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Detection of Concentration of CagA Protein of H Pylori in Both Dental Caries Patients and Non -Caries Patients by ELISA

Shadha Alqaysi[1], Jehan Yawez Al-Demirchi[2], Aisha Hasan Mohamed[3]

[1,2,3] Department of Dentistry Al-Hadi University Collage, Baghdad -10011, Iraq

Abstract: Introduction: CagA protein, is a marker for the cag pathogenicity island which plays an important role in the etiology of H. pylori‐induced gastric pathologies. H pylori are detected in dental plaque, saliva and dental pulp. Saliva sample of 80 subjects with dental caries and free of caries is collected to detect the CagA level by ELISA technique. The results revealed no significant difference between two groups.

Conclusion: H-pylori are present in both groups which confirms that these bacteria alone cannot cause caries but it may cause later gastric ulcer.

Keywords: CagA protein, H pylori, ELISA, dental caries.

INTRODUCTION

H pylori is a gram-negative, microaerophilic, motile rod, with a distinctive spiral shape, and long flagella that assist with both motility and attachment within the stomach mucosal layer. H. pylori are carcinogenic bacteriapylori has been detected in samples of dental plaque, saliva, and dental pulp [1, 2,3,4]. Some studies have reported an association between H. pylori infection and its presence in the oral cavity. Okuda et al. reported that 12 of 54 H. pylori-infected subjects (22%) expressed H. pylori in their dental plaque. Similarly, Bagot et al. reported that 12 of 56

H. pylori-infected subjects (21%) possessed the bacterium in their saliva Thus, the presence of H. pylori in the oral cavity appears to be indicative of H. pylori infection.(1)

Evidence suggests that oral cavity temperature, pH, and microaerophilic conditions are suitable for *H. pylori* colonization, especially in dental biofilm [5,6]Therefore, simultaneous colonization has been reported in dental plaques and gastrointestinal biopsies [7]. However, the presence of this bacterium in dental plaque is associated with poor oral hygiene and could increase the risk of recurrence of gastrointestinal problems. Studies have also reported the incidence of *H. pylori*-associated halitosis, glossitis, aphthous stomatitis, and dental decay [8,9]

CagA H. pylori is genetically more diverse than most other bacterial species and the genetic diversity of several virulence factors, such as CagA and VacA, can be used as a tool for predicting the risk of developing various diseases. CagA encodes a 120‐ to 145‐kDa CagA protein, and is a marker for the cag pathogenicity island, this protein plays an important role in the etiology of H. pylori‐induced gastric pathologies. The severity of disease outcome could be attributed to possession of the Cag pathogenicity island, which encodes a type IV secretion system that facilitates translocation of the CagA protein [10]

MATERIAL AND METHOD

Subjects

This study was conducted at the University of Alhadi/ Dentistry departments from December 2022 until May 2023. The Ethics committee of the dentistry department, University of Alhadi approved the protocol. Before participating in the study; all patients were asked to sign an informed consent form after providing all information describing the purposes and aims of the study. The number of participant was about 90 students male and female divided into two groups 40 caries free and the other group with DMF more than one. their age between 20 and 25 years.

Saliva sample collection

Subjects were asked to rinse their mouth with tap water and then drooled the whole saliva into sterile tubes while seated in an upright position. A micropipette was used to aspirate a measured volume of the saliva of 500µl into a plastic Eppendorf tube. After collection, samples were centrifuged at 3000 rpm for 10 minutes by a Centrifuge machine to separate the cellular debris from the salivary supernatants. After being centrifuged and separated from the cellular debris, the salivary fluid was aspirated again, stored in a clean Eppendorf tube, and then frozen at -20^{0}C until analyzed by ELISA**.**

Measurement of CagA

Measurement of CagA: Detection of the levels of salivary CagA were determined by the commercially-available ELISA kit and done according to the guidelines present in the attached leaflet Shanghai/China. In brief, this assay uses a quantitative sandwich enzyme immunoassay method to evaluate human CagA in saliva. There is a pre-coated 96-well microplate with a CagA monoclonal antibody on it. The streptavidin-peroxidase combination detects CagA in samples and standards sandwiched by the first immobilized antibody in the well and a biotinylated antibody specific for CagA. A peroxidase enzyme substrate is then administered after any unbound material has been washed away. Within 10 minutes of applying the stop solution, the optical density OD value of each well was calculated using a microplate reader set to 450 nm.

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means in this study.[11]

RESULT

The level of CagA protein in both groups are shown in the table 1 and figure 1, which illustrates that mean level of salivary CagA was shown no statistically difference between two group.

Table 1: Comparison between Caries and Free groups in Detection of CagA

Group	No	Mean ±SE of Detection of CagA (ng/ml)	Range (Min. – Max.)
Caries	45	0.974 ± 0.037	0.0898 – 1.337
Free	45	1.041 ±0.031	0.340 – 1.309
T-test	---	0.0961 NS	---
P-value	---	0.169	---
NS: Non-Significant.			

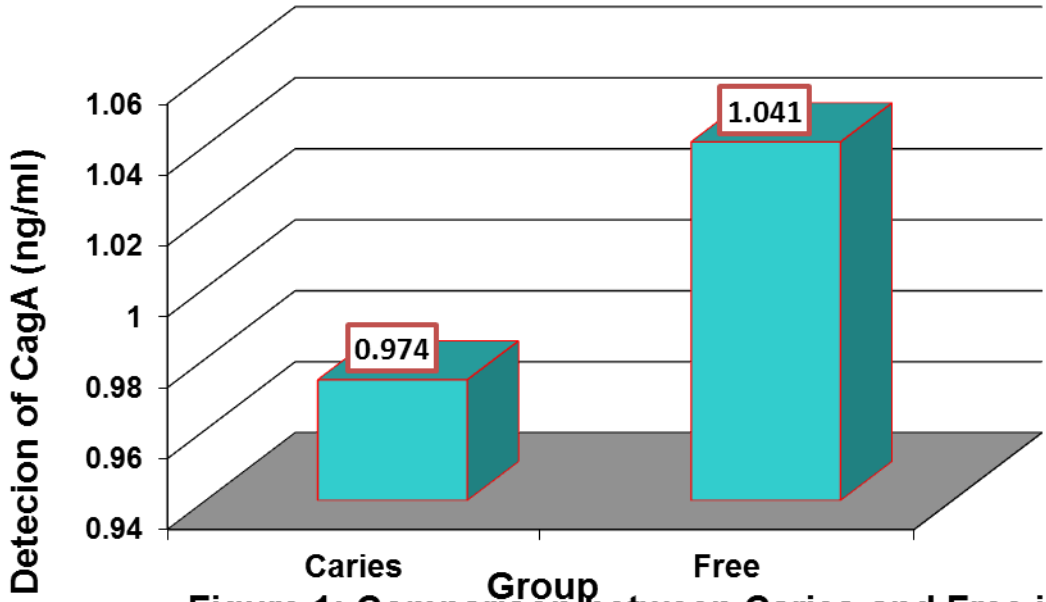

Figure 1: Comparison between Caries and Free in Detecion of CagA

DISCUSSION

The presence of CagA protein of H.pylori strains in both groups in saliva indicated that the saliva is potential reservoir of this bacterium. although the presence of this bacteria is increase the presence of dental caries according to Mehdipou et al [8], Zang et al [12] Sayud Abdul et al [13].

The reason for those subjects who had no caries with high CagA in their saliva is that the dental caries causes are multifactorial and many strains of bacteria are a causative factor of dental caries [14] these findings agreed with the finding done by Mehdipour *et al.* in addition there are certain strains are more virulent and cause more severe diseases than others [15].

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