop 2017 classification[8] of periodontal and peri-implant health and diseases. Periodontitis was defined as the presence of interdental loss of attachment present in ≥2 non-adjacent teeth and/ or buccal loss of attachment ≥3 mm with pocketing >3 mm detectable at ≥2 teeth as per the 2017 classification.[8] The patients with systemic disease, patients consuming proton pump inhibitors, and patients who had taken antimicrobials within the previous two months are excluded from the current study.[9]

The sampling method used was purposive convenient sampling. The sample size was determined based on the prevalence method using data derived from a similar study done by Akshit et al., 83.3%.[10] The sample size obtained was 105 using a 94% confidence interval. The sample size was calculated using the standard formula as mentioned below:

$$\text{Prevalence} = \frac{z^2pq}{e^2}$$

z= 1.645 at 94% confidence interval,
p= 0.833 (83.3%),
q= 1 – p,
e = margin of error= 0.06 (6%).

$$n = \frac{(1.645)^2 \times 83.3 \times 16.7}{6^2}$$

$$= 104.5 \sim 105$$

Subgingival plaque samples were collected from an interproximal area of the posterior tooth of a diagnosed periodontitis case using area-specific curette (Figure 1). Study participants were screened and diagnosed as periodontitis cases by the principal investigator and consultant periodontists of KUSMS. Informed consent was obtained from the participants agreeing for participating to the study. The area of sample collection was first air-dried using dry cotton gauze and three-way syringe to avoid contamination. Any samples contaminated with blood were discarded.

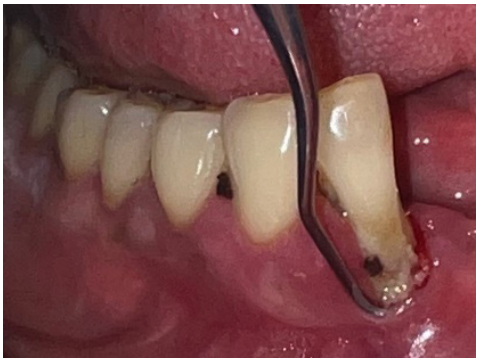

Figure 1: Collection of plaque sample for rapid urease test.

The urease activity was measured using Rapid Urease Test (RUT) kit from Gastrohub, Kolkata, India with an ISO certification ISO 13485: 2016 (Figure 2). First, the sample was collected, and then the label of RUT kit was peeled off to place the plaque sample in the urea broth. The urea broth was then moistened with the addition of 1-2 drops of distilled water provided in the test kit (Figure 3). The test kit was covered with the label again as it was at the beginning. Finally, the evaluation of colour change was done at 10-180 minutes time frame as per the manufacturer's instruction. The colour changes from yellow to pink or red if the test is positive and remains yellow if

Figure 2: Rapid urease test kit from Gastrohub, India.

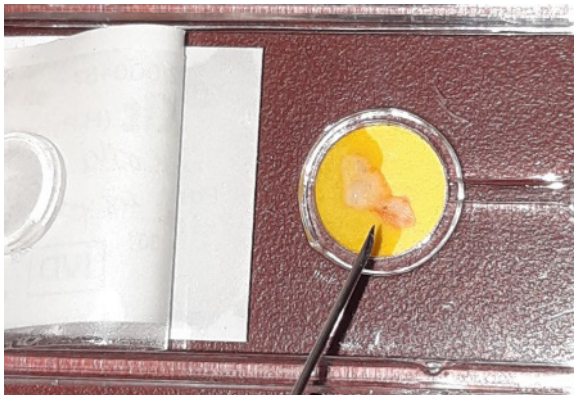

Figure 3: Process of sample evaluation in urea broth.

Figure 4: Colour change- pink indicating positive and yellow indicating negative results.

urease activity is absent in the plaque sample (Figure 4). In every individual test, age, gender, and time of sample collection was recorded which was printed on the backside of the label of the test kit itself.[11] All the data were kept in a separate folder in password-protected computer in Department of Periodontology and Oral Implantology, KUSMS. Data were entered in Microsoft Excel version 2016 and then analysed using IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA).

RESULTS

The study evaluated the urease activity in 105 patients of age ranging from 21-67 years with a mean age of 38.11 ± 12.41 years. Out of 100 patients' sample, five samples were not included as the results were inconclusive as suggested by manufacturer's guideline for colour change. So, 100 results were finally analysed for tests of significance. There were 62 (62%) male patients and 38 (38%) female patients with male to female ratio of 1.63 (Table 1). Rapid urease tests were found to be positive in 85 (85%) of the total patients and negative in the rest 15 (15%). There were no statistically significant differences in the urease activity in males and females (P value >0.05). RUT was also compared with age groups ≤40 and >40 years where there were almost equal numbers of patients (age group ≤40 = 54 patients and age group >40 = 46 patients). However, the results were again statistically not significant which suggests that there is a high prevalence of urease activity in human dental plaque samples of periodontitis patients, irrespective of gender and different age variations (Tables 2, 3). The comparison was done using Pearson

Table 1: Distribution of frequency of demographic parameters.

Variables	Frequency	
Gender	Male	62 (62%)
	Female	38 (38%)
	Male: Female	1.63
Age (in years)	Range	21-67
	Mean	38.11 ± 12.41

Table 2: Association between rapid urease test results and gender.

Gender	Positive count within gender n (%)	Negative count within gender n (%)	P value
Male	52 (83.90)	10 (16.10)	0.163
Female	33 (86.80)	5 (13.20)	
Total	85 (85)	15 (15.00)	

Chi-square tests.

Table 3: Association between rapid urease test results and age groups.

Age groups	Positive count within age groups n (%)	Negative count within age groups n (%)	P value
≤40 years	47 (87.00)	7 (13.00)	0.382
>40 years	38 (82.60)	8 (17.40)	
Total	85 (85.00)	15 (15)	

DISCUSSION

Periodontal medicine was popularised and added as an essence of an hour as a separate branch of periodontology by Steven Offenbacher. It was defined as a broad term that defines a rapidly emerging branch of periodontology focussing on the wealth of new data establishing a strong relationship between periodontal health or disease and systemic health or disease. Periodontal medicine has a history of almost 100 years and the impact of periodontal infection has been linked with more than 50 systemic diseases.[12] The dental plaque is a complex microbial structure which can harbour plenty of microorganisms that could lead to serious systemic complications.[13, 14]

Urease is an enzyme that hydrolyses urea into ammonia and carbon dioxide.[7] Urease activity is shown by numerous microorganisms including those present in the oral cavity.[1,2] A few of the important aspects that have to be cautious is the possibility of *Helicobacter pylori* and *Haemophilus parainfluenzae* colonisation in dental plaque which could be threatening.[5,15-17] In addition, urea is believed to increase the baseline pH of dental plaque.[18,19] The pH of around 7.6 is required for the growth of dental plaque crystals causing periodontal disease thus suggesting the alkaline nature of plaque is important for disease causation.[20]

Urease activity in dental plaque is measured by three common methods i.e., RUT, Culture, and polymerase chain reaction (PCR) among which rapid urease test is a simple, reliable, and cheap method for detection of urease activity. RUT has a sensitivity and specificity rate of around 80-100% and 97-99% respectively.[21,22] It is commonly used for the detection of *H.* pylori in gastric mucosa as it could lead to gastric ulcers and gastric carcinomas. They are even more dangerous as they are similar to other chronic diseases that remain in the body for longer period before they present clinically.[23,24] Though, RUT is primarily used for *H.* pylori detection, it has been used recently for the detection of other capable oral microorganisms. The study done by Dahlen et al in 2018 found that apart from *H. pylori*, microorganisms like *H. parainfluenzae* of various strains show strong urease activity using RUT whereas major periodontal pathogens did not demonstrate urease activity.[7] Other oral microorganisms like *S. salivarius* and *A. naselundii* who are considered to have urease activity demonstrated a weak activity. Their weak activity probably does not justify the previous school of thought of the anticariogenic properties of these bacteria.[25,26] Hence, one should be worried and take a quick consideration for dental plaque removal for potential infections with major pathogens like *H.* pylori (causing gastric disease) and *H. parainfluenzae* (causing cardiovascular and pulmonary disease).

The results of the current study found 85 (85%) positive for urease activity was in accordance with the previous study done by Al-Refai et al. 52 (87%) and Anand et al. 65 (80%) using the RUT method[27,28] and a few other studies yielded a prevalence of RUT positivity of around 50% which is contradictory to the results of our current study.[29,30] The high urease activity might be because of above-mentioned pathogens like *H.* pylori and *H. parainfluenza*. Thus, every attempt should be made for dental plaque removal as soon as possible to remove any infectious and inflammatory pathway that can hamper other distant body parts. There are certain limitations of this study like we could not isolate and identify the possible microorganisms leading to the urease activity in dental plaque. Further, culture-based methods and polymerase chain reaction techniques should be used in future studies.

CONCLUSIONS

Periodntal systemic connections are closely related as many diseases are chronic having similar infectious and inflammatory pathogenesis. As a periodontist, we should be able to connect every minute dots that the periodontal infection of the oral cavity can lead to serious complications in the human body. Thus, we should eliminate dental plaque and try to reinforce good oral hygiene to every patient that we encounter. Furthermore, studies focussed on microorganism isolation using standardised culture techniques and PCR technology should be conducted in multiple centres as a lack of such facilities and manpower would result in huge social and financial burdens in underdeveloped and developing countries.

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Conflict of interest: None.

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