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Molecular Detection of *DupA*-Positive *Helicobacter Pylori* from Dental Plaque of Periodontal Patients: A Prospective Study

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ABSTRACT

Introduction: This study aimed to detect *H. pylori* genome in dental plaque samples and determine the frequency of the *dupA* after bacterial culture and PCR assay.

Materials and Methods: In this prospective study, the inclusion criteria were as: age greater and equal to 20; the need for dental examination performed by a clinician, and finally no record of antibiotic uptake against H. pylori 30 days before sampling. All demographic information of included subjects such as gender, age, history of smoking, and anti-H. pylori therapy was also recorded. DNA was extracted and bacterial culturing was performed within 4 hours of sample receipt. A patient was declared positive for H. pylori when the confirmatory glmM PCR result.

Results: Of 40 symptomatic subjects, thirty-five were *H. pylori* positive (87.5%). Among the *H. pylori*-positive subjects, 28 (80%) were *dupA* positive. Meantime, for the subjects in the healthy group, 15 cases were *H. pylori* positive, and among this population 6 patients (40%) were *dupA* positive (*P* value <0.05). According to this study, it has been shown that in both groups with a periodontal pocket depth of 3-4 mm and without this depth *H. pylori* was detectable in the dental plaques, but this rate was significantly higher in the patient group (P=0.023).

Conclusions: Conclusively, the study can be the initial step in the designation of a specific bacterial gene to track the H. pylori infection and chance of associated diseases in clinical samples while discarding the endoscopic examination as the invasive method is the highest priority.

Keywords: Helicobacter pylori, dupA, dental plaque, human reservoir.

INTRODUCTION

Helicobacter pylori (*H. pylori*), a microorganism commonly found in human gastric mucosa, is closely linked with gastric epithelial cells (1-3). Recent cumulative shreds of evidence have emphasized that *H. pylori* can be isolated from about 50% of cases including antral biopsies and less than 40% from the esophageal biopsy sections (3, 4). Moreover, the human oral cavity acts as the potential reservoir for *H. pylori*; thus, the detection of *H. pylori* in the oral cavity mostly from symptomatic subjects also has undeniable clinical value for clinicians. It is now well-documented that *H. pylori* persists in the oral cavity for days to weeks. Nonetheless, various findings show that eliminated H. *pylori* infection in the human cavity is positively associated with reduced severity of gastroduodenal diseases in subsequent subjects (5-7). In 2005, the first virulence factor of *H. pylori*, which causes duodenal ulcers was discovered (8-10). Expressing the *dupA* gene was frequently associated with increased levels of the production of DupA, showing the release of inflammatory factors from mononuclear cells (11-15). Dental plaque is actually a bacterial mass found on the tooth surface due to the accumulation of hundreds of different bacterial species, such as Gram-negative and Gram-positive bacteria (16, 17). The

composition of dental plaque is an important ecological site for hosting different bacterial species through the formation of biofilms (18-20). In the context of periodontitis-associated biofilms, inflammation of the periodontal tissue causes the creation of pores for anaerobic bacteria to grow and increases the secretion of nutrient-rich gingival fluid. On the other hand, biofilms also contain a large number of Gram-negative anaerobic bacteria (21-23). Low oxygen levels, microaerophilic environments, and average oral temperatures of 35 to 37°C are ideal conditions for *H. pylori* growth. (24, 25). As we know, the presence of *H. pylori* in the stomach is undeniable, in parallel, the abundance of the bacterium in dental plaque has also been proven (26-28). The PCR technique was one of the diagnostic methods and the DNA of the bacteria can be detected regardless of whether it is alive. Also, with this method, the organism can be specifically detected (29-31). Cultivation of dental plaque samples to detect *H. pylori* is often of low efficacy because the main biotype of *H. pylori* in dental plaque is the coccid form and due to its microaerophilic nature and structure, it leads to unacceptable culture efficiency (32, 33). Some researchers have suggested that dental plaque can be used to definitively diagnose *H. pylori* colonization in the stomach, but others have suggested that its presence in the oral cavity is only transient and does not cause adverse effects (34, 37). Accordingly, *H. pylori* is a common pathogen in dental plaque. *H. pylori* in the oral cavity can fight those antibiotics prescribed to eliminate this bacterium from the stomach (39, 40). Current failed therapeutic regimens against *H. pylori* show that carrier subjects have a dramatically high rate of re-infection which highlights the risk of recurrence (41,42). In this regard, many studies have been conducted to determine the relationship between dental plaque and periodontal disease with *H. pylori* infection, the same as our study group aimed (43, 44).

MATERIALS AND METHODS

Patients and dental plaque samples

In this prospective study, the inclusion criteria were as: age greater and equal to 20; the need for dental examination performed by a clinician, and finally no record of antibiotic uptake against *H. pylori* 30 days before sampling. 80 patients referred to the periodontal department of the dental hospital were selected. All demographic information included subjects such as gender, age, history of smoking, and anti-*H. pylori* therapy was also recorded. These 80 patients were divided into two groups of 40: the healthy group (with a good gum condition) and the sick group, which had improper periodontal pockets and the depth of the gingival groove (the distance between the gum and the tooth due to infection and gingivitis) of about 3-4 mm or more. Subjects in this category were usually in the age group over 40 or under 12 years old. The depth of the gum in normal inflammations is about 2 mm, but in a sick group it is much more and it is to the extent that in some cases the gum is separate and free, in this model of people, we can also see the erosion of the gum. The healthy group includes people who do not have the characteristics of the disease group, that is, do not have deep pockets of 3-4 or more, this group of people usually does not have periodontal pockets. After a general examination of the internal oral hygiene, specimens were taken from the supragingival and subgingival plaque of the molars and canines with a sterile periodontal probe and transferred to physiological saline at 4 degrees Celsius. Thioglycollate broth (Merck, Germany) was used to ship the dental plaque microbiology laboratory at 4°C within 4 hours. To perform bacterial culture, the specimen was crushed by a surgical razor and pulled in to the Columbia agar medium (Himedia, India) supplemented with 10% fetal bovine serum (Sigma, USA), defibrinated sheep blood 8% (Himedia, India), Campylobacter supplement (Merck, Germany), amphotericin B, vancomycin, and trimethoprim. The inoculated agar plates were put at microaerophilic conditions 7% carbon dioxide at 37°C in an incubator. The methods, including dental plaque (Figure 1) collection, and the study protocol were reviewed by the institutional review board and approved by the medical ethics committee of the Tarbiat Modares University, Tehran, Iran (IR. MODARES. REC. 1399. 123). Written informed consent was obtained from all admitted subjects.

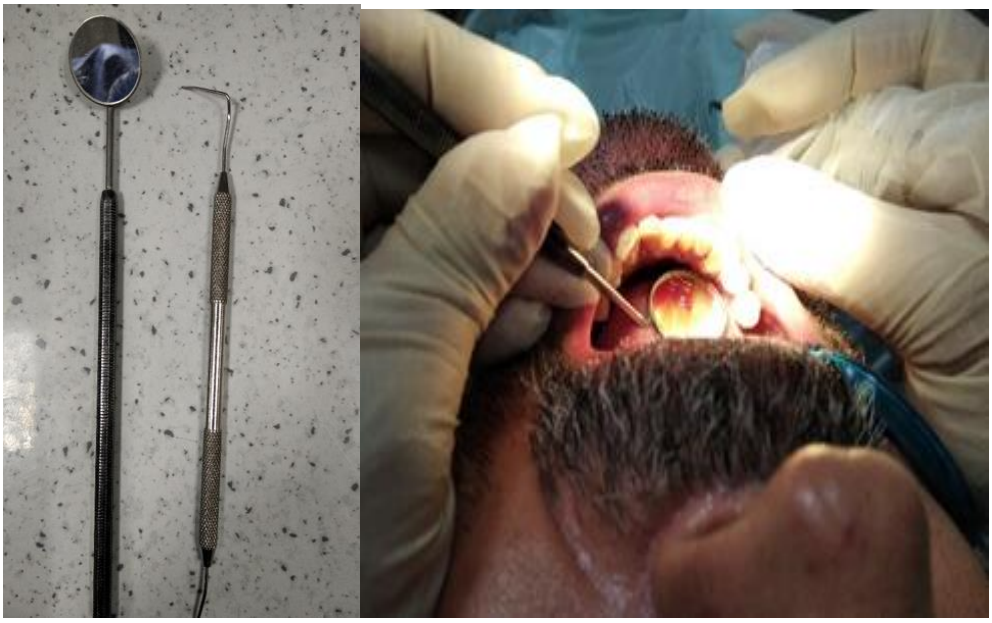

Figure 1: Sampling the dental plaques in clinic

Table1- Primer sequences and PCR cycles

Genes	Primer sequences	PCR condition	Product size
glmM	GCT ATG ACG GGT ATC C GAT TTT ACC CCT ACA CCA	94 ◦C, 5min; 94 ◦C, 1 min; 60 ◦C, 1 min (36 cycles);72◦C, 60 min; 72, 10 min	298 bp
dupA	TTC ATC AGT ATC TTT TGT GG CAT GGC GTT CAA AAA ATA T	94 ◦C, 5min; 94 ◦C, 1 min; 60 ◦C, 1 min (36 cycles);72◦C, 60 min; 72, 10 min	112 bp

DNA extraction and *dupA* PCR

DNA was extracted and bacterial culturing was performed within 4 hours of sample receipt. A patient was declared as positive for *H. pylori* when the confirmatory *glmM* PCR result. We did not further process any dental plaque specimen which had a negative bacterial culture. The plates were incubated at 37 ◦C under a microaerophilic condition (5% O2, 10% CO2, and 85% N2 with up to 95% humidity) for 7–10 days. *H. pylori* growth was confirmed by visualization of translucent, bacterial colonies and Gram-negative staining *glmM* PCR was used to confirm the genetic identity of the selected colonies. A commercial DNA-extraction kit (Roje, Tehran, Iran) was used according to the manufacturer's instructions. The concentration of the purified DNA was measured at 260 nm using a spectrophotometer. Extracted DNA was stored at − 20 ◦C and all extracted DNA was applied to determine existence of *glmM*-specific PCR to genetically confirm *H. pylori* colonization. PCR setups for *dupA* and *glmM* were showed as detailed in Table 1. After the completion of PCR, the amplified samples are taken for electrophoresis on a 1% gel and the result is matched with DNA Ladder 100 bp molecular marker to confirm the presence of 112 and 298 bp fragment.

Statistical Analyses

Data for each group were initially collected and analysed by SPSS version 22.0. A $P < 0.05$ was taken as statistical significance for our analysis.

RESULT

In this study, we found no positive bacterial cultures, prompting us to shift to molecular analysis of the *dupA* gene using PCR (Tables 1, 2). There was no significant differences between

sex distribution, age groups and disease background among the all included subjects (P value > 0.05). During the testing process, the conditions for both groups are considered to be almost the same, and the dental plaque samples of each patient were separately subjected to DNA extraction and finally examined in terms of the frequency of the *dupA* gene (Figure 2).

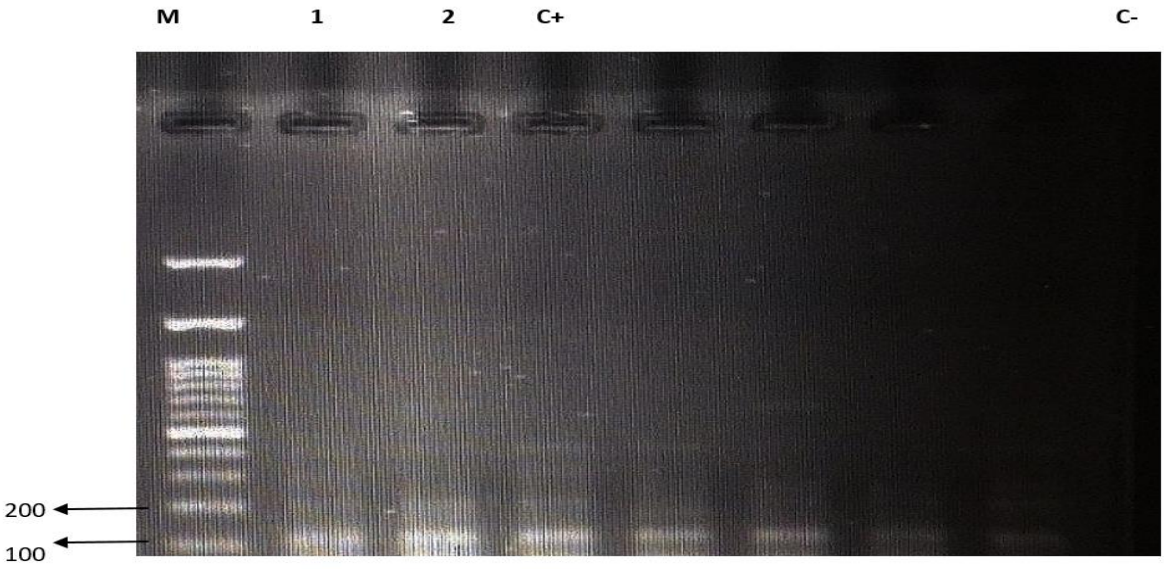

Figure 2: Gel-electrophoresis of *dupA* positive *Helicobacter pylori* isolates

(Lanes 1 and 2 are clinical samples)

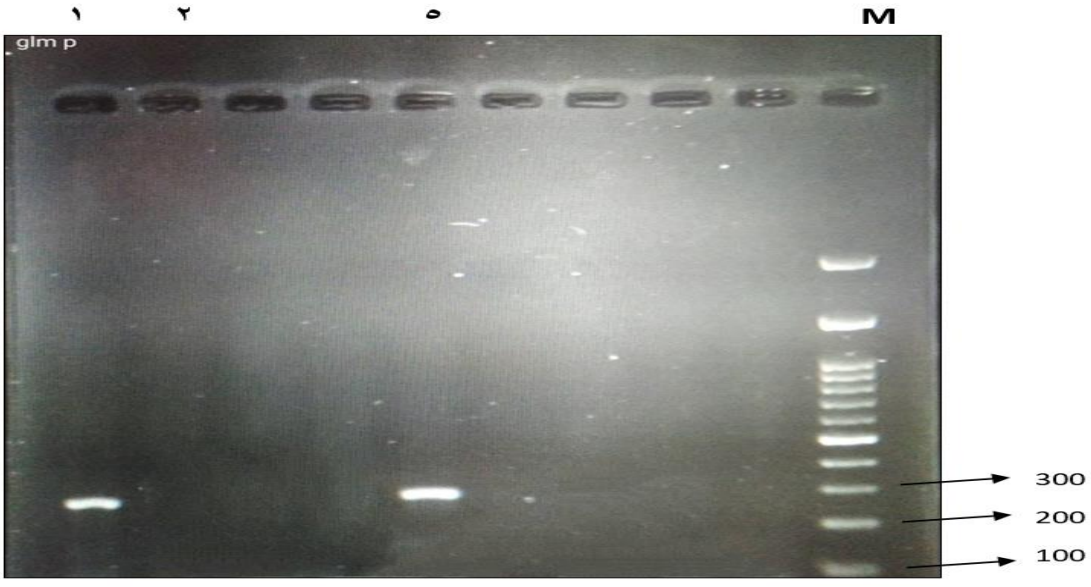

Figure 3: Gel-electrophoresis of *glmM* (298bp) as genetic control for all *H. pylori* clinical samples in our study

Lane 1,2,3,5 are as positive and negative clinical samples,

According to the findings in the PCR assay, it was observed that in each patient and healthy group, *H. pylori* was detected in the dental plaque of both groups. Still, this rate was significantly higher in the patient group. Additionally, the presence of *H. pylori* in the patient group was estimated at 87.5% and in the healthy group at 37.5%. Also, in the population of 40 people in the patient group, we saw that 35 of them were *H. pylori* positive, and among this positive population, 80% were *dupA* positive. Similarly, in the healthy group, among the population of forty cases, 15 samples were H. pylori positive which included 40% *dupA* positive, and these reports indicate that the presence of *H. pylori* in the mouth leads to the recurrence of infection.

Number	*H.pylori* positive	*dupA*
Patient group (n=40)	87.5% (35/40)	80%(28/35)
healthy group (n=40)	37.5% (15/40)	40%(6/15)

Table 2: Distribution of *dupA*-positive *H. pylori* isolates among the 80 cases

DISCUSSION

H. pylori, a spiral-shaped Gram-negative bacterium is one of the hard-to-cultivate bacteria that survive in harsh gastric microniche attributed with a wide range of digestive diseases. It is also frequently repeated by the World Health Organization (WHO) that *H. pylori* is one of the carcinogenic agents that need intensive attention in treatment and management (45,46). The prevalence of *H. pylori* has been reported in developed countries and mostly in developing countries (47). It is now agreed that this bacterium is usually transmitted during childhood (48,49). To now, the stomach was assumed as the main reservoir of this bacteria, while forthcoming data indicates that there are several potential reservoirs for long-life colonization; this was a major assumption in this study (50,51). This bacterium can be present in the depth of the gum groove and dental plaque in anaerobic or microaerophilic conditions along with other bacteria present in the mouth and can interact with other gram-positive and negative bacteria in the mouth. The mouth slows down the creation of a microbial biofilm, although in this case the bacteria was in its non-cultivable and coccid form and was not able to be cultivated (52,59). Regardless of the result of the current project, the presence and participation of *H. pylori* infection in the mouth is still controversial (53,45). Some studies show various results, for example, in the study of Shams and colleagues, using the RFLP technique, it was shown that gastric and oral *H. pylori* strains have the same genetically root, whilst, Song et al found completely different results and showed that gastric and oral *H. pylori* strains are fed by at least two different ancestral strains (57,58). According to our findings, it was observed that in both groups with a periodontal pocket depth of 3-4 mm and without this depth, *H. pylori* was detectable in the dental plaques, but this amount was significantly higher in the patient group (P=0.023). As such, the presence of *dupA* as *H. pylori* virulence gene could be used to predict certain periodontal diseases, but pending further molecular confirmatory studies. On the other hand, it should be noted that the presence of this bacterium alone in the mouth is not pathogenic, unless it enters into the stomach and experiences successful colonization, digestive symptoms will not appear. Our main study limitation was the lack of full demographic and digestive complaints reports by the patients. If so, we could have made a strong statistical analysis to determine the association between the presence of certain pathogenic *dupA*-positive *H. pylori* and different gastroduodenal disorders. No doubt that some recent meta-analyses showed the fact that human periodontal diseases and *H. pylori* infection can correlate but the ultimate conclusion needs further molecular and wide-range genotypic analysis.

CONCLUSION

It has been confirmed that *H. pylori* exists only in dental root canals, and not in the plaque. According to the report, the relatively high rate of *dupA* as a virulence gene in the *H. pylori* DNA

recovered from dental plaque might potentially be used as a marker or predicting tool for certain periodontal diseases. Therefore, the study can be the initial step in the designation of a specific bacterial gene to track the *H. pylori* infection. Studying *H. pylori* infection and associated disease risk in clinical specimens, while eliminating endoscopic examination as an invasive method, is a priority. Furthermore, our results highlight the potential for *H. pylori* to contribute to recurrent infection and persistent disease in some subjects after repeated antibiotic treatment.

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