Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 31 (3), July, 2024
Pages: 41–47.
www.Ejimmunology.org
https://doi.org/10.55133/eji.310304

Helicobacter pylori present in caries sample among dental caries patients

Zainab K. Ahmad[1], Baha H. H. Al-Amiedi[1], Thulficar G. H. Al-Khafaji[2], and Sinan A. Shwailiya[2]

[1]Department of Microbiology, College of Dentistry, University of Babylon, Hilla, Iraq.
[2]Department of Conservative Dentistry, College of Dentistry, University of Babylon, Hilla, Iraq.

Corresponding author: Zainab K. Ahmad, Department of Microbiology, College of Dentistry, University of Babylon, Hilla, Iraq.
Email: dent.zainab.almahdi@uobabylon.edu.iq

Abstract

Helicobacter pylori is Gram negative bacteria, the reason for causing peptic ulcer. There is suggestion between the presence of *H. pylori* in oral cavity and gastritis. The present study aimed to detect *H. pylori* in dental caries samples. The study included 29 dental caries patients from both sexes (13 males and 16 females), with different age groups (children and adult), and nine apparently healthy subject as a control group (2 males & 7 females). Dental caries samples were collected and investigated for this study from patients with dental caries who visited the Dental Faculty in the College of Dentistry, University of Babylon, Iraq. *H. pylori* antigen was detected using an enzyme linked immunosorbent assay (ELISA) technique. Of the 29 dental caries patients, 19 (65.51%) patients were positive for *H. pylori* antigen test. Most of them were in the age group 20-30 (9 patients) & 30-40 (8 patients). The age groups (10-20) & (40-50) years shows 100% positivity for *H. pylori* antigen. Also, result was recorded significant higher difference's between *H. pylori* positive antigen between dental caries patients and *H. pylori* positive antigen among control group. (t=2.697,df=5, $p \leq 0.05$). Pearson correlation recorded significantly higher association between the presence of *H. pylori* antigen and the dental caries infection among test group (r=1, $p \leq 0.000$), 4 (44.5%) of the 9 control subjects, without dental caries, were positive for *H. pylori* antigen test. In summary, the *H. pylori* positive antigen test was recorded in both dental caries patients (65.51%) and in the control group (62.5 %). In conclusion, *H. pylori* antigen was present in dental caries patients. This could indicate that the bacteria *H. pylori* present in dental caries samples may contribute to caries processes.

Keywords: *Helicobacter pylori*, antigen, caries sample.

Date received: 06 October 2023; **accepted:** 28 November 2023

Introduction

Helicobacter pylori is Gram negative bacteria, microaerophilic, *H. pylori* overlies gastric-type epithelial cells.[1] It is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.[1] *H. pylori* was successfully isolated and cultured from the human stomach.[2] Recently, many research studies investigated that *H. pylori* is present in environment of oral cavity and may contribute in some oral disease such as caries, or the oral cavity act as reservoir for *H. pylori* infection.[3-5] The bacteria were isolated from saliva, feces, vomitus[4,5] and dental plaque.[5]

Dental caries is a disease in which oral parenchymal defects in the tooth structure due to the acid produced from carbohydrates, and it is the most common cause of pulp infection.[6] Research shows that the presence of *H. pylori* in dental plaque is associated with systemic *H. pylori* infection. Two different modes of transmission may be hypotheses, these include fecal-oral transmission and oral-oral infection.[7]

Previous studies reported an association between *H. pylori* infection and its presence in the oral cavity.[8, 9] A study by Nomura et al., 2018, showed that *H. pylori* possessed both adhesion and invasion ability.[10] Therefore, it can be expected that *H. pylori* colonizes the dental pulp and teeth utilizing this ability.[10] *H. pylori* in dental pulp might remain viable after eradication because antibiotics are difficult to penetrate dental pulp.[11]

In a report by Hirsh *et al*., 2012, viable *H. pylori* was detected in the root canal of deciduous teeth.[12] Previously, *H. pylori* had been found in saliva in a Japanese report, Brazilian report, and Iranian report.[13-15] Previous studies reported that people who suffer from sever dental caries have a high *H. pylori* detection rate in their saliva in comparison with those who do not.[16,17]

Recently, *H. pylori* was detected in saliva, dental plaque, and pediatric dental caries.[17-19] The oral cavity is colonized by various microorganisms, and interspecies co-aggregation is thought to be important for bacterial colonization.[20] A study by Ishihara et al., 1997, reported that *Fusbacterium nucleatum* and *Prophyromans gingivalis* were found to be co-aggregated with *H. pylori*.[21]

Metabolic activity of bacteria in oral environment supports *H. pylori* making a network of action in oral cavity. Some dental infection bacteria can be isolated easier than others such as *Streptococci*, *Lactobacilli*, *Staphylococci*. However, *H. pylori* requires enrichment medium supplemented with blood and/or blood products and antibiotic-containing media such as Skirrow's medium, in order to suppress overgrowth by other competing bacterial flora.[1] Identification tests such as serological tests are used for detection of anti *H. pylori* IgG antibodies in peptic ulcer patients.[22]

Molecular techniques such as the polymerase chain reaction (PCR) were used for detection of *H. pylori* in oral cavity.[23] A study by Eskandari *et al*., 2010, investigated the presence of *H. pylori* in dental plaque from patients with or without gastritis using PCR.[18] The present work aimed to detect the presence of *H. pylori* in dental caries lesion among dental caries patients and in dental plaque or surface of teeth in caries free subjects using an enzyme linked immunosorbent assay (ELISA) technique for detection *H. pylori* antigen.

Subjects and Methods

The study included 29 dental caries patients identified by clinical examination, from both sexes (13 males and 16 females) with different age groups (children and adult). Samples were collected from the surface of the teeth and from deep caries lesion from the patients and from nine (2 males & 7 females) apparently healthy subjects as a control group.

Sample collection: Using forceps, small pieces from dental caries were taken by a specialist dentist and transferred to a 10 ml tube containing normal saline and freezed directly. While collection of samples from the control group was done by taking swabs from the surface of teeth.

Detection of *H. pylori* antigen: an ELISA technique was used for detection the presence of *H. pylori* antigen by commercial test kits (AccuDiag TM *H. pylori* antigen, Diagnostic Automation/ Cortez Diagnostics, Inc/USA), according to the manufacturer's instructions.

Statistical Analysis

The analysis was performed using the GraphPad Prism, version 8.3.4. released in 2020, California, USA. The two-way ANOVA test was performed to determine the significant difference between the means of two or more groups, the Tukey's multiple comparisons test was done to determine which groups were significantly different from each other and for comparing the incidence of *H. pylori* antigen among different age groups. t-test was done for

estimation the deference of the presence positive value of *H. pylori* antigen between dental caries and control group, Pearson correlation was done for evaluate how strong the relation between the No. of dental caries patients and the No. of *H. pylori* positive antigen among dental caries group. *p* value ≤0.05 considered as significant.

Results

Our data recorded that 19 (65.5%) cases out of the 29 cases with dental carriers were *H. pylori* antigen positive and of the 9 controls cases 4 (44.5%) subjects were positive for *H. pylori* antigen. Also result was recorded significant higher difference's between *H. pylori* positive antigen between dental caries patients and *H. pylori* positive antigen among control group. (t=2.697, df=5, *p*≤ 0.05). Pearson correlation recorded significantly higher association between the presence of *H. pylori* antigen and the dental caries infection among test group (r=1, *p*≤ 0.000) Figure 1, while there was non significant correlation related with the presence *H. pylori* antigen in control group (r=0.05. *p* ≤ 0.05).

Our study samples (dental caries patients and control groups) were grouped according to the age group and according to *H. pylori* antigen positivity (Table 1). Our result indicated that the most dental caries patients with *H. pylori* antigen positive were within the age group (20-30) & (30-40) years. There were significant differences between these groups and > 10 years age group, similar result was recorded for *H. pylori* positive antigen among control subject (*p*≤ 0.05) (Figure 2). The total *H. pylori* positive antigen cases were 24 (64.86%) cases, with 73.3% in males and 68.4% in females, among dental caries patients and control group (Table 1 and Table 2). Among control group, *H. pylori* positive antigen was higher in women.

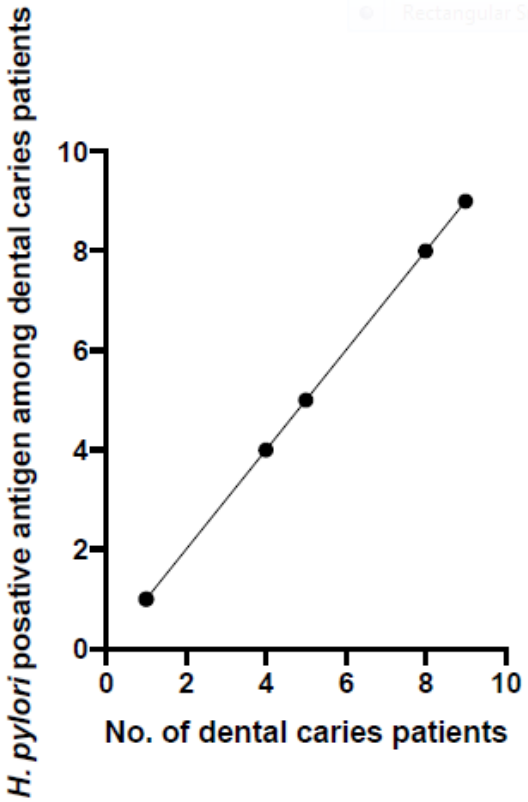

Figure 1. Pearson correlation shows positive correlation ((perfect line), r= 1, *p*=0.000)) between *H. pylori* positive antigen among dental caries patients and No. of dental caries patients.

Table 1. Distribution of *Helicobacter pylori* positive antigen test in dental caries patients and in control group according to their age groups.

Age group (Years)	No. of patients	*H. pylori* positive antigen	Control group	*H. pylori* positive antigen
10<	1	1	2	2
10-20	4	4 (100%)	1	0
20-30	9	4(44.4%)	3	1
30-40	8	5 (62.5%)	1	0
40-50	5	5 (100%)	1	1
50>	1	0	1	0
Total	29	19 (65.5%)	9	4(44.5%)

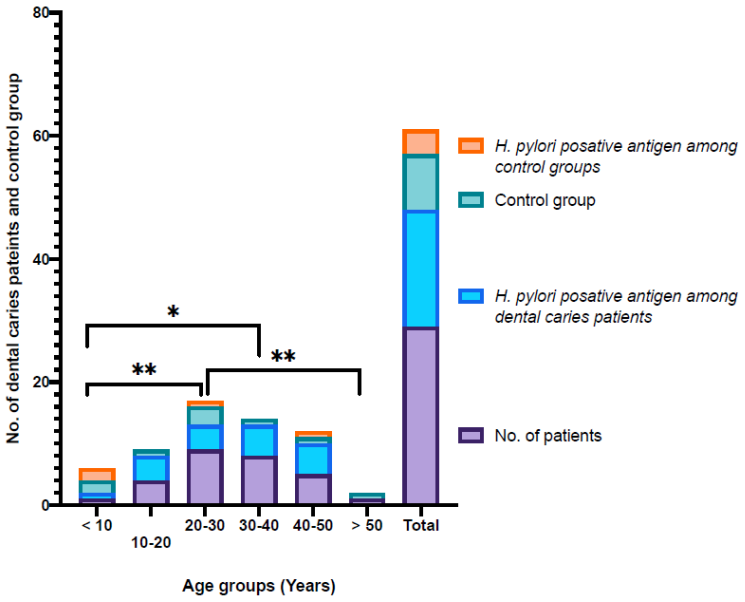

Figure 2. Distribution of dental caries patients in to the different age groups. *H. pylori* positive antigen among dental caries patients and control groups within different age groups (< 10 ; 10-20; 20-30; 30-40; 40-50 and > 50 years). Most dental caries patients within age groups (20-30) & (30-40) years. (10-20) and (40-50) years age groups recorded the highest positivity percentage for *H. pylori* antigen among dental caries patients (100%). (*: Significant ($p \leq 0.05$).

Table 2. Distribution of *Helicobacter pylori* positive antigen test in dental caries patients and in control group according to their gender.

No. of cases	Gender	
	Male	Female
Dental caries patients 29	13	16
+ve *H. pylori* antigen: 19 cases (65.51%)	9 (69.2%)	10 (62.5%)
Control group: 9	2	7
+ve *H. pylori* antigen:4 (44.5%)	2 (100%)	2 (28.6%)

Discussion

Oral environment contains different species of bacteria both in health and disease status.[24] *Streptococcus mutants* and *Lactobacillus* have had high record in oral cavity of patients with dental caries and subjects without dental caries.[25-29] Enterobacteriaceae groups such as *E. coli and Klebsiella* were also isolated with other bacteria in caries and periodontal disease and in subject without dental caries.[25,26, 28- 30] There is a state of equilibrium of occurrence of these bacteria in health which change and shift under special circumstances leading to disease status.[24]

Dental caries occurs by action of many bacteria, the metabolite product from some bacteria can support the environment for growth other bacterial species until reaching to caries processes. This network may be strong enough to overcome the oral cavity immune response until initiate disease, since the work of Al-Mahdi & Abood, 2021, did not record significant differences in immune responses in health and disease for some immune parameters.[25]

Among dental caries patients visited dental clinic, patients within age group (20-30) & (30-40) years were the highest No. among other age groups. The age group (10-20) & (40-50) years shows 100% positivity for *H. pylori* antigen. In addition, 44.5 % of subjects without dental caries also showed positivity for the *H. pylori* antigen test.

Similar results by the study of Rowland *et al*, 2005, recorded that children get *H. pylori* infection at a very young age, and the threat of infection dropped quickly after 5 years of age. *H. pylori* infection was nearly undetectable (0.6%) among the young children (0-11 years), whereas the prevalence elevated to reach 20% in adolescents (12-17 years) and reached a peak of 45% in adults (≥18 years).[31]

Oral mucosal epithelium is colonized with normal oral flora which represent an essential

resistance mechanism for prohibition potential pathogens from colonizing the oral cavity. The normal resident oral flora secretes metabolic by-products, competes for receptors and nutrients, alters the conditions in the oral environment by its metabolic activity (e.g., oxygen, pH) to limit the growth of potential pathogens. Structural components of the normal flora such as lipopolysaccharides motivate non-specific innate immune defense mechanisms (e.g., activation of phagocytes, production of protective antibodies). When the oral normal flora is exhausted (e.g., during using broad-spectrum antibiotic), providing a chance for potential pathogens that may cause oral disease. Such example is the infection by *Candida albicans*, the oral fungal pathogen, when most of the normal commensal bacteria are killed by taking wide broad-spectrum antibiotics such as tetracycline.[24]

Biochemical activities of *H. pylori* such as urease production[1] change the pH of oral environment toward encourage growth of some species of bacteria or inhibit other groups. Also, urease has immunogenic properties since natural dental plaque demonstrates significant ureolytic activity.[32]

Salivary glands secrete urea in oral cavity at concentrations parallel to the concentration of urea in the blood, about 3 to 10 mM in healthy individuals.[33] Urease enzyme destroys urea and produce ammonia, causing pH elevation in the dental biofilms, lead to neutralize the acids production from the glycolytic activity for dietary carbohydrates by bacteria in dental plaque[34–37] then provide protection for acid-sensitive bacteria.[38, 39]

A previous study indicated that strains of urease producer bacteria *Actinomyces naeslundii* possess significant contributors to a total plaque ureolysis, mostly in the course of there is an increased threat for development of caries. There is also evidence that metabolism of urea may support the formation of calculus and ammonia released from urea could aggravate the periodontal diseases.[40]

A study by Morou-Bermudez *et al*., 2011, suggests that reduction the ability of ammonia production from urea in dental plaque can be an essential risk factor for caries and suggested an important clinical role. There are remarkable and complex interactions between the activity of urease in oral cavity and caries development and could be a sign for dental infection with *Streptococcus mutans* in children.[41]

The presence of *H. pylori* in oral cavity influence for induction peptic ulcer. The present study recorded that *H. pylori* positive antigen was higher among male (69.2%) than among female (62.5%) among dental caries group and for control group the percentage was 100% among males and 28.6% among females.

A study in Diyala Governorate/ Iraq recorded that the prevalence of anti-*H. pylori* antibody of blood samples was 75.2% and the infection with *H. pylori* among males were higher among female as the rate among male was 78.1% while the infection rate among females was 70%.[42] A study in Kurdistan recorded that *H. pylori* infection was higher among females (62.8) than among males (37.2%) and the most incidence of infection was in age group 31-40 years.[43] Zamani *et al*., 2018, mentioned that the worldwide *H. pylori* infection rate was 42.7% among females and 46.3% among males.[44]

Based on our results, we conclude that the bacteria *H. pylori* are present in oral cavity in dental caries patients. Our records improve significant contribution for *H. pylori* in caries processes. The study also recorded that *H. pylori* positive antigen was higher among males than among females.

Acknowledgment

The authors would like to thank the Collage of Dentistry/ Babylon University for providing service and support for completion the present work.

Author Contributions

ZKA, Lab work, statistical analysis and writing. BHHA, Writing the paper. TGHA, Clinical diagnosis of patients and collection of samples. SAS, clinical diagnosis of patients and samples collection.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed by the scientific Committee, and approved by head of Microbiology and Biomedical safety trainer of College of Dentistry, University of Babylon, Hilla, Iraq.

Informed consent

The biomedical samples were collected under acceptance from all participants by specialist dentist prior to their participation in the study.

ORCID iD

Zainab K. Ahmad **iD** https://orcid.org/orcid.org/0000-0002-4057-8267

References

1. Brooks G F, Carroll K C, Butel J S, et al. (2013). Jawetz, Melnick, & Adelbergs, *Medical Microbiology, 26th edition*. Section II, PP 130-140. McGraw-Hill Education. U.S.A.

2. Marshall B, Warren R. (1984). Unidentified bacilli in the stomach of patients with gastric and peptic ulceration. *Lancet* 1: 1311-1315.

3. Payao L, Rasmussen, L. (2016). *Helobacter pylori* and its reservoirs: A correlation with the gastric infection. *World journal of gastrointestinal pharmacology and therapeutics*; 7 :126-132.

4. Ahmed Ks, Khan AA, Ahmed I et al. (2006). Prevalence study to elucidate the transmission pathways of *Helicobacter pylori* at oral and gastroduodenal sites of a south Indian population. *Singapore Medical Journal*. 47:291-296.

5. Momtaz H, Souod N, Dabri H, et al. (2012). Study of *Hrlicobacter pylori* genotype status in saliva, dental plaques, stool and gastric biopsy samples. *World Journal Gastroenterol*. 18 (17): 2105-2111.

6. Rodopi E, Evangelos P, Christina P. (2021). Impact of Epigenetic Alterations in the Development of Oral Disease. *Bentham Science*; 28 (6):191-1103. [Pub Med] [Google Scholar]

7. Brown LM. (2000). *Helicobacter pylori*. Epidemiology and routes of transmission. *Epidemiol Rev.* 22(2):283-297.

8. Okuda K, Ishihara K, Miura T, et al. (2000). *Helicobacter pylori* may have only a transient presence in the oral Cavity, and on the surface of oral cancer. *Microbial Immunol*. 44 (5): 385-388.

9. Bago l. Bago J, Plecko V, Aurer A, et al. (2011). The effectiveness of systemic eradication therapy against oral *Helicobacter pylori*. *Journal Oral Pathol Med*. 40(5):428-432.

10. Nomura R, Ogaya Y, Matayoshi S, et al. (2018). Molecular and clinical analysis of *Helicobacter pylori* colonization in inflamed dental pulp. *BMC Oral Health.* 18(1): 64.

11. Bouad AF. (2002). Are antibiotics effective for endodontic pain? Evidence–based review. *Endod Top.* 3(1):52-66.

12. Hirsh C, Tegtmeyer N, Rohde M, et al. (2012). Live *Helicobacter pylori* in the root canal of endodontic–infected deciduous teeth. *Journal Gastroenterol.* 47(8) :936-940.

13. Umeda M, Kobayashi H, Takeuchi Y, et al. (2003). High prevalence of *Helicobacter pylori* detected by PCR in the oral cavities of periodontitis patients. *Journal Periodontal.* 74(1): 129-134.

14. Amiri N, Abiri R, Eyvazi M, et al. (2015). The frequency of *Helicobacter pylori* in dental plaque is possibly underestimated. *Archives of Oral Biology* 60 (5): 782-788.

15. Saliva DG, Stevens RH, Macedo JM, et al. (2009). Detection of cytotoxic genotypes of *Helicobacter pylori* in stomach, saliva, and dental plaque. *Arch Oral Biol.*; 54: 684-688.

16. Ying Liu. (2008). Study on the Relationship Between *Helicobacter pylori* in the Dental Plaque and the Occurrence of Dental Caries of Oral Hygiene Index. Aug; 13 (4):256-260.

17. Kolho K L. (2001). Dental Caries is Common in Finish Children Infected with *Helicobacter pylori Research Article Dis.*

18. Eskandari A; Mahmoudpour A, Abolfazli N, and Lafzi. A. (2010). detection of *Helicobacter pylori* using PCR in dental plaque patients with and without gastritis. Med Oral Patol Oral Cir Bucal.15(1): e28-31.

19. Abo –Lubad M, et al. (2017). Molecular Epidemiology of Helicobacter in Dental Plaque among Jurdanians; Aprobabol source for infection and treatment failure. *Journal Pure Appl Microbiolo*. 11(1):9-14.

20. Kolenbrander PE, London J. (1993). Adhere today, here tomorrow, Oral bacterial adherence. *Journal Bacteriol.* 175:3247-3252.

21. Isihara K, Mira T, Kimizuka R, et al. (1997). Oral bacteria inhibit Helicobacter *pylori* growth. *FEBS Microbiol Lett.* 152:355-361.

22. Madloom BM, Umran HH. (2021). *Helicobacter pylori* serology in a sample of Iraqi patients with chronic renal failure. *Med J Babylon* 18:28-31.

23. Clayton CL, Kleanthous H, Coates PJ, et al. (1992). Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. *J Clin Microbiol*. Jan; 30 (1):192-200. doi: 10.1128/jcm.30.1.192-200.1992. PMID: 1734052; PMCID: PMC265019.

24. Samaranayke L. (2012). Essential Microbiology for Dentistry. 4th ed. *Elsevier Ltd*. P: 279-285.

25. Al-Mahdi Z & Abood F. (2021). Systemic immunological responses among dental infection patients. *Inter J Immunol*. 9(1): 6-12

26. Al-Janabi1 A S; Al-Mahdi Z K; Alhamadi, W W et al. (2019). Fixed Orthodontic Appliance Associated with Change in Bacterial Diversity During First Stage of Active Orthodonotic Treatment. *Journal of Global Pharma Technology*. 11(03): 439-448.

27. Abas, K, A; Al-Mahdi Z; Merza I et al. (2022). Mutans Streptococci and Removable Orthodontics. *Indian Journal of Forensic Medicine & Toxicology. Vol. 14, DOI.No. 192964.*

28. Mohammed, Z H (2022). Study the bacterial profile and humoral immune response among patients with dental infection. Theses. College of Dentistry, University of Babylon.

29. Al-khafaji S F H, Al-mahdi Z K A & Alhamadi W W. (2023). Microbial distribution and secretory IgA level among crossbite patients at an early stage of comprehensive orthodontic treatment. 20(1): 160-167. DOI: 10.4103/MJBL.MJBL_326_22

30. Abd RS. (2017). Bacterial and immunological status of patients with orofacial. Infections in Babylon province. Theses. Collage of Dentistry, University of Babylon

31. Rowland, M., Daly, L., Vaughan, M., et al. (2006). Age-specific incidence of *Helicobacter pylori. Gastroenterology*, 130(1), 65–211. https://doi.org/ 10.1053/j.gastro.2005.11.004

32. Sissons, C. H., E. M. Hancock, and T. W. Cutress. (1988). The source of variation in ureolysis in artificial plaques cultured from human salivary bacteria. *Arch. Oral Biol.* 33:721–726. The regulation of production of urease by *H. pylori* by nitrogen availability

33. Kopstein J, Wrong OM. (1977). The origin and fate of salivary urea and ammonia in man. *Clin Sci Mol Med.* Jan; 52(1):9–17. *PubMed PMID:* 23916. [PubMed: 23916]

34. Dibdin GH, Dawes C. (1998). A mathematical model of the influence of salivary urea on the pH of fasted dental plaque and on the changes occurring during a cariogenic challenge. *Caries Res*. 32(1): 70–74. PubMed PMID: 9438574. [PubMed: 9438574]

35. Imfeld T, Birkhed D, Lingström P. (1995). Effect of urea in sugar-free chewing gums on pH recovery in human dental plaque evaluated with three different methods. Caries Res.; 29(3):172–180. *PubMed PMID*: 7621491. [PubMed: 7621491]

36. Kleinberg I. (1967). Effect of urea concentration on human plaque pH levels in situ. *Arch Oral Biol.* Dec; 12(12):1475–1484. *PubMed PMID*: 5237332. [PubMed: 5237332]

37. Sissons CH, Cutress TW. (1987). *In-vitro* urea-dependent pH-changes by human salivary bacteria and dispersed, artificial-mouth, bacterial plaques. *Arch Oral Biol.;* 32(3):181–189. *PubMed PMID*: 3478020. [PubMed: 3478020]

38. Morou-Bermudez E, Burne RA. (1999). Genetic and physiologic characterization of urease of *Actinomyces naeslundii*. *Infect. Immun*. Feb; 67(2):504–512. *PubMed PMID*: 9916052; PubMed Central PMCID: PMC96348. [PubMed: 9916052]

39. Shu M, Browngardt CM, Chen YY, et al. (2003). Role of urease enzymes in stability of a 10-species oral biofilm consortium cultivated in a constant-depth film fermenter. *Infect Immun*. Dec; 71(12):7188–7192. PubMed PMID: 14638814; *PubMed Central PMCID*: PMC308945.

40. Morou-Bermudez, E., & Burne, R. A. (2000). Analysis of urease expression in Actinomyces naeslundii WVU45. *Infection and immunity*, *68*(12), 6670–6676. https://doi.org/10.1128/IAI.68.12.6670-6676.2000

41. Morou-Bermudez, E., Elias-Boneta, A., Billings, R. J., et al. (2011). Urease activity in dental plaque and saliva of children during a three-year study period and its relationship with other caries risk factors. *Archives of oral biology*, 56(11), 1282–1289. https://doi.org/10.1016/j.archoralbio.2011.04.015

42. Abdulrahman,S M; Alzubaidy, Z M; Almashhadany, D A et al. (2022). Serological Diagnosis and Epidemiological impact of *Helicobacter pylori* infection on human health in Diyala Governorate, Iraq. *Tikrit Journal of Pure Science*, 27(3), 6–10. https://doi.org/10.25130/ tjps.v27i3.57 (Original work published November 30, 2022).

43. Ali, S H R; Salih S S; Sour T A H; Raouf G M & Rahim A L. (2021). Prevalence of *Helicobacter pylori* infection and its Associated Risk Factors among symptomatic Residents of Sulaimani city, Kurdistan region, Iraq, 2020. Kurdistan Journal of Applied Research (KJAR). 6 (1): pp. 1-12, https://doi.org/10.24017/science.2021.1.1

44. Zamani, M., Ebrahimtabar, F., Zamani, V., et al. (2018). Systematic review with meta-analysis: the worldwide prevalence of *Helicobacter pylori* infection. *Alimentary pharmacology & therapeutics*, *47*(7), 868–876. https://doi.org/10.1111/apt.14561