Dental risk factors associated with oral *Helicobacter pylori* infection: a cross-sectional study based on saliva antigen test

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Abstract

Introduction: Besides stomach, the oral cavity is the second reservoir of *Helicobacter pylori* (*H. pylori*) that plays an important role in oral diseases and recurrent gastric infection. This study aimed to determine the risk factors of oral *H. pylori* infection for better human health.

Methodology: Saliva samples from 280 subjects who visited the dental clinics were collected for the *H. pylori* antigen test. The data regarding age, gender, residence, frequency of tooth brushing, presence of dental caries and/or periodontitis were reported for each participant. Stool antigen *Helicobacter pylori* test was used to detect gastric infection.

Results: The overall prevalence of *H. pylori* in oral cavity and stool were 40.4%; 95% CI [34.8–46.2], and 36.4%; 95% CI [31–42.2], respectively and were not statistically significant (*p* = 0.546). The differences of positive rates of *H. pylori* infection according to the presence of periodontitis (54.7% vs 30.1%, *p* < 0.001, OR; 95% CI: 2.8 (1.7-4.6)) and dental caries (47.1% vs 32%, *p* = 0.001, OR; 95% CI: 2.2 (1.3-3.5)) were statistically significant. Living in rural areas increases the risk of acquiring *H. pylori* infection compared with urban areas (46.9% vs 34.7%, *p* < 0.001, OR; 95% CI: 0.6 (0.4-1)).

Conclusions: The *H. pylori* antigen test on saliva could be used as evidence of gastric infection. Oral diseases including periodontal diseases and caries are important risk factors for *H. pylori* colonization, so the professional treatment of these diseases may reduce the rate of new and recurrent gastric infection by *H. pylori*.

Key words: *H. pylori*, oral cavity, saliva periodontitis, oral diseases.


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Introduction

*Helicobacter pylori* (*H. pylori*) is one of the most common human bacterial pathogens worldwide, with 4.4 billion infected individuals in 2015 [1]. A higher incidence was reported in unhygienic and economically poor areas; the rate of *H. pylori* infection in Africa, South America, and Asia was significantly higher than that in Western Europe, North America, and Australia [2].

*H. pylori* is a motile, microaerophilic Gram-negative rod that has a specific spiral shape. Although over 80% of the infected individuals are asymptomatic [3,4], *H. pylori* infection may play an important role in many gastric diseases according to numerous studies that have confirmed its contributions to gastritis, peptic ulcers, and gastric malignancies [5]. Recent studies have shown that the eradication of *H. pylori* in infected individuals of all ages can reduce the occurrence of gastric cancers [6,7].

Several scientists have successfully isolated *H. pylori* from many oral specimens such as dental plaque, saliva, and dental pulp [8–10]. Nowadays, the oral cavity is considered as the second most significant reservoir for *H. pylori* preceded by the stomach [11]. Consequently, *H. pylori* infection can be acquired through the oral-oral route or the fecal-oral route [12]. Furthermore, some studies suggest cross-infection between gastric *H. pylori* and oral *H. pylori* based on the similarities between the strains of *H. pylori* in the mouth and stomach [13,14]. Additionally, the existence of *H. pylori* in the oral cavity per se can cause different diseases including periodontitis and oral mucosal ulcers [15]. Therefore, this cross-sectional study was conducted to understand the risk factors of oral *H. pylori* infection. The understanding of risk factors is a prerequisite for preventing oral and gastric infections and their consequences.

Methodology

Study design and subjects

All the participants in this study were subjects that had their dental examination at different dental clinics in Erbil-Kurdistan Region between July and December 2019. Those subjects who had at least one of the
following criteria were excluded from the study: treatment with antibiotics or proton pump inhibitors (PPI) within the last four weeks, and oral diseases treatment during the last six months. Participants of similar age, gender, and residing in nearby locations were grouped together.

All participants were informed about the objectives and the protocols of the study and were assured that all data will be treated confidentially. Thereafter, the participants gave their consent to be included in the study. The study was approved by the Dental Clinics Ethics Committee and was conducted according to the Helsinki ethical principles declaration.

A structured questionnaire was used to collect information about gender, age, residence site, and the frequency of tooth brushing. The diagnosis of oral cavity diseases (periodontitis, caries) was based on the report of the dentist.

Detection of \textit{H. pylori} antigen in the saliva

Saliva samples were collected from each subject according to the Manual of Procedures for the Human Microbiome Project [16]. Briefly, the subject was asked to retain saliva in the mouth for at least 1 minute before spitting at least 0.5 mL saliva into a sterile 50-mL collection tube (Falcon, conical polypropylene tube with flat-top screw cap).

The saliva \textit{H. pylori} antigen Test (Ameritek Diagnostic Reagent Co., Ltd, Jiaxing, China) is a rapid immunochromatographic assay that uses a monoclonal antibody to detect urease in saliva [17]. According to the manual, the test should be done within five minutes by adding saliva and buffer solution into the sample well and observing the result within 20-30 min. As the test kit begins to work, the red color moves across the result window in the center of the test strip. The presence of 2 red-colored bands (‘T’ band and ‘C’ band) within the result window indicates a positive result. The presence of only 1 color band (C band) indicates a negative result. If there is no control band, the sample is invalid [18,19].

Stool collection and detection of \textit{H. pylori} infection

Detection of \textit{H. pylori} antigen in stool samples was done by the One-Step \textit{H. pylori} Antigen Test Card (CTK Biotech, Inc., California, USA). Briefly, using the cap-attached applicator stick, approximately 100-200 mg of stool sample were transferred into the test buffer bottle and mixed thoroughly by shaking the bottle for a few seconds. The bottles were held properly in a vertical position to drop about 120-150 μL (3 drops) of diluted stool sample into the sample hole on the test card. The result was read within 10-15 minutes. A positive result is indicated by the appearance of two red lines (T and C bands), while only one red line at the C band is interpreted as a negative result. A strong positive sample may show the result in less than 10 minutes [18].

Statistical analysis

We used the statistical software program VassarStats [19] to calculate Odds Ratio (OR); 95% Confidence Intervals (CI); and Risk Ratio (RR). Chi-square test was used to compare groups; \(p\) values less than 0.05 indicated statistical significance.

Results

A total of 280 participants were involved in this study, including 140 males and 140 females. Their mean age was 51.79 ± 9.1 years, and the age group between 45 to 60 years was the most represented (40%; 112/280). 53.6% of the subjects were living in the urban zone and 46.4% were living in rural areas. One hundred and fifty-five subjects (53.8%) had dental caries, and 117 (41.8%) had periodontitis. Regarding the tooth brushing habits, 92 (32.9%) brushed once daily, 65 (23.2%) brushed twice daily, and 123 (43.9%) did not brush at all.

The percentages of positive participants for \textit{H. pylori} antigen in the saliva and the stool were 40.4% (113/280; 95% CI [34.8–46.2]), and 36.4% (102/280; 95% CI [31–42.2]), respectively. There was no significant difference between the subject-wise positivity via saliva and stool (\(p = 0.546\)) (Figure 1).

The results of \textit{H. pylori} detection showed that 37.1% (52/140) males and 43.6% (61/140) females were positive for \textit{H. pylori} antigen in the saliva. Although female subjects had higher positivity than males, \(\chi^2\) test demonstrated that the difference was not

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Positive rate of \textit{H. pylori} infection by stool antigen test and saliva antigen test.}
\end{figure}
The presence of *H. pylori* in different age groups were: 40.4% (19/47) in the < 30 years age group; 43.8% (28/64) in subjects of 30 to 44 years age group; 41.1% (46/112) in subjects of 45 to 60 years age group; and 35.1% (20/57) in subjects > 60 years of age. The differences among age groups were not statistically significant ($p = 0.391$). Furthermore, 34.7% (52/150) of urban residents and 46.9% (61/130) of rural residents were positive for *H. pylori*. This difference between urban and rural residence was statistically significant ($p = 0.038$).

Regarding the association between saliva antigen positivity and oral diseases, *H. pylori* was detected in 54.7% (64/117) among subjects with periodontitis, and in 47.1% (73/155) among subjects with dental caries. On the other hand, the prevalence of *H. pylori* among participants without periodontitis and caries were 30.1% (49/163) and 32% (40/125), respectively. This difference in the prevalence was statistically significant ($p < 0.001$).

There was no statistically significant difference between positive *H. pylori* antigen results in the saliva of subjects with different frequency of tooth brushing. The prevalence of *H. pylori* was 22.1% (25/92), 28.3% (32/65), and 49.6% (56/123), respectively among subjects who brushed once, twice, and no zero times daily ($p = 0.7$) (Table 1).

**Discussion**

The oral cavity is considered to be a gateway that allows materials to reach the stomach from the outside environment. Poor oral cavity health has been associated with a high risk of oral and gastric cancer [20,21]. Studies have also identified the oral cavity as a reservoir for microorganisms such as, *H. pylori*, *Neisseria* spp, and *Streptococci*, all of which are responsible for many diseases inside and outside the gastrointestinal tract. Miyabayashi et al. [22] demonstrated that *H. pylori* in the oral cavity is a risk factor for recurrent gastric infection. Furthermore, Tongtawee [23] suggested that adjunctive periodontal therapy improves the treatment efficiency of *H. pylori* and decreases the recurrence of gastric infection. Therefore, identification of the risk factors of oral *H. pylori* infection provides a good understanding of the best ways to control this infection and prevent its consequences.

The Food and Drug Administration (FDA) accepted the *H. pylori* stool antigen test (HpSA) as a non-invasive test for rapid diagnosis and confirmation of *H. pylori* infection [24]. This test is non-invasive, easy-to-perform, and has sensitivity and specificity similar to other diagnostic assays [25]. In the current study, we used a stool antigen test as evidence of gastric infection. The prevalence was 40.4% and 36.4%, based on saliva antigen test and stool antigen test without a statistically significant difference between both tests. This finding indicates that a positive saliva antigen test could be used as evidence of *H. pylori* gastric infection. Wang et al. reported that the sensitivity, specificity, and accuracy of the *H. pylori* saliva antigen test when compared with PCR (Polymerase Chain Reaction) were 98.7%, 68.8%,

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Positive <em>H. pylori</em> antigen in the saliva %</th>
<th>OR$^1$ (95% CI)$^2$</th>
<th>RR$^3$ (95% CI)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>37.1</td>
<td>0.8 (0.5-1.2)</td>
<td>0.9 (0.6-1.1)</td>
<td>0.427</td>
</tr>
<tr>
<td>Female</td>
<td>43.6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>40.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-44</td>
<td>43.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-60</td>
<td>41.1</td>
<td>0.9 (0.4-1.9)</td>
<td>0.9 (0.6-1.4)</td>
<td>0.391</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>35.1</td>
<td></td>
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</tr>
<tr>
<td>Residence</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>34.7</td>
<td>0.6 (0.4-1)</td>
<td>0.7 (0.6-1)</td>
<td>0.038</td>
</tr>
<tr>
<td>Rural</td>
<td>46.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental caries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47.1</td>
<td>2.2 (1.3-3.5)</td>
<td>1.6 (1.2-2.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>1.6 (1.2-2.1)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Periodontitis</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54.7</td>
<td>2.8 (1.7-4.6)</td>
<td>1.8 (1.4-2.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No</td>
<td>30.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of teeth brushing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once daily</td>
<td>22.1</td>
<td>0.4 (0.2-0.8)</td>
<td>0.6 (0.4-0.8)</td>
<td>0.7</td>
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<tr>
<td>Twice daily</td>
<td>28.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>None</td>
<td>49.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Odds Ratio; $^2$Confidence Intervals; $^3$Risk Ratio.
and 88.7% respectively, indicating a satisfactory sensitivity and accuracy [26].

Conflicting results were reported regarding the relationship between the presence of *H. pylori* in the stomach and the oral cavity. Asikainen et al. [27] failed to detect *H. pylori* in the oral cavity of 336 periodontitis patients although no assays were performed to detect *H. pylori* in the gastrointestinal tract. Another study conducted by Ji et al. [9] concluded that *H. pylori* commonly exists in the oral cavity, with no clear link between this existence and gastric infection by *H. pylori*, as the treatment of gastric infection failed to change the rate of oral infection. On the other hand, Miyabayashi et al. [22] found the eradication rate of *H. pylori* decreases in the case of positive oral infection. Moreover, Sert et al. [28] illustrated that the key point for achieving a successful treatment of gastric *H. pylori* infection is maintaining a healthy oral cavity by professional treatment of oral diseases and practicing good personal hygiene. These conflicting results may be due to the differences in the subjects studied, methods of specimen collection, and specificity of assays employed for *H. pylori* detection.

This study found 40.4% of the Erbil population to be infected with *H. pylori* based on saliva antigen test. These findings are consistent with previous studies from India (42.8%) [29]. However, a higher prevalence was reported from Thailand (64%) [10] and China 51.3% [17]. In contrast, a very low rate was recently reported in Japan (6%) [8]. Therefore, oral *H. pylori* infection could be affected by the socio-economic status and environmental hygiene [30].

In this study, results showed that different age groups have no statistical differences for *H. pylori* infection, which means that age is not a risk factor. This result is contrary to other studies that found the rate of *H. pylori* infection to increase with age [30,31]. This could be interpreted by the fact that all the subjects included in this study were adults, whereas *H. pylori* infection starts at an early age (generally before 10 years age) and persists for life in the absence of treatment or treatment failure [32].

Data on gender also demonstrated no association with *H. pylori* infection. This result is consistent with the study of Al-Mashhadany et al. [33]. However, this association is controversial as Kouitcheu et al. [34] demonstrated that males have a higher risk of *H. pylori* infection than females. In contrast, another study demonstrated female gender is a risk factor of *H. pylori* infection [35]. The causes of these conflicting results are still unknown.

In the present study, the prevalence of *H. pylori* infection is statistically higher in rural areas due to socioeconomic factors. This result is consistent with previous reports [36,37].

Participants with periodontitis had a higher number of *H. pylori* infections compared with non-periodontitis subjects. This correlation is consistent with Silva et al. [38], and Ding et al. [39] who suggested a positive association of *H. pylori* with periodontal diseases. The higher prevalence of *H. pylori* infection among periodontitis patients may be due to the periodontal pockets which form a typical environment for bacterial growth.

Furthermore, in the current study, dental caries had a positive effect on *H. pylori* infection. Indeed, a retrospective study carried out in Finland suggested that *H. pylori*-infected children have a higher risk of dental caries [40]. These results raise doubts whether the dental caries is a cause or a result of *H. pylori* infection.

Lastly, this study did not find an association between the frequency of tooth brushing and *H. pylori* colonization, which is consistent with other studies [10,41]. The *H. pylori* tends to accumulate in the periodontal pockets that are difficult to clean with the toothbrush.

The current study has some limitations. Firstly, only two oral cavity diseases (periodontitis and caries) were included. Insufficient number of other diseases may result in inaccurate statistical analysis. Secondly, the cross-sectional study design is not the best method to assess the risk factors; however, it could be considered as a foundation for designing case-control studies or cohort studies to get further information. Finally, the strains of *H. pylori* in the stomach and the oral cavity in this work were not identified or compared.

**Conclusions**

The current study highlighted the importance of the oral cavity as a reservoir for *H. pylori* and supported the link between *H. pylori* in the oral cavity and gastric infection. An important association between *H. pylori* infection and oral diseases including periodontitis and caries was detected. A combination of good prevention methods and professional treatment of oral diseases maybe necessary for preventing *H. pylori* infection and its consequences.

**Acknowledgements**

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