**The Seroprevalence of *Helicobacter pylori* Infection in Tripoli (Libya)**

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**Abstract:** *Helicobacter pylori* (*H. pylori*) is one of the most common chronic bacterial infections in developing countries. H. pylori infection has been established firmly with the development of gastric neoplasia, including gastric adenocarcinoma and gastric mucosaassociated lymphoid tissue lymphomas. The aim of this study was to investigate the seroprevalence of Helicobacter pylori infection in symptomatic patients and to determine the relationship between H. pylori and ABO/Rhesus blood groups, age, gender, socio-economic status and smoking in Tripoli, Libya, using three different commercial serological test methods. One hundred patients from Alhanan Specialized Medical Clinic in Tripoli were included in the study. A total of 100 samples were collected from these patients undergo serologic testing for the presence of anti–*H pylori* antibodies in serum or whole blood. Results revealed that by using (ABON test method) antibodies were seen in 36% of the patients and 29% by using ADVANCE QUALTY test method and 35% in **(**Helicobacter pylori IgG Enzyme immunoassay test method) which was nearly similar to the result obtained by ABON test. We found that the seroprevalence of H. pylori infection was not significantly associated with ABO blood groups, age, sex, occupation, socioeconomic condition and smoking (P- value > 0.05).

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**1. Introduction**

*Helicobacter pylori* represent one of the most common and medically important infections worldwide. *H. pylori* is Gram-negative, microaerophilic, spiral, motile bacterium that resides in the gastric pits and the overlying mucus blanked. (Versalovic, 2003) (Blaser, 1990). It is recognized as the major cause of gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The prevalence of *H pylori* infection is 70%-90% in developing countries and 25%-50% in developed countries (Demiray et al., 2006). Person-to-person spread is the most probable mode of transmission. Faecal-oral and oral-oral transmissions have been reported (Allaker et al,2002).

*H. pylori* infection is usually acquired during the early years of life and persists for several years. The prevalence of *H. pylori* infection has been reported to increase with age. It seems to be an association between the prevalence of *H. pylori* in adults and the risk of acquisition of *H. pylori* infection during childhood (Ghanaei et al., 2009). The prevalence of *H pylori* infection varies among countries and within a country it is dependent on socio-economic conditions especially poverty, overcrowding, poor sanitation and hygiene. Also, within countries, there may be considerable variation in prevalence by ethnic group (Whittle et al., 2010). It was also shown that the frequency of O blood group and non-secretor phenotype of ABO antigens are higher among patients with peptic ulcers. However, several studies reported absence of association between H. pylori infection and ABO blood groups (Tadesse et al., 2014).

*H pylori* and its association with multiple gastroduodenal diseases have emphasized the importance of accurate and prompt diagnosis of symptomatic individuals. The “test and treat” approach may be justified on the basis of a significantly increased lifetime risk of chronic peptic ulcer disease and gastric cancer (Graham and Rabeneck, 1996) (O’Connor et al, 1996). It is well known that a successful eradication of H. pylori dramatically reduces the rate of recurrence of gastric and duodenal ulcers in affected children. An accurate *H. pylori* test is crucial for initiation of appropriate treatment. Numerous diagnostic tests are available to detect *H. pylori* infection and are divided into either invasive (histology, rapid urease test (RUT) and bacterial culture) or noninvasive tests (serology, 13C-urea breath test (13C-UBT), and stool antigen test) (Guarner et al., 2010). Effective antimicrobial treatment depends on sensitive and accurate diagnostic approaches (Warren and Marshall, 1983). However, there has been no single test available that can be used as a gold standard to detect *H. pylori* infection reliably and accurately (Frenck et al., 2006) (Koletzko et al., 2011). Seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H. pylori* infection in a population, but the assays used need to be validated in the population studied (Hoang et al.,2004)**.** Because assays validated in one region may yield variable diagnostic performances in others. These variations may be attributed to many factors, including the source of antigen used, the prevalence of infection in each population studied, and the reference method used to determine true *H. pylori* infection status. Therefore, reevaluation is needed before implementing a test in different populations (Orrawadee et al., 2004).

The aim of this study was to investigate the seroprevalence of Helicobacter pylori infection in symptomatic patients and to determine the relationship between H. pylori and ABO/Rhesus blood groups, age, gender, socio-economic status and smoking in Tripoli, Libya. We therefore evaluated the performance of three different commercial diagnostic serological test methods used for detecting of immunoglobulin G (IgG) antibody to *H. pylori* and evaluated the possible risk factors for the *H. pylori* infection.

**2. Material and methods**

This study was carried out at the Specialized Alhanan Medical Clinic in Tripoli Libya. A total of 100 symptomatic patients selected randomly (39 males and 61 females; age range, 9 to 89 years) were studied. Written informed consents were obtained from all patients before the study.

**2.1 Data Collection**

On the enrollment date, questionnaire forms were collected back and dates of birth and other data of each patient were noted. All subjects were interviewed and personal, socioeconomic status, profession’ educational level, environmental, and geographical data were obtained.

**2.2 Serological tests:**

Three ml (of clotted) venous blood was drawn from each patient and serum was stored refrigerated at (4–8 °C) for up to 48 hours. For a longer storage they were kept at -20 °C until analyzed for anti-*Helicobacter pylori* antibodies - IgG detection. Other 3ml of blood was drawn and put in tubes with anticoagulant (EDTA) to detect the blood groups (ABO). We used three different commercial test methods kits for detection of immunoglobulin G (IgG) antibody to *H. pylori*:

**(1**) **(**Helicobacter pylori IgG Enzyme immunoassay (EIA) test KIT: Enzyme Immunoassay for the detection of IgG Antibodies to Helicobacter pylori in human serum) (Biocheck, Inc. 323 Vintage Park Dr. Foster City, CA 94404). **(2)** (ABON: A rapid one step test for the qualitative detection of antibodies to Helicobacter pylori in human serum or plasma) (Abon Biopharm (Hangzhou) Co., Ltd. Hangzhou, 310018, P. R. China)-Wellkang Ltd SuiteB, 29 Harley Street, London, WIG 9QR, UK. **(3**) Advanced quality: Rapid Anti-H. pylori Test (Whole blood/ serum / plasma) (InTec products, INC.). All the tests were performed according to the manufacturer’s instructions and without the knowledge of the status of the patient’s infection.

**2.3 Data analysis**

Data analysis was made by using SPSS version 14 software. Chi-square test was used to test for presence of association between prevalence of *H. pylori* and age, sex and standard of living. P-values <0.05 were considered to show significant difference.

**3. Results**

A total of 100 patients (39 Males and 61 Females) attending the Specialized Elhanan Medical Clinic in Tripoli Libya included in this study. Helicobacter pylori infection was detected by three serological test methods.

Results revealed that antibodies were detected in 36% of the patients by using (ABON test method), in 29% by using (ADVANCE QUALTY test method) and in 35% by using (Helicobacter pylori IgG Enzyme immunoassay test method) which nearly similar to the result obtained by ABON test (table 1). The infection rate was most similar between males and females which was 35.9% and 36% respectively and there was no statistically significant difference in the prevalence of *H. pylori* with respect to gender (p=0.991) (table 2). The infection rate in patients with blood group AB was 40%, O was 38% and those with blood group B was 35% and A was 32.4%.

The prevalence of H. pylori infection according to age, ranging from (6.3%) in the 9-19 year old group, to (55%) in the 40-49 year old and (0%) in the 80-89 year old and there was no statistically significant difference in the prevalence of *H. pylori* among age groups (p=0.243). (table 3). The seroprevalence according to occupation or profession was shown in table (4). Also we found that patients with low standard of living were associated with a higher prevalence of *H. pylori* (47%), Moderate 34% and those with high standard of living was 20% and there was no statistically significant difference (p=0.199) (Tables 5). As for Predisposing factors which lead to infection we found that 29.4% of the smokers were infected with H. pylori compared with 37.3% of non smokers infected with H. pylori and 34.7% of the patients who always drink coffee were infected and 40% of the patients who do not drink coffee were infected (Table 6).

**(Table 1) Seroprevalence by using three different test methods**

|  |  |  |  |
| --- | --- | --- | --- |
| **EIA** | **ABON** | **ADVANCE QUALITY** | **Test**  **Method** |
| 35% | 36% | 29% | Percentage |

**(Table 2) Seroprevalence of H. pylori infection according to gender**

|  |  |  |  |
| --- | --- | --- | --- |
| **(%)** | **No. Infected** | **No. Tested** | **Sex** |
| 35.9% | 14 | 39 | **Males** |
| 36 % | 22 | 61 | **Females** |
| 0.991 | | | *P-value* |

**(Table 3) Seroprevalence according to age**

|  |  |  |  |
| --- | --- | --- | --- |
| **(%)** | **No.Infected** | **No.Tested** | **Age** |
| 6.3% | 1 | 16 | **9-19** |
| 32.6 % | 10 | 31 | **20 – 29** |
| 47% | 8 | 17 | **30 – 39** |
| 55 % | 11 | 20 | **40 – 49** |
| 50% | 4 | 8 | **50 – 59** |
| 0% | 0 | 2 | **60 – 69** |
| 50 % | 2 | 4 | **70 – 79** |
| 0% | 0 | 2 | **80 – 89** |
| 0.243 | | | *P-value* |

(Table 4) Seroprevalence according to occupation

|  |  |  |  |
| --- | --- | --- | --- |
| **(%)** | **No. infected** | **No. tested** | **Profession** |
| 48.4% | 15 | 31 | Employee |
| 17.6% | 2 | 17 | Students |
| 25% | 5 | 20 | Workers |
| 43.8% | 14 | 32 | Housekeepers |
| 0.213 | | | *P-value* |

Table (5) Prevalence of infection according to standard of living

|  |  |  |  |
| --- | --- | --- | --- |
| **Percentage of infection** | **No. infected** | **No. Tested** | **Standard of living** |
| 47% | 8 | 17 | Low |
| 34% | 27 | 78 | Moderate |
| 20% | 1 | 5 | High |
| 0.199 | | | *P-value* |

Table (6) percentage of infection according to smoking and drinking coffee

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *P-value* | **Infection rate** | **No. infected** | **No. Tested** | **Predisposing factor** |
| 0.708 | 29%  36% | 5  30 | 17  83 | Smokers  Non smokers |
| 0.192 | 42.9%  24.3% | 27  9 | 63  37 | Coffee drinker  Non coffee drinkers |

**4. Discussions**

Non-invasive tests are easier to accomplish but need appropriate equipment and validation of methods for each region, population and age. (Feldman and Evans, 1995).

Serologic testing represents a primary screening approach for evaluation of *H pylori* status in patients not immediately requiring Esophagogastroduodenal (EGD) endoscopic studies. (Kimmel et al., 2000). Overall, the medians of the sensitivity and specificity for *H pylori* serology kits have been reported as 92% and 83%, respectively (Laheij et al., 1998).

In this study we used three different commercial serological test methods for the detection of immunoglobulin G (IgG) antibody to *H. pylori* and evaluated the performance of these three commercial test kits. A total of 100 patients enroled in the study. Results revealed that antibodies were detected in 36% of the patients by using (ABON test method), in 29% by using (ADVANCE QUALTY test method) and in 35% by using (Helicobacter pylori IgG Enzyme immunoassay test method) which nearly similar to the result obtained by ABON test. We observed that 7 out of 36 seropositive samples (tested by ABON test method) gave negative results when tested by (ADVANCE QUALTY test method). We found that 6 of these 7 samples were positive when tested by using **(**Helicobacter pylori IgG Enzyme immunoassay test method). Rates of positive test results between (ABON test 36%) and **(**Helicobacter pylori IgG Enzyme immunoassay test 35%) were most similar 35% this mean that they were most sensitive than ADVANCE QUALTY test method). Performance varies significantly for commercial serologic kits, with top performers exceeding 90% in sensitivity and specificity and bottom performers having less than 90% in sensitivity and less than 80% in specificity (Laheij et al., 1998). Additional studies reported variable sensitivities (86%-100%) and specificities (76%-98%) of assays measuring anti–*H pylori* serum IgG (Meijer et al., 1997).

Compared to rates of infections found in other countries, the prevalence of Helicobacter pylori infection in this study was lower than that obtained by other workers. Alo et al., (2013) in their study in Ethiopie found that the prevalence of Helicobacter pylori infection was 38.3%. Our results are also much lower than those found in other studies conducted in some developing countries. A prevalence of 62% was found among Chinese populations (Shi et al., 2008), 63%-70% was found in Bahir Dar, Ethiopia (Tadege et al., 2005), while a prevalence of 64% was found in a study conducted in Ibadan, Nigeria (Jemilohun et al., 2011). Hoang et al., (2005) in their study, using a validated ELISA assay, reported a high overall seroprevalence of *H. pylori* infection of 74.6% in a Vietnamese population sample of 971 individuals aged 1 to 88 years. The variability of *H. pylori* seropositivity in different populations is likely partly explained by technical factors. Many of the studies used commercial kits that have been validated in developed countries but not in the populations investigated. These kits are usually based on strains from developed countries, while recent studies by us and others have shown that using local strains led to a significantly improved sensitivity and specificity (Romero-Gallo, et al**.**,2002). In addition, even if validated with a local population, some of the assays used in seroepidemiological studies had been validated with patients undergoing endoscopy for gastrointestinal diseases. This approach allows determination of the sensitivity of the assay against the “gold standard,” i.e., culture of *H. pylori*. It entails, however, the risk of setting an erroneous cutoff level if antibody concentrations differ significantly between symptomatic and asymptomatic individuals (Hoang et al.,2004).

As to the relation between ABO blood groups and H. pylori infection we observed that ABO was not significantly associated with infection in our study population, many epidemiologic studies had found that nonsecretors of ABO blood group antigens and individuals of blood group O were overrepresented among patients with peptic ulcers. These studies encouraged many researchers to investigate the relation between ABO blood groups and their secretor status with peptic ulcer. Many authors report an association between blood group O and *H. pylori* infection, while others failed to find such an association (Jaff, 2011)*.*

In this study the prevalence of *Helicobacter pylori* infection in males 35.9% and females 36% and there was no statistically significant difference in the prevalence of *H. pylori* with respect to gender. Previous investigations have shown the importance of sex and age in the acquisition of *H. pylori* infection. (Goodman and Correa, 1995). Some studies have shown no sex difference in the prev­alence of *H. pylori*. (Altuglu et al., 2001). but in a study in Iran, *H. pylori* prevalence was higher in males than in females.(Jafarzadeh et al., 2007). Also Kanbay et al., (2005) in their study reported that H. pylori infection can be related to ABO blood group, age, gender, and smoking.

In our study we found that the seroprevalence of H. pylori infection progressively increased with age, ranging from (6.3%) in the 9-19 year old group, to (55%) in the 40-49 year old, then it drop ranging from 50% in the age groups (50-59 and 70-79) to 0% in the age groups (60-69 and 80-89) There was no statistically significant difference in the prevalence of *H. pylori* among all age groups (p=0.243). This is in agreement with the results obtained by Tadesse et al., (2014) and in contrast to the results obtained by Moujaber et al., (2008).

Low socioeconomic conditions and occupation were not associated with the prevalence of *H. pylori* infection in our studied popu­lation. This is in contrast to the results obtained by other workers who found that low socioeconomic status is associated with increase in prevalence of *H. pylori* infection (Banatvala et al., 1993) and (Goldman et al., 2006**)**. Analysis of the relationship between smoking and H. pylori infection in recent studies has provided conflicting results. While some results have reported significantly elevated risks of H. pylori infection with smoking and one reported significantly reduced risk, most, similar to ours, found no significant association with current smoking (David and Hoda, 2002).

**Conclusion**

H. pylori infection was detected using serological test, which is acceptable for epidemiological studies. Serological detection of IgG antibody against H. pylori provides a reliable assessment of current or past H. pylori infection. H. pylori infection is usually lifelong therefore a positive test usually denotes active infection unless the patient has received eradication therapy. The results of this study demonstrate that the prevalence of infection with H. pylori in Libia was lower than rates reported in other developing countries, at 29-36.%. H. pylori infection positivity rate was not related to ABO blood groups, gender, age, low socioeconomic conditions and smoking. Further studies to determine subgroups at higher risk of infection may help target the more susceptible populations to establish the prevalence pattern in recent times and to determine if there is a change in the prevalence of Helicobacter pylori infection. Accurate information about the incidence and prevalence of *H.* pylori infection will help in the planning of community-wide H. pylori eradication programs.

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