



Research Article

Relationship between ABO Blood Groups and Helicobacter Pylori Infection among Patients with Dyspepsia

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Abstract

The aim of this study was the detection of H. pylori infection in patients with various gastrointestinal symptoms as related to blood groups and Rhesus factor. A total of 200 patients, 110 males and 90 females, whose age ranged from 18-75 years were included in this study. Gastric biopsies were taken from all patients for detection of H.pylori by RUT, culture and PCR. Blood samples were tested for H.pylori antibodies by RDT, and subjected to ABO/Rhesus blood group antigen typing. Results showed that 66.5%, 69.5%, 71% and 67.5% of patients were positive for H.pylori infection by RUT, RDT, culture and PCR, respectively. According to gender, 84.5% males and 58.9% females were H. pylori positive. The frequencies of H.pylori infection among patients with gastritis, gastric ulcer, duodenal ulcer, and gastric cancer were 73.4%, 83.3%, 68.6% and 50%, respectively; but the difference was not significant ($P > 0.05$). Patients of blood group O were more prone to H. pylori infection (88.9%) than patients in other blood groups, and patients in the AB blood group were less prone to H. pylori infection (35.3%) as compared with patients in other blood groups ($P < 0.001$). In this study H.pylori was found in 74.2% Rh+ and in 57.1% Rh- patients without statistical difference ($P > 0.05$). The results of this study suggest that ABO blood groups influence positivity for H. pylori infection

Keywords: Helicobacter pylori, Blood groups, PCR.

Introduction

Helicobacter pylori infection is a high prevalence infection worldwide, and it is

more common in developing countries than developed ones (Magalhães and Luzzi, 2006). It is a major risk factor for chronic gastritis, peptic ulcer and gastric cancer

(Fock and Ang, 2010). The ABO blood group system is the most important in human blood transfusion. The blood group antigens present on RBC are permanent, fixed and lifelong biological markers of any individual, they are almost as individual as fingerprints (Ravn and Dabelstein, 2000). The distribution of the ABO blood groups varies in populations throughout the world (Garratty et al., 2004). In addition to clinical significance for transfusion and transplantation, it is becoming increasingly apparent that ABO antigens are of biological significance and may be associated with predisposition to, or protection from many diseases (Reid and Bird, 1990).

Many authors reported an association between blood group O and *H. pylori* infection (Kanbay, et al., 2005). While, others failed to find such an association (Niv et al., 1996). The ABO phenotype has been linked with stomach ulcers, which are more common in group O individuals and gastric cancer, which is more common in group A individuals (Iodice et al., 2010).

The aim of this study was to report for the first time the incidence of *H. pylori* infection among patients with dyspepsia symptoms, and to determine the relationship between the ABO blood groups and *H. pylori* infection in patients with various gastrointestinal symptoms, from Basrah-Iraq.

Materials and Methods

Sample Collection

Two hundred (200) patients with various gastrointestinal symptoms attending endoscopy unit at Teaching Hospital in Basrah, during the period from March 2011 to October 2012, were studied after obtaining their consent. These consecutively chosen patients were 110 males and 90 females whose age ranged from 18-75 years. Patients were diagnosed as having gastritis, gastric ulcer, duodenal ulcer and gastric cancer using specimens from the antrum of the stomach of each patient. Diagnosis of

gastric carcinoma was based on the WHO classification (Hamilton and Aaltouren, 2000) while, gastritis was characterized by chronic inflammatory cells infiltration with degeneration and detection of microorganisms. One of the specimens was used for the rapid urease test (RUT) immediately after collection. The other two gastric biopsy specimens were placed in transport medium, brain heart infusion broth for bacterial culture and PCR.

Three ml of venous blood sample was drawn from each patient, then 2ml of it transferred into plain tube and centrifuged at 5000 rpm for 5-10 minutes. The serum was used for rapid diagnostic test in screening for the detection of antibodies against *H. pylori* (IgG). The remaining 1 ml of the blood was emptied into disposable tube, and used for determination of ABO blood group antigens by a standard hemagglutination test.

Rapid Urease Test (RUT)

One-minute ultra-rapid urease test (URUT) was performed according to (Thillainayagam et al., 1991). A positive RUT result was noted immediately by a change in the color of the solution from yellow to deep pink within the first minute.

Rapid Diagnostic Test (RDT)

This one step test is used for qualitative detection of IgG antibodies of *H. pylori* in the serum by using Rapid Diagnostic Test Kit (ACON, USA) (Ansorg et al., 1991). All the 200 collected sera from patients were examined for the presence of antibodies against *H. pylori*.

Bacterial Culture

Gastric biopsy samples after homogenate were cultured on MCUA slant tubes under microaerophilic condition at 37°C for 1-2 days by using MDCS method (AL-Sulami et al., 2002). Colonies of primary isolates of *H. pylori* were subcultured on MCUA medium plates and incubated at 37°C under

microaerophilic conditions for 3-5 days. Suspected colonies were identified as *H. pylori* on the basis of the color of the medium changing from yellow to pink indicating urease activity, and further confirmed for positivity of urease, oxidase and catalase traits.

DNA Extraction and PCR

Genomic DNA was extracted directly from all biopsy specimens by using Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until used. PCR was performed on extracted DNA from gastric biopsies directly using *H. pylori* 16SrRNA specific primers (Liu et al. 2008). The identification of the isolates have been confirmed by using the primer of the 229 pb of the 16S rRNA composed of forward 5'- TCG GAA TCA CTG GGC GTA A - 3' and reverse 5'-TTC TAT GGT TAA GCC ATA GGA TTT CAC -3'. For the detection of *H. pylori* by PCR, amplification conditions were carried out in a 20 µl of reaction mixture containing 5 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl DNA samples, 8 µl free deionized water. PCR conditions include: denaturation at 95°C for 5 min, followed by 30 cycles at 94°C for 30 sec, annealing at 52°C for 30 sec and extension at 72°C for 45 sec, and an additional extension step at 72°C for 10 min. After completing PCR cycles, the amplified PCR products were electrophoresed in 2% agarose and 2000 bp ladder was used as DNA molecular weight standard. Then, the product was detected and examined under ultraviolet transilluminator, where a 229-bp band was considered a positive PCR result.

Determination of ABO Blood Group Antigens

The ABO blood group was determined for each patient by the conventional hemagglutination test using the anti- A, anti-B and anti-D sera. The ABO blood grouping procedure is based on the principle of agglutination or clumping as the patient's blood is reacted with anti-A, anti-B and anti-Rh antibodies separately.

Statistical analysis

Statistical analysis was carried out by the use of the computer program, Statistical Package for Social Science (SPSS) version 21. The relationship between the parameters was determined using Chi-Square (X²) test. P value less than 0.05 was considered as statistically significant, and P-value less than 0.001 considered as highly significant.

Results

Of the two hundred patients with various gastrointestinal symptoms, 110 were males and 90 were females. The age range of patients was 18-75 years with the highest percentage >50 years old (29 %). The distribution of the ABO blood groups of the patients was O (40.5 %) followed by A (30 %), B (21 %) and AB (8.5 %), while 186 (93%) of patients were Rh-positive and only 14 (7%) of patients are Rh-negative. The Rh+ distribution among blood groups was as follow: 57 (30.6%), 37 (19.9%), 15 (8.1%) and 77 (41.4%) for blood groups A, B, AB, and O respectively. While, Rh- distribution among blood groups was 3 (21.4%), 5 (35.7%), 2 (14.3%) and 4 (28.6%) for blood groups A, B, AB, and O respectively as shown in (Table. 1)

Table 1: ABO/Rh blood group distribution in patients

ABO system	Blood groups				Total
	A No. (%)	B No. (%)	AB No. (%)	O No. (%)	
Rh+	57 (30.6)	37 (19.9)	15 (8.1)	77 (41.4)	186

Rh-	3 (21.4)	5 (35.7)	2 (14.3)	4 (28.6)	14
Total	60 (30)	42 (21)	17 (8.5)	81 (40.5)	200

$\chi^2=3.042$, $p>0.05$

200 patients with various gastroduodenal symptoms were tested for *H.pylori* infection by using four different methods as shown in Figure 1. Of these patients, 133(66.5%) were positive for RUT and 139 (69.5%) were positive RDT. By culture method, *H.pylori* was isolated from 142 (71%) patients by using differential and selective MCUA and MDCS method, and identified by Gram staining and biochemical tests. The colonies of the isolated *H.pylori* were small to middle in size, rounded and creamy in color. All these isolates were gram negative, spiral or curved in shape. The biochemical tests include positivity of oxidase, catalase, and urease. In addition, 200 biopsy samples were subjected to 16S rRNA PCR targeting the 229 bp domain of *H.pylori* resulting in 135 (67.5%) positive samples.

There are variations in *H.pylori* positivity among patients through using these different methods. Accordingly, the total incidence of *H.pylori* positivity was 146 (73%) in those patients as shown in (Fig.1).

This study reveals that the incidence of *H.pylori* infection in male (84.5%) was significantly higher than in female patients (58.9%) ($p<0.001$).

The frequency of *H.pylori* infection among patients with gastritis, gastric ulcer, duodenal ulcer, and gastric cancer was 73.4%, 83.3%, 68.6% and 50%, respectively (Table 2). Although, the frequency was higher in gastric ulcer patients and lower in gastric cancer patients, the difference was not significant ($P>0.05$).

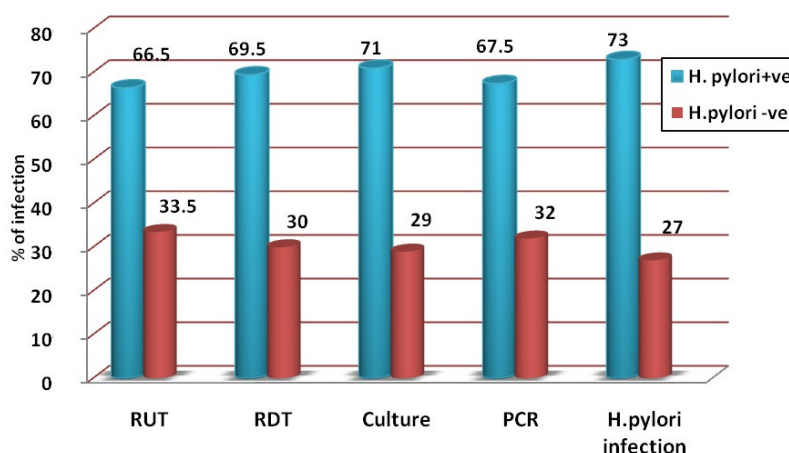


Figure 1: Total incidence of *H. pylori* infection by using four different methods.

Table2: The association between H.pylori infection and their clinical diagnosis among patients.

Clinical diagnosis	No. patients	H.pylori +ve NO. (%)	H.pylori -ve NO. (%)
Gastritis	139	102 (73.4)	37 (26.6)
Gastric ulcer	30	25 (83.3)	5 (16.7)
Duodenal ulcer	19	13 (68.4)	6 (31.6)
Gastric cancer	12	6 (50)	6 (50)

$\chi^2=5.058$, $P>0.05$

The distribution of ABO blood groups and H. pylori infection demonstrated that positivity rate of H. pylori was 88.9 % in blood group O, 78.3 % in blood group A, 50 % in blood group B and 35.3 % in blood group AB (Table 3). Thus, the frequency of blood group O in H. pylori infected patients was the highest, and

blood group AB was the lowest. There was statistically highly significant difference in positivity rate of H.pylori infection among ABO blood groups ($p< 0.001$). However, the pattern is quite different in case of non-infected patients with AB being the highest and A the lowest.

Table 3: Incidence of ABO blood groups among H. pylori infected and non-infected patients

ABO blood group	No. patients	H.pylori +ve NO. (%)	H. pylori -ve NO. (%)
A	60	47 (78.3)	13 (21.7)
B	42	21 (50)	21 (50)
AB	17	6 (35.3)	11 (64.7)
O	81	72 (88.9)	9 (11.1)

$\chi^2=34.776$, $P<0.001$

From the total number of Rh+ patients and Rh- patients 138/186 (74.2%) and 8/14 (57.1%) were respectively positive for H.pylori as shown in Table 4. Chi square test

showed no statistically significant difference ($P>0.05$) in the above mentioned groups, indicating that the presence of H.pylori did not relate to the Rh factor.

Table 4: Incidence of Rh factor among H. pylori infected and non-infected patients

Rhesus System	No. patients	H. pylori +ve No. (%)	H. pylori -ve No. (%)
Rhesus positive	186	138 (74.2)	48 (25.8)
Rhesus negative	14	8 (57.1) 8	6 (42.8)

Discussion

The ABO blood group system is the most important in human blood transfusion. This system is used as a genetic marker in studies of associations with infectious and non-infectious diseases (Mattos et al., 2002). The ABO blood group antigens confers advantage of resistance against certain infectious disease (Reid and Bird, 1990). The risk of gastric cancer was found higher among those with the blood group A, while the gastric ulcer was more common in blood group O persons (Alkout et al., 2000 and Nakao et al., 2011).

Various diagnostic tests for H. pylori infection may have false negative results, and the use of multiple tests may help to provide a more accurate diagnosis of H. pylori infection (Akbar and El Tahawy, 2005 and Laheij et al., 2000). However, this study showed that H. pylori detection rate by RUT, RDT, culture and PCR were 66.5%, 69.5%, 71% and 67.5% respectively with no significant difference. Thus, a patient may be negative by culturing, but positive by PCR or other methods making the total positivity 73%. . The culture method was the most sensitive one; to detect this bacterium is ascribed to culturing on MCUA medium and applying the MDCS (Al-Sulami et al., 2002). Results on gender agree with some studies that indicated the males were associated with a higher risk of acquiring H. pylori infection than females (Ndip et al., 2004 and Ndip et al., 2009) while, disagree with other studies as males and females are infected at the same rate (Nwodo et al., 2009 and Rasmi et al., 2009).

Studying the distribution of H. pylori infection and their clinical diagnosis among patients reveal that H.pylori positivity among patients with gastritis, gastric ulcer, duodenal ulcer, and gastric cancer were 73.4%, 83.3%, 68.6% and 50%, respectively. These results showed that the difference was statistically not significant ($P>0.05$) between H. pylori infection and the degree of clinical status of these patients.

The results of this study showed that there was a significant association between ABO blood groups and H. pylori infection, in which type O has a greater tendency towards infection and type AB to non-infection. These results are reinforced by the data obtained from other researchers showing the greater susceptibility of blood group O to H. pylori infection (Kanbay et al., 2005 and Mattos et al., 2002). While, the results disagree with some previous studies which demonstrated that the O blood group did not represent a risk factor for H. pylori infection (Seyda et al., 2007 and Sharara et al., 2006).

The H type 2, Lewis (a) can act as receptors for H. pylori on the gastric mucosa (Alkout et al., 2000). Additionally, it is demonstrated that epithelial cells of persons of group O bound significantly more H. pylori than did cells of persons of other blood groups (Alkout et al., 1997 and Kanbay et al., 2005). H. pylori express lipopolysaccharides on its outer membrane including blood group antigen-binding adhesion A (BabA adhesin) which causes adhesion of bacteria to gastric epithelium and allow persistent colonization (Linde 'N et al., 2002).

The present study demonstrated higher incidence of *H. pylori* among patients of blood group O with gastric ulcer (46.7%) as compared with A (20%), B (13.3%) and AB (3.3%) blood groups where that differs significantly ($P < 0.05$). Also, a higher incidence of *H. pylori* among blood group O with duodenal ulcer (47.4%) as compared with other blood groups, but the difference was statistically not significant ($P > 0.05$). These findings indicate that people of blood group O with gastric ulcer are more susceptible to infection with *H. pylori* as compared with other blood groups.

Previous studies demonstrated that individuals with blood group O have a higher risk of developing duodenal ulcers and also a higher incidence of gastric ulcers (Dhillon, B.S. and Shergill, 2004 and Martins et al, 2006). The increased susceptibility to peptic ulceration among persons with blood group O was due to density of colonization of epithelial cells and higher inflammatory responses to *H. pylori* (Alkout et al., 2000).

Regarding Rh status, 74.2% Rh+ patients and 57.1% Rh- patients were positive for *H. pylori*. The present study showed no significant differences between the positive patients and negative patients, indicating that the presence of *H. pylori* did not relate to the Rh factor, which is in an agreement with previous study by Petrovic et al (2011).

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