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Molecular Epidemiology of *Helicobacter pylori* in Dental Plaque among Jordanians; A Probable Source for Infection and Treatment Failure

Mohammad Abu-Lubad[1]*, Hamed Alzoubi[1], Dua'a Jarajreh[1], Alaa Al Sawalqa[2] and Munir Al-zeer[3]

[1]Department of Medical Microbiology and Immunology,
Mutah University Faculty of Medicine, Jordan.
[2]Royal medical services, Department of Dentistry, King Hussein Medical Center.
[3]Department of Molecular Biology, Max Planck Institute of Infection Biology, Berlin, Germany.

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The human pathogen *Helicobacter pylori* infects around 50% of the global population. Significant proportion of infected individuals with *H. pylori* can undergo gastritis or gastric adenocarcinoma. The major source and reservoir of infection and transmission with *H. pylori* is not fully understood, yet. Oral cavity has been proposed to be a reservoir for *H. pylori* and possibly a major source of gastric reinfection; however, the results are still controversial. In this study a total of sixty Jordanian individuals were tested for the presence of *H. pylori* in their dental plaques. Samples were analyzed by PCR to detect for the presence of *H. pylori* using specific primers for *H. pylori* 16s ribosomal RNA gene. Our data show that all individuals tested in this study were found positive for *H. pylori*. Although, individuals tested in this study were heterogeneous in term of age, sex, education, and geographical region but this did not influence the presence of*H. pylori* in the dental plaque of Jordanians. The results of the present study suggest that the oral reinfection route of transmission of *H. pylori* should be taken into consideration because it may explain the high prevalence of *H. pylori* associated gastritis in Jordanian people.

Keywords: DNA: deoxyribonucleic acid, MALT: Mucosa associated lymphoid tissue lymphoma, PCR: polymerase chain reaction, bp: base pair.

Helicobacter pylori is a Gram negative spiral-shaped, microaerophilic bacterium[1,2].*H. pylori* infection is a major factor in the development of chronic gastritis, gastric and duodenal ulcers[3,4]. *H. pylori* is classified as a Group one Carcinogen based on the world health organization because it is associated with gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma[5,6]. Therefore, it has been suggested that the proper eradication of *H. pylori* is an important tool for a successful treatment of many *H. pylori* related diseases[2,7,8]. The risk of *H. pylori* infection is associated with several factors including the age, hygienic conditions, geographical region, and socioeconomic status[9]. It is estimated that 50% human population are infected with *H. pylori* (25-40% in developed and almost 100% in developing countries)[10,11]. Despite the high prevalence of *H. pylori* in the stomachs of the world's population, the mode of transmission is not yet completely known[12]. Evidences for the possible route of *H. pylori i*nfection are including oral-oral and fecal-oral are provided, but the predominant route of infection has not been proved[13].About 30 years

* To whom all correspondence should be addressed.
Tel.:+962 3 238 6287; fax: +962 3 239 7180;
E-mail: Abu_lubbad@yahoo.com

ago, *H. pylori* was successfully isolated and cultured from the human stomach[14]. *H. pylori* was then isolated from extra-gastric regions such as tonsillar and adenoid tissues[2], saliva, feces, vomitus[15], and dental plaque[16].

Dental plaque is a biofilm of bacterial growth on the surfaces within the mouth[17]. Plaque is associated with oral diseases such as cavities and periodontal diseases and its formation is inevitable process. Bacteria utilize biofilms as a barrier to protect themselves from the attack of the host immune response and from the effect of antibiotics[18]. *H. pylori* is considered a component of the dental plaque which contains more than 600 different microorganisms[13,19-22]. However, several studies have concluded that the oral cavity might be the primary reservoir of *H. pylori* infection. These findings are still controversial, and the exact route and source of *H. pylori* infection remains elusive[15]. *H. pylori* isolation and culture from the oral cavity is difficult due to the limitation of the use of microaerophilic condition, the long incubation period, the over growth of other oral bacteria, and the inhibitory effect of the oral cavity bacteria on *H. pylori*. Therefore, polymerase chain reaction (PCR) has been utilized as a reliable tool to detect *H. pylori* to overcome the difficulties of isolating and culturing *H. pylori* from the oral biofilm[23,24]. Nowadays, PCR is considered an acceptable method for *H. Pylori* diagnosis and detection form oral cavity samples[21]. Different genes can be utilized to screen for *H. pylori* form oral cavity using PCR; including urease, the 16S ribosomal RNA, and the adhesion genes[25,26].The prevalence of *H. pylori* in the oral cavities in subjects has not yet been investigated in Jordan. In this study, we aimed to determine the prevalence of *H. pylori* in oral biofilms among Jordanian volunteers to better understand the correlation of *H. pylori* transmission to explain the high percentage of *H. pylori* associated gastric disease in Jordan[27].

MATERIALS AND METHODS

Study population

A total of 60 dental plaque samples were collected from Jordanian individuals who attended outpatient dental clinic at the medical center of Mutah University. Thirty samples out of 60 were collected from students and the remaining samples were collected from employees working at Mutah University and other visitors. The study population comprised individuals aged 18-52 years. All subjects were interviewed by a questionnaire for socioeconomic and health information. The selection criteria for the sixty volunteers excluded smokers and those who were under antibiotics treatment during the previous four weeks of the sample collection. All volunteers showed good oral hygiene and no oral ulcers. Individuals were asked for the frequency of teeth brushing which was ranging from none to three times daily. Samples were collected after signing an informed consent from all participants of this study which was approved from the scientific research ethical committee in the faculty of medicine at Mutah University.

Dental plaque sample collection

Samples were collected from the surface of teeth with sterile curettes[28] from all participants who did not brush their teeth one morning before the samples collection. The collected samples were transferred into 1.5 ml sterile Eppendorf tubes containing 1ml sterile physiological saline solution. Samples were stored at -20p C until tested.

DNA extraction

Genomic deoxyribonucleic acid DNA was isolated and purified using extraction mini kit (OMEGA, Bio-TEK) according to the manufacturer's instructions. Briefly, samples were thawed then centrifuged at 12,000rpm for 2 min at room temperature to precipitate the dental plaque materials. Supernatants were discarded and the genomic DNA was isolated from the sediments according to the manufacturer's instructions. DNA was eluted in 100 ìl nuclease free water, quantified on a UV-nanospectrophotometer (Quawell Technology Inc, USA), and stored at -20°C until tested. Genomic DNA from *H. pylori* (P12 strain) was used as a positive control.

PCR primers and amplification

The bacterial 16s ribosomal RNA gene was used as a target gene for amplification to detect the presence of *H. pylori* using PCR. Forward primer 5'-GAAGATAATGACGGTATCTAAC-3 and the reverse primer 5-ATTTCACACCTGACTGACTAT-3' were used to amplify a 16s ribosomal RNA segment of 150bp (figure 1). Each amplification

reaction was performed using Applied Biosystems thermal cycler in a volume of 50 ìl containing 40 ng of the extracted DNA and 1 ìl of each primer with a final concentration of 10 pmol. The amplification cycling consisted of initial denaturation at 98p C for 30 sec then by40 cycles as follow: denaturation at 98p C for 7sec, annealing at57p C for 30 sec, and extension at 72p C for 30 sec. The ûnal DNA extension cycle was performed at 72p C for 7 min. *H. pylori* DNA positive control was used as in each PCR reaction. All amplification products were analyzed by agarose gel electrophoresis, and the DNA bands were then visualized using Thermo Fischer Scientific gel documentation system.

RESULTS

In this cross sectional study, a total of 60 dental plaque samples were collected from healthy Jordanian participants. The ages of the participants were between 18 and 52 years old with a mean age of 25.95. Forty one samples were males (68.3%) with a mean age of 23.1 years, while 19 samples were females (31.7%) with a mean age of 25.4 years. Samples were collected randomly from the participants taking into consideration the variation in the social, economic, age, sex, occupational, and educational background as well as the hygienic conditions of the volunteers' teeth. The PCR results showed that all dental plaque samples were positive for *H. pylori* as shown in table 1.

DISCUSSION

H. pylori infection is one of the most common bacterial infections in human. The role of *H. pylori* infection has been proved in the development of chronic gastritis, peptic ulcer, gastric cancer, and MALT lymphoma[3,4]. Therefore, the proper control of *H. pylori* infection would reduce the rate of the gastric associated diseases[3]. One of the most important control approaches is identifying the mode of transmission[3] because the failure in *H. pylori* treatment has been found to be in part due to reinfection from extragastric sources[12,29]. The human stomach was considered to be the only reservoir for *H. pylori* until pathogen was detected in extragastric sources such as the dental plaque, water, and saliva [6,30,31].

Several difficulties limit the specificity and accuracy of *H. pylori* identification and diagnosis from oral samples using conventional methods such as bacterial culture. Urease assay was considered as a useful tool to test for the presence of *H. pylori*, however this test is not indicative for *H. pylori* infection as other bacteria produce urease such as *Streptococcus* spp., *Hemophilius* spp., and *Actinomyces*spp.[12]. In addition, *H. pylori* can transform from its normal helical morphology to a coccoid form which cannot be cultured *in vitro*. This unique life style of *H. pylori* is very important determinant for the underestimated prevalence of

Table 1. The frequency of *H. pylori* in dental plaque samples in Jordanian participants in relation to age and gender

Age	Number	Gender	Percent of *H. pylori*
15-29	30	M: 20 F: 10	100%
30-44	17	M: 12 F: 5	100%
45-60	13	M: 9 F: 4	100%

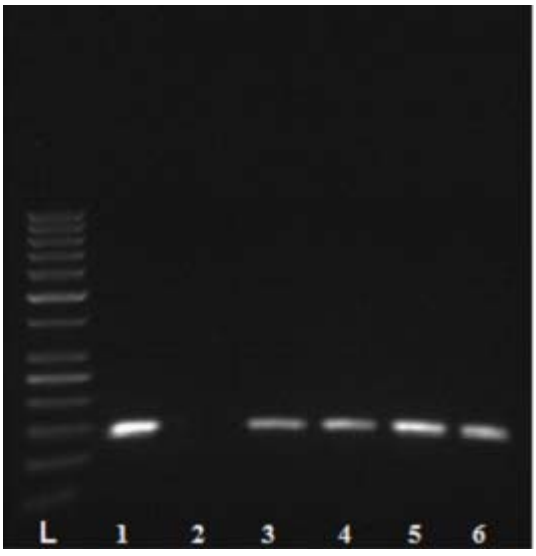

Fig. 1. PCR products gel electrophoresis for the detection of *H. pylori* in the dental plaque samples. L: 50 bp DNA ladder, lane 1: positive control, lane 2: negative control, lanes 3 to 6: random samples from some participants. PCR: polymerase chain reaction, L: Marker, bp: base pair

H. pylori among human using the conventional culture methods[32]. Based on that, PCR is considered a useful tool for *H. pylori* diagnosis from the oral cavity as it is highly sensitive compared to the other conventional methods[23,24]. Many studies have demonstrated the sensitivity and specificity of PCR for the detection of *H. pylori* in clinical samples. PCR was able to detect *H. pylori* in four biopsies that were tested negative using the conventional culture *in vitro*[33]. Further, using PCR confirmed that 15 out of 23 gastric biopsies were positive for *H. Pylori* compared with only seven samples confirmed positive for *H. pylori* using the conventional culture techniques[34].

In the present study, the molecular prevalence of *H. pylori* among Jordanian individuals was 100% regardless of age, gender, hygienic parameters, and geographic distribution. In agreement with our data, *H. pylori* was detected in the dental plaque of 40/40 (100%) asymptomatic Indian volunteers[35]. Similar data was found among German patients tested for *H. pylori* in the oral cavity (97%)[31]. Other studies showed different prevalence rate in the presence of *H. pylori* in the dental plaque samples, for example 73% of dyspeptic Pakistani patients were positive for *H. pylori*[28]compared with 65.6% in Polish volunteers[36] and 65% in Saudi patients with dyspepsia[37]. Lower prevalence rate was found (37.5%) in Venezuelan patients who had chronic gastritis[38].On the contrary, *H. pylori* was not detected in 52 Sweden patients who had positive culture for *H. pylori* from gastric biopsies[39]. Similarly, *H. pylori* was not detected from any of the 290 oral samples of non-dyspeptic French population[40]. Further, dental plaque samples were analyzed from 43 Brazilian patients with gastric disease and found to be *H. pylori* negative[41].

There is a controversy in the importance of mouth hygienic conditions in the colonization of *H. pylori* in the buccal cavity. It was found that the occurrence of *H. pylori* antigens in dental plaque of natural teeth is not associated with oral health status[36]. While, others contraindicated that and found the oral health parameters are determinants for the colonization of *H. pylori* in the dental plaque[42].Some reports suggested that the failure in *H. pylori* treatment has been linked to be in part to the reinfection from the oral cavity[12,29,38]. It has been demonstrated that the dental plaque unaffected by the triple drug therapy of *H. pylori* which might raise an alarm for the possibility of gastric reinfection from the dental plaque after a successful therapy[43]. Therefore, the detection of *H. pylori* in dental plaque samples is considered a noninvasive procedure compared to the endoscopy method and it might be used as an important tool to monitor the efficiency of treatment of *H. pylori* associated gastritis[43].

Although the prevalence of the *H. pylori* in dental plaque was shown to be very low in some studies, our current study showed that the prevalence of *H. pylori* in the dental plaque samples from Jordanian people is unexpectedly high and unexplained. Our data might explain the high prevalence (78%) of *H. pylori* among Jordanian patients with gastric diseases[27]. It is necessary to pay close attention to the dental plaque as a possible reservoir of *H. pylori* and a possible source of reinfection and infection among Jordanians.

In conclusion, our study proposes the significance of the oral-oral route of *H. pylori* transmission among Jordanian people due to the 100% prevalence of H. *pylori* in the selected dental plaque samples which mandates protective measures to prevent the transmission to possibly people with negative dental plaque for H. pylori and to follow new treatment strategies for patients with gastritis and positive for *H. pylori* in their dental plaques.

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