Study of the effect of hypoxia on the prevalence of helicobacter pylori infection among Saudi Students at Taif University

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Accepted 14 June, 2017

ABSTRACT

Background: H. pylori is one of the most important human pathogens, infecting more than 50% of the world’s population. While over 80% of infected individuals are asymptomatic, it can lead to intestinal and extra-intestinal manifestations. Intestinal manifestations include peptic ulcer disease and some gastrointestinal malignancies. Aims of the study: We aimed in this study to determine the prevalence of H. pylori among Taif University students by measuring H. pylori antibody (in serum) and antigen (in stool) and comparing between them. Also, we aimed to estimate the hematological effects of H. pylori.

Materials and Methods: A cross-sectional study was conducted on 280 male students at Taif University from different colleges. A self-administered questionnaire was filled by each student that included questions about intestinal and extra-intestinal manifestations of H. pylori. Three mLs of venous blood was collected for qualitative detection of IgG antibodies against H. pylori and for complete blood count (CBC) analysis. A stool sample was taken for detection of H. pylori antigen in stools. Results: There was statistical positive association between H. pylori prevalence and larger family size, lower family income, frequent fast food eating, smoking, latency, and abnormal body mass index (BMI). The prevalence of H. pylori antibody in serum was 37.5% compared to 26.8% antigen in stool (P<0.0001). There was no statistical significance of hematological parameters between infected and non-infected groups (P>0.05). Conclusions and recommendations: H. pylori is more prevalent in Taif City compared to western areas of KSA. Stool antigen test is more specific than serum antibody for detection of H. pylori.

Keywords: BMI, CBC, H. pylori, Leukopenia, Thrombocytopenia.

INTRODUCTION

Helicobacter pylori (H. pylori) is a helix shaped, microaerophilic, Gram-negative, flagellated bacteria. It is one of the most important human pathogens, infecting more than 50% of the human population. H. pylori and
mankind have had an ancient relationship for at least 50,000 years (Atherton and Blaser, 2009). Infection with H. pylori is usually acquired in early childhood and persists for life (Kusters et al., 2006). While over 80% of infected individuals are asymptomatic (Blaser, 2006), the infection can lead to peptic ulcer, gastritis, and gastric cancer. H. pylori has been classified as a class I carcinogen for gastric cancer since 1994 by the International Agency for Research on Cancer (IARC/OMS), and if not treated, is associated with a 1-2% lifetime risk of stomach cancer (Khalifa et al., 2010).

The mode of H. pylori transmission is unknown, but it is thought to be mainly through the fecal-oral route. Oral-oral and water-borne transmissions are also possible (Al-Moagel et al., 1990). Although immune cells that normally recognize and attack invading bacteria accumulate near sites of H. pylori infection, they are unable to reach the stomach lining. In addition, H. pylori has developed ways of interfering with local immune responses, making them ineffective in eliminating the bacterium (Kusters et al., 2006).

The 2005 American College of Gastroenterology (ACG) guidelines for the management of dyspepsia recommended testing for H. pylori infection among dyspeptic patients even without alarming features (Gunaid et al., 2004).

H. pylori is the major cause of gastritis, that plays a key role in the etiology of peptic ulcer and it is a risk factor for gastric cancer. However, detailed information on the prevalence of the bacteria in developing countries and on the factors that may influence the pattern of distribution remains scanty (Kamal and Shadi, 2001).

H. pylori infection is widespread throughout the world, and it is present in about 50% of the global human population (Howden, 1996). Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors. It is higher in developing than developed countries. In general, however, in the recent years there has been a decreasing trend in the prevalence of H. pylori infections in many parts of the world (WGO, 2010). In developing countries the prevalence of H. pylori ranges between 70-90% (Al-Moagel et al., 1990), while in developed countries it is approximately 50% (Graham et al., 1991).

H. pylori prevalence in developed countries is slowly increasing during childhood, which continues through adolescence and early adulthood, with an abrupt increase around 50-60 years of age (Torres et al., 2000). In the non-industrialized countries, H. pylori prevalence increases more rapidly during childhood and most adolescents and adults are infected. Thus, differences in H. pylori prevalence between industrialized and non-industrialized countries are greater at younger ages and get smaller at older ages (Bardhan, 1997). It has been reported in Saudi Arabia that infection is acquired at an early age and reaches up to 36.9% as age advances (Hanafi and Mohamed, 2013). Differences in prevalence within populations are due to a variety of factors, primarily relating to socioeconomic status and geographic origin (Abdulaziz, 2009).

The prevalence rate of H. pylori infection in patients undergoing endoscopy for upper gastrointestinal symptoms has now been reported from many Middle Eastern countries, including Egypt, Iran, Israel, Oman, Saudi Arabia, the United Arab Emirates, and Yemen (Novis et al., 1998). It has been reported in Yemen that prevalence of H. pylori infection among patients underwent upper GI endoscopy in Sana’a major hospitals was very high (99.6%) (Huda, 2002). Another study showed that patients with gastritis and peptic ulcer disease had similar rates of infection as reported from Europe, United States, and Africa 71-92%. However, patients with non-ulcer dyspepsia had higher rates of infection 61-89% (Novis et al., 1998).

In 1998, 88.5 % of patients in Kuwait with dyspeptic symptoms who were referred for endoscopy proved H. pylori-positive (Waleed et al., 2010). Another study showed that 82.2% of dyspeptic patients in Yemen have H. pylori infection (Huda, 2002). It has been shown that H. pylori-positive patients tend to have dyspepsia, but the relationship between H. pylori and dyspepsia remains controversial (Waleed et al., 2010).

H. pylori infection has been reported to be hyper-endemic in Saudi Arabia (Khan, 1998). Reports in the 1990s have shown a prevalence of 68-82.2% in various age groups of patients including those with non-ulcer dyspepsia. This high prevalence has been attributed to several factors including sanitation and socioeconomic status. However, reports in the 2000s have shown marked reduction in the prevalence to 35-55% (Almadi et al., 2007).

In another study done on 62 patients referred for upper GI endoscopy for pre-bariatric surgery evaluation, only 6 patients had mild dyspepsia, the mean age was 34 years, and a mean body mass index (BMI) was 55 kg/m², the majority were females (female/male ratio was 42/20). H. pylori infection was detected histologically in 53 patients (85.5%). All patients with positive H. pylori organisms in their biopsy specimens had chronic active gastritis of variable severities (i.e., mild, moderate, or severe). The study demonstrated a very high prevalence of H. pylori infection in morbidly obese Saudi patients who underwent bariatric surgery (Ahmad, 2010).

Serum antibody testing (serology) for H. pylori has previously been recommended as the most appropriate test in New Zealand. However, with the improved availability and accuracy of fecal antigen tests, serology is no longer the preferred test, and it is no longer funded in New Zealand. Serological testing detects the presence of IgG antibodies to the H. pylori bacteria. Although the sensitivity of the test is comparable with the other non-invasive tests, the specificity is variable and when
prevalence of *H. pylori* is low the positive predictive value of the test declines (Gisbert and Calvert, 2013).

Serology also cannot distinguish between infection that is past or current, and because antibody levels decrease slowly over 6-12 months or longer after eradication treatment, it cannot be used as a test of cure (Malfertheiner et al., 2012). The fecal test detects the presence of antigens to *H. pylori* in a fecal sample and can be used to diagnose active infection and, if required, to confirm that eradication treatment has been successful. Sensitivity and specificity of fecal antigen testing is similar to that reported for carbon-13 urea breath testing “¹³C UBT” (Shimoyama, 2013).

**Aims of the work**

1. To determine the prevalence of *H. pylori* infection among Saudi students at Taif University in the age range ~ 18-24 years (by positive serum antibody test).
2. To confirm the prevalence of active infection among them (by positive stool antigen test).
3. To compare between the two methodologies in diagnosis of *H. pylori* regarding specificity and sensitivity.
4. To compare the prevalence of the disease in Taif City with other western areas of KSA.
5. To estimate the impact of *H. pylori* on hematological parameters values of CBC in infected students compared to the non-infected one.
6. To determine some socioeconomic and personal conditions that may be related to *H. pylori* infection through a questionnaire.

**MATERIALS AND METHODS**

**Ethical considerations**

Each studied student was informed about the study objectives with stressing on confidentiality of the collected data and sample results, and also on getting a verbal consent to share in the study.

**Study settings**

The study was conducted in Taif University, which is a large sized public University that is located at Taif governorate in the Western region of Saudi Arabia and that consists of thirteen faculties. Taif City is a high altitude area that is more than 2400 meters above the sea level (Adnan and Ali, 2011). At this altitude level oxygen is less readily available (a hypoxic state). The importance lies in that the organism is a microaerophilic one and prefers to live and grow at low oxygen tension that is found in Taif City. So, it is expected to be highly prevalent in Taif more than other Saudi low land areas.

**Subjects of the study**

A cross-sectional study was carried out on 280 male volunteer students 18-24 years old from different Colleges of Taif University.

Subjects were classified according to their hemoglobin (Hb) concentration (Conc.) into two groups according to Barbara et al. (2012) into:

- Group 1: with normal Hb Conc. (i.e., 13-17 g/dL).
- Group 2: with high Hb Conc. (i.e., >17 g/dL).

**Inclusion criteria**

All students involved in the study must be born and living in Taif City and in a good health, with no symptoms referable to upper gastrointestinal tract.

**Exclusion criteria**

Subjects were excluded if they has had a history of *H. pylori* infection, treatment with proton pump inhibitors, H₂ blockers, and regular use of antacids, bismuth, or antibiotics 2 weeks before sampling, or had a history of peptic ulcer or frequent upper gastrointestinal tract symptoms as dyspepsia, heart burn, abdominal pain, loss of appetite, etc.

**Data collection (questionnaire)**

All students included in the study were interviewed personally and asked to fill the self-administered structured questionnaire after initial training. The questionnaire was developed, pretested, and validated in a pilot study. The questionnaire included questions about the following items:

1. Residence.
2. Family size.
4. Eating rapid meals (outside home).
5. Smoking.
7. Exclusion criteria (mentioned above).
8. BMI was used to classify students into; underweight, normal weight, overweight, and obese groups according to WHO (1995). It was calculated from the formula (BMI = weight in kg/height in m²).

**Sampling**

A. Venous blood samples (3 mLs) were drawn from each
Table 1. Factors associated with prevalence of positive H. pylori antigen in stools among studied students

<table>
<thead>
<tr>
<th>Variables</th>
<th>Variable 1</th>
<th>Variable 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family size:</td>
<td>&lt;5 members</td>
<td>≥5 members*</td>
<td>0.03</td>
</tr>
<tr>
<td>Family income / month:</td>
<td>&gt;5000 SR</td>
<td>&lt;5000 SR*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Frequent eating of fast food:</td>
<td>No</td>
<td>Yes*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cigarette smoking:</td>
<td>No</td>
<td>Yes*</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Physical exercise:</td>
<td>Yes</td>
<td>No*</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Abnormal BMI:</td>
<td>No</td>
<td>Yes*</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

*Significant increase in H. pylori prevalence. SR: Saudi Riyal.

Table 2. Comparison between blood parameters of both groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (No. = 183)</th>
<th>Group 2 (No. = 97)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (×10^6/µL)</td>
<td>5.5742 ± 0.38893</td>
<td>5.9172 ± 0.28162*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>16.1596 ± 0.65709</td>
<td>17.7031 ± 0.54876*</td>
<td>0.0001</td>
</tr>
<tr>
<td>PCV%</td>
<td>45.953 ± 1.82265</td>
<td>49.4196 ± 1.70835*</td>
<td>0.0001</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>82.7448 ± 4.14695</td>
<td>83.6237 ± 3.184*</td>
<td>0.045</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.1262 ± 2.01837</td>
<td>29.9649 ± 1.19914*</td>
<td>0.0001</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.1781 ± 1.28746</td>
<td>35.8351 ± 0.85074*</td>
<td>0.0001</td>
</tr>
<tr>
<td>RDW%</td>
<td>13.2492 ± 0.90602</td>
<td>13.1856 ± 0.64226</td>
<td>0.2312</td>
</tr>
<tr>
<td>WBCs (×10^3/µL)</td>
<td>5.5497 ± 1.56763</td>
<td>6.1959 ± 1.82585*</td>
<td>0.0055</td>
</tr>
<tr>
<td>Neut (×10^3/µL)</td>
<td>49.9831 ± 10.43836</td>
<td>52.1969 ± 11.69031*</td>
<td>0.0217</td>
</tr>
<tr>
<td>Lym (×10^3/µL)</td>
<td>45.1514 ± 9.96482</td>
<td>42.9268 ± 11.22533</td>
<td>0.1063</td>
</tr>
<tr>
<td>Mxd (×10^3/µL)</td>
<td>4.8656 ± 2.63759</td>
<td>4.8763 ± 2.47634</td>
<td>0.8888</td>
</tr>
<tr>
<td>Plt (×10^3/µL)</td>
<td>196.2022 ± 71.91125</td>
<td>180.8969 ± 64.95904</td>
<td>0.0654</td>
</tr>
</tbody>
</table>

*Significantly statistically increased difference

Statistical analyses

Data entry and statistical analyses were performed using the Statistical Package for Social Science (SPSS) program for windows version 20. Mean and standard deviation was used for quantitative variables. Percentage was used to determine the prevalence rates of H. pylori antibodies and antigens. P-values of <0.05 was considered statistically significant.

RESULTS

As shown in Table 1 there was a positive significant association between increased family size, decreased family income/month, frequent eating of fast foods, cigarette smoking, lesser physical activity, and abnormal BMI (underweight, overweight, and obesity).

Table 2 revealed comparison between blood parameters...
Table 3. Prevalence of H. Pylori (antibody and antigen) in groups 1 and 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>H. Pylori IgG (Serum Antibody)</th>
<th>H. Pylori (Stool Antigen)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive No.</td>
<td>%</td>
<td>Negative No.</td>
</tr>
<tr>
<td>Group 1: Hb Conc. 13-17 g/dL</td>
<td>70</td>
<td>38.3</td>
<td>113</td>
</tr>
<tr>
<td>Group 2: Hb Conc. &gt;17 g/dL</td>
<td>35</td>
<td>36</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>37.5</td>
<td>175</td>
</tr>
<tr>
<td>P-Value</td>
<td>&gt;0.05</td>
<td></td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 4. Comparison between detection of H. pylori using serum antibody and stool antigen

<table>
<thead>
<tr>
<th>Variable</th>
<th>H. Pylori IgG (Antibody +ve.)</th>
<th>H. Pylori (Antigen +ve.)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>105/280</td>
<td>75/280</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>%</td>
<td>(37.5%)</td>
<td>(26.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 showing a comparison between detection of H. pylori IgG antibody (serum) and antigen (stool) using the two qualitative chromatographic immunoassay techniques for serum antibody and stool antigen.

DISCUSSION

H. pylori infection is considered as one of the most common chronic bacterial infections worldwide. The infection constitutes a public health problem often with a fatal sequela (Sayed et al. 2007).

This study was conducted in Taif governorate in the Western region of Saudi Arabia. We determined the prevalence of H. pylori infection among 280 Saudi students at Taif University in the age range ~ 18-24 years using two qualitative methodologies depending on rapid chromatographic immunoassay techniques for serum antibody IgG and stool antigen.

Regarding the antibody, H. pylori was positive in 105 cases with an overall prevalence of 37.5%. Regarding the antigen, H. pylori was positive in 75 cases with an overall prevalence of 26.8%. There was a highly statistically significant difference (P<0.0001) of H. pylori detection between both methodologies. This can be explained by the high sensitivity and low specificity of the antibody detection due to the possible detection of new and old infection, especially with the increased awareness of the importance of eradication of H. pylori infection. Another explanation might be due to false-positive results that might be produced by the high carriage of other cross-reacting intestinal pathogens in developing countries, such as Campylobacter species. Gamma globulin administration also can cause false-positive results. On the other hand, stool antigen detection is more specific but less sensitive. So, it can be used to diagnose active recent infection, and to confirm successful eradication of H. pylori (Jeongmin et al., 2011).

Taif City is a high altitude area that is more than 2400
meters above the sea level (Adnan and Ali, 2011). This high altitude cause a state of hypoxia and since H. pylori is a microaerophilic organism, we aimed to compare the prevalence of the organism in Taif with other coastal (low land) level Saudi governorates near Taif (i.e., western region) to conclude if this hypoxic condition will favor its prevalence.

Our results were in agreement with a study done in Makkah area (low altitude) which found that the prevalence of H. Pylori was 27.4% in which 14C urea breath test was used for H. pylori detection. However, our results were more than that study when we used the IgG antibody H. pylori test for detection (Telmesani, 2009).

Our study was in agreement with (Senra et al., 1998) who studied the prevalence of IgG H. pylori antibody in the healthy population of Ubrique and Grazalema (mountain location) and in Barbate (coastal location) and found positive titers of 30% in the coastal population and 54% in the mountain location.

In addition, a high rate of H. pylori infection (using microbiology culture of gastric/duodenal aspirates) at high altitudes in comparison to coastal areas was also reported from Peru (Sharma et al., 2015). However, in a study done by Eric et al. (2013) high altitude was not associated with high prevalence of duodenal ulcer or frequency of H. Pylori infection compared to low altitude areas in the kingdom of Saudi Arabia.

In the recent years, prevalence of H. pylori infection had been decreasing in many parts of the world. In the Czech Republic, between 2001 and 2011, the prevalence decreased from 30% to 10% in young adults, and from 60% to 40% in older subjects. In the Republic of Korea, the prevalence decreased between 1998 and 2005 from 50% to 20% at 20 years. Small declines were also observed in the United States of America between 1988-1991 and 1999-2000 at =60 years (Barbara et al., 2014).

Many studies has revealed the relationship between H. pylori infection and socioenvironmental factors. In our study, there was a positive significant association between increased family size (P<0.03), decreased family income/month (P<0.001). This finding was in accordance with those of Cheng et al. (2009) who investigated the prevalence of H. pylori in one rural and one urban region of Beijing, China and Hasosah et al. (2015) who carried out a similar study in Saudi Arabia.

There was also a significant relation between H. pylori infection and frequent eating of fast foods (P<0.001), lesser physical activity (P<0.04), and abnormal BMI i.e., underweight, overweight, and obesity (P<0.02). These findings were in match with Erol et al. (2009) who found increased prevalence of H. pylori in subjects with obesity, and considered that obesity can be a risk factor for H. pylori infection.

Our study found also a significant association with cigarette smoking (P<0.03) which come in agreement with Ogihara et al. (2000) who stated that current smokers had a 0.82 (0.74-0.91)-fold greater risk of H. pylori seropositivity than those who had never smoked.

No anemic cases were found because hypoxia in Taif City is expected to result in secondary polycythemia in some people, especially in young adult males. The importance lies in that the organism is a microaerophilic one and prefers to live and grow at low oxygen tension (i.e., hypoxia) that is found in Taif City. So, it is expected to be highly prevalent in Taif more than other Saudi areas. Therefore, our subjects were classified according to their Hb Conc. into two groups according to Barbara et al. (2012); into Group 1 with normal Hb Conc. (i.e., 13-17 g/dL) and Group 2 with high Hb Conc. (i.e., >17 g/dL) in order to compare the effect of H. pylori infection on both groups. There was a statistically significant difference between both groups regarding; RBCs, Hb Conc., PCV%, MCV, MCH, MCHC, WBCs, and Neut. However, there was no significant difference between groups regarding RDW%, Lym, Mxd, and Plt. These results were expected due to secondary polycythemia.

There was no impact of H. pylori on all CBC parameters when positive and negative H. pylori students were compared i.e., P>0.05. This indicated no effect of H. pylori infection on hematological parameters. There were no students suffering of anemia, leucopenia, or thrombocytopenia.

CONCLUSIONS

H. pylori prevalence was higher in Taif City compared to western areas of KSA; this could be contributed to its hypoxic state. The prevalence was affected with some socioeconomic states and personal factors and showed no significant deteriorating effects on the blood parameters. Stool antigen test was found to be more specific and less sensitive in detection of H pylori compared to serum IgG antibody test.

RECOMMENDATIONS

Serious measures should be taken to implement good sanitary conditions and provide health education against the transmission of H. pylori to block the infectious process to students and their communities. Also, it is preferable to use stool antigen test for detection and follow up of H. pylori cases instead of serum IgG antibody for its specificity. More studies are needed to confirm the positive relation between H. pylori infection (incidence) and prevalence in high altitude areas (i.e., hypoxic regions) like Taif City.
Study limitation

The subjects involved in this study were volunteers in a good health and not randomly selected. All subjects were males (females were not involved in this study due to difficulty to take samples from female students in the Taif University).

ACKNOWLEDGEMENT

We would like to express our deep thanks and gratitude to the Taif University, Research Deanship for encouragement and financial support of this project to its end.

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