Original article

Seroprevalence of *Helicobacter pylori* Infection in and its Relationship with ABO Blood Groups

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Abstract

Background: *Helicobacter pylori* infection occurs worldwide, but the prevalence varies greatly among countries and among population groups studied. People with blood group O have been noted to be more susceptible to *H. pylori* infection.

Objectives: To determine the magnitude of *H. pylori* infection in adult patients with and without dyspeptic symptoms attending Felege Hiwot Hospital, Bahir Dar, North West Ethiopia. We also prospectively examined the relationship between *H. pylori* infection and ABO blood groups in those subjects to clarify the possible association.

Methods: A case-control study was conducted between dyspeptic (n=100) and non-dyspeptic patients (n=100) for the presence of *H. pylori* infection between mid November 2002 and March 2003 by using serology.

Results: Of the total 200 subjects, 128 (64%) were males and 72 (36%) females with mean age of 32.7, range 15-75, years. Of these, 112 (56%) and 66 (33%) were found positive and negative for *H. pylori* infection, respectively, by EIA. Borderline values were measured in 22/200 (11%). *H. pylori* infection was detected in 124/200 (62%) of study subjects by immunoblot. The most prevalent blood group was type O (43%), followed by A (23.5%), B (22.5%) and AB (11%). In comparison of the subjects with blood group O and the other blood groups, no statistical significance could be identified in the seroprevalence of *H. pylori* ($p>0.05$).

Conclusion: The overall seroprevalence of *H. pylori* infection in Bahir Dar in this study was between 63% and 70% in dyspeptic patents and 49% and 54% in non-dyspeptic patients by EIA and IB, respectively. There were no statistically significant differences in the frequency of *H. pylori* seroprevalence between dyspeptic and non-dyspeptic patients. There was no statistically significant association between *H. pylori infection* and sex, age and ABO blood groups. Thus, further investigations along this line are recommended. [*Ethiop.J.Health Dev.* 2005;19(1):55-59]

Introduction

Helicobacter pylori infection is the most common chronic bacterial infection in the world. Previous seroepidemiologic studies indicated that about 50% of adults in the developed countries and nearly 90% of adults in developing countries were seropositive for *H. pylori* (1). Chronic *H. pylori* infection may be associated with chronic gastritis (2), peptic ulcer disease (3), mucosal associated lymphoid tissue (MALT) lymphoma (4) and gastric adenocarcinoma (5).

Dyspepsia is one of the commonest complaints in any Ethiopian outpatient department (6-8). Although a number of epidemiological studies have suggested a higher prevalence of *H. pylori* infection in patients with dyspepsia, only few high quality therapeutic trials have specifically investigated whether *H. pylori* infection causes dyspepsia. The majority of dyspeptic patients are not simple to diagnose, and may need several empirical trials of therapy, or more specific diagnostic assessment (9). Recent studies in Ethiopia showed that the overall prevalence of *H. pylori* infection in adult dyspeptic patients, as found by the different diagnostic methods, varied between 69 and 91% (10).

People with blood group O have been noted to be more susceptible to peptic ulcer disease for decades without appropriate explanations (11). In 1993, Boren *et al.* (12) reported that people with blood group O had more *H. pylori* receptors, and Lewis b antigens mediated the attachment of *H. pylori* to the gastric mucosa. Furthermore, higher density of colonization of *H. pylori* was noted in the gastric mucosa of people with blood group O (13). However, absence of correlation between *H. pylori* infection and ABO blood groups was reported in some subsequent studies (14-15).

At present there are only few studies addressing the possibility of an association between *H. pylori* infection and dyspepsia and/or non-dyspepsia in Ethiopian patients (10, 16-17). There is no published information concerning the relationship between *H. pylori* infection and ABO blood groups in the Ethiopian setting. Therefore, we conducted a further study in an Ethiopian setting to ascertain the importance of *H. pylori* infection in dyspeptic and non-dyspeptic patients and to compare the relationship of ABO blood groups and *H. pylori* infection.

Methods

A case-control study was conducted among randomly selected dyspeptic (cases) and non-dyspeptic patients (controls) from outpatient and in-patient departments of Felege Hiwot Hospital, Bahir Dar, northwest Ethiopia. Consecutive informed and consenting adult patients (15 or more years of age) with dyspeptic symptoms (n=100)

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and without dyspeptic symptoms (n=100) were investigated for *H. pylori* infection between mid November 2002 and March 2003. Sample size (n) was calculated according to a previous study of prevalence (p) of *H. pylori* infection in Ethiopian patients with dyspepsia and without dyspepsia (60-80%), marginal error (d) 3.5%, and 5% level of confidence.

Dyspepsia has been defined as pain or discomfort centred in the central portion of the upper abdomen associated with a variety of symptoms such as fullness in the upper abdomen, early satiety, bloating or nausea or vomiting.

All appropriate information (demographic, clinical, and laboratory data) was recorded on the questionnaire prepared for this study. Patients and controls have been taking antibiotics including treatment for *H. pylori*, non-steroidal anti-inflammatory drugs (NSAIDs) or steroids in the past one-month were excluded.

Five to ten ml venous blood was collected from each informed and consenting adult dyspeptic patients and non-dyspeptic patients. The sera were obtained from the blood by centrifugation (3000 RPM for 10 minutes). Sera were aliquoted and immediately stored at -20^{o}C until tested for serology. ABO blood groupings were performed using a commercial kit (Gamma Biologicals, Houston, Tx, USA).

All the 200 sera collected from patients were examined for the presence of IgG antibodies against *H. pylori* pooled antigens by using an enzyme immuno assay (EIA) method as previously described (10). Briefly, microtiter plates (Maxisorp immunoplate NUNC, Rosklide, Denmark) were coated overnight with acid glycine extracted cell surface proteins from *H. pylori* reference strain (CCUG 17874) and incubated at 4°C for 16 h. Plates were washed three times in washing-buffer (Phosphate buffered saline or PBS, 0.05% and Tween-20, PBS-T). Antigen-coated wells were then blocked with blocking-buffer (bovine serum albumin in PBS-T), and incubated for 1h at 22 °C. To this 100 µl of test serum diluted 1:800 in PBS-T was added and incubated for 90 min at 37 °C. Serum was tested in duplicate and a PBS-T control for each sample was tested to monitor non-specific background readings. After washings, 100µl alkaline phosphatase labeled goat antihuman IgG antibody (Sigma, St Louis, USA) was added and plates incubated for 60 min at 37 °C. After washing, 100 µl of a substrate solution containing p-nitrophenyl phosphate (Sigma) was added and incubated for 30-60 min at 37 °C. A reference standard, human gammaglobulin (Pharmaca & Upjohn, Stockholm Sweden), was run in parallel throughout the procedure. The absorbance was read by using a microplate reader (Bio Rad) at 405nm and the EIA results were calculated as relative antibody activity (RAA). RAA values >35 and <25 units were considered as positive and negative, respectively. RAA values between 25 and 35 were regarded as low positive (borderline). A known positive and negative serum for *H. pylori* was run in parallel throughout the procedure as a quality control (provided from Lund University, Sweden).

All sera were tested with immunoblot (IB) to confirm EIA results. The same glycine extracted antigen used for EIA was taken for immunoblot. Immuno assay was done according to the procedures described previously (10). Briefly, immunoblot strips containing *H. pylori* acid glycine extracted cell surface proteins (provided from Lund University, Sweden) were probed with patient sera diluted 1:100 and incubated at 4°C for 16 h under gentle shaking. After repeated washings with washing buffer, strips were incubated with horseradish peroxidase labelled rabbit antihuman IgG antibodies (Dako, A/S, Glostrup, Denmark) diluted 1:600 and incubated at 22°C for 2 h. Membrane bound antibodies were detected by adding a substrate solution containing 50 mM sodium acetate buffer, 1% 3-amino-9-ethy1carbazole (Sigma) and 30% H_2O_2. Strips were incubated in darkness at room temperature for 30 min. The intensity of the band obtained with the patient sera were compared to that obtained with a strip containing molecular mass markers and the results were interpreted positive for *H. pylori* infection based on the presence of antibody reactivity to high molecular mass proteins (120 kDa) and or at least two to five of the low molecular mass proteins (33, 31, 30, 29, 26 kDa) as previously reported (10).

EPI-INFO Version 2000 (CDC, Atlanta, Georgia, USA) with Yates's correction and Pearson Chi-square were applied and p values < 0.05 were considered as statistically significant.

The study has been approved by the ethical committee of Department of Biology, Science Faculty, Addis Ababa University.

Results

The age and sex distribution of dyspeptic and non-dyspeptic patients is shown in Figure 1. Of the total 200 (100 dyspeptic and 100 non-dyspeptic) subjects 128 (64%) were male and 72 (36%) female with mean age 32.7, range 15-75 years, respectively. Subjects between age 15 and 44 years comprise 81.5%. The male to female sex ratio was 1.8:1.

Of the total 200 studied subjects, 112 (56%) and 66 (33%) were found seropositive and seronegative for *H. pylori* infection by EIA, respectively (Table 1).

Borderline values were measured in 22/200 (11%) (Table 1). All 200-serum samples were further analysed by immunoblot (IB). IgG to *H. pylori* was detected in 70 (70%) and 54 (54%) from dyspeptics and non-dyspeptics, respectively (Table 1). There was no statistically significant difference in seroprevalence of *H. pylori* infection between dyspeptics and non-dyspeptics (p>0.05). There was no statistically significant difference in *H. pylori* seropositivity between males and females (data not shown).

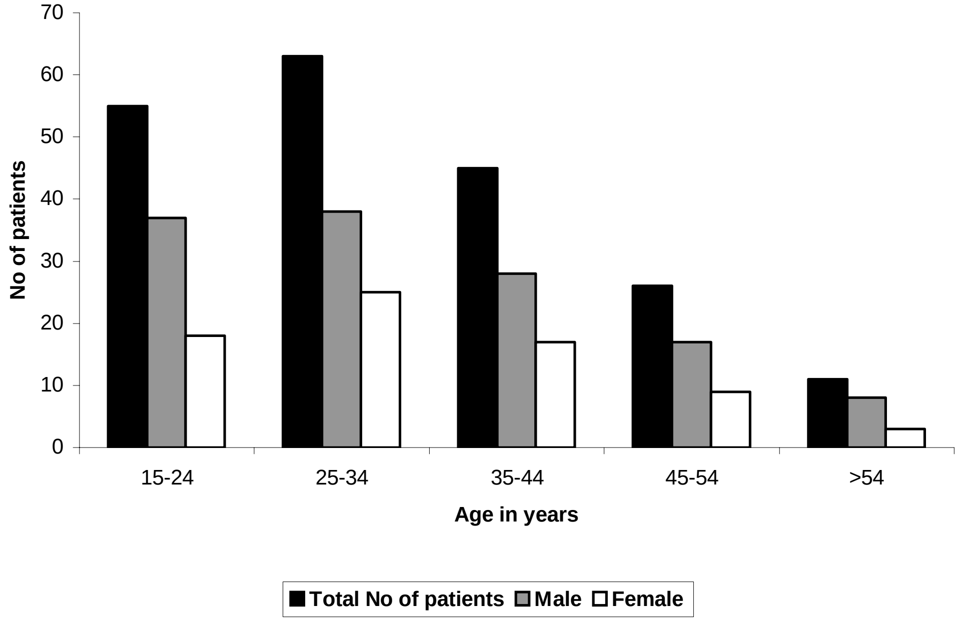

Figure 1: **Age and sex distribution of adult dyspeptic and non-dyspeptic patients in Felege-Hiwot Hospital investigated for Helicobacter pylori infection between November 2002 and March 2003.**

Table 1: **Frequency of detection of *H. pylori* (HP) infection in dyspeptic and non-dyspeptic patients attending Felege Hiwot Hospital, North-West, Ethiopia.**

Serological Methods	HP Positive No. (%)	HP Negative No. (%)	Total No. (%)
Enzyme immuno assay (EIA)			
Dyspeptic patients (n=100)	63 (63)	37 (37)[1]	100 (100)
Non dyspeptic patients (n=100)	49 (49)	51 (51)[2]	100 (100)
Immunoblot (IB)			
Dyspeptic patients (n=100)	70 (70)	30 (30)	100 (100)
Non-dyspeptic patients (n=100)	54 (54)	46 (46)	100 (100)

[1] Borderlines are included (n=13); [2] Borderlines are included (n=9)

Blood groups and H. pylori infection

The most prevalent blood group was type O (43%) followed by A (23.5%), B (22.5%) and AB (11%) as shown in Table 2. Further analysis on the ABO blood groupings and seroprevalence of *H. pylori* demonstrated that seropositive rate of *H. pylori* was 66.3% in blood group O, 59.6% in blood group A, 57.8% in blood group B and 59.1% in blood group AB (Table 2). In comparison of the subjects with blood group O with the other blood groups, no statistical differences could be identified in the seroprevalence of *H. pylori* (p>0.05).

Neither was the difference significant among the blood groups in being susceptible to *H. pylori* infection.

Table 2: **Relationship of ABO blood groups and seropositivity to *Helicobacter pylori* (HP) infection**

Blood group	Total tested No. (%)	HP Positive No. (%)	HP Negative No. (%)
O	86 (43.0)	57 (66.3)	29 (33.7)
A	47 (23.5)	28 (59.6)	19 (40.4)
B	45 (22.5)	26 (57.8)	19 (42.2)
AB	22 (11.0)	13 (59.1)	9 (40.9)
Total	200 (100)	124 (100%)	76 (100%)

Discussion

The overall prevalence of *H. pylori* infection in Bahir Dar in this study was 49-70%. This is in agreement to that reported in the previous studies done in Ethiopia (56-70%) (17-18) and in some other developing countries (36-81%) (19-20), but lower than that reported in recent studies in Ethiopia (69-91%) (10, 21). However, there are reports from different parts of the world with lower prevalence of *H. pylori* infection e.g. 27% in Kuwait (19) and 15-46% in the Gambia (19). The low prevalence in this study may be attributed to differences in study area, subjects, sample size or elimination of *H. pylori* infection as a result of antibiotic treatment in occasion of concomitant diseases, such as giardiasis, amoebiasis, and respiratory diseases, etc., as these diseases are reported to be more prevalent in the study area (22). In this study, there were no statistically significant differences between dyspeptics and non-dyspeptics in the frequency of detection of *H. pylori* by serological methods, which is in agreement with the results obtained from other studies (23-25). There was no significant difference in the overall prevalence of *H. pylori* infection between males (53.1%) and females (61.4%), p>0.05. This finding is compatible with other studies (1, 10, 24, 26-28). However**,** some studies have found a higher prevalence of *H. pylori* infection in males, which may be related to higher exposure of the males to potential environmental sources of infection (29-30).

In general, results of worldwide epidemiological studies have been unconvincing. No clear-cut link has been documented concerning the rate of seroprevalence of *H. pylori* between individuals with and without dyspeptic symptoms. In the randomized controlled trials, methodological weaknesses may explain in part the conflicting results, but even in the well-conducted trials controversy persists. The possible reasons for these variations could be, misdiagnosis of patients, differences in dyspepsia scoring systems, sample size, lack of clear-cut definition of dyspepsia, methodological weaknesses, including low study power, a lack of randomization and interplay of various confounding factors such as social, economic and demographic factors.

Individuals with blood group O were found to be more susceptible to peptic ulcer disease for decades without known cause until the relationship between Lewis^b antigens and the attachment of *H. pylori* to gastric mucosa was observed (11-13). However, the correlation between *H. pylori* infection and ABO blood types was not supported in some reports (14-15). In our study, although the most prevalent blood group was blood type O (43%), subjects with blood group O don't show an increased susceptibility to *H. pylori* infection than those with other blood groups (p>0.05).

In conclusion, the seroprevalence of *H. pylori* infection in Felege Hiwot Hsopsital, northwest Ethiopia, is similar to that in the developed countries. Although blood group O was reported to be related to *H. pylori* infection in some studies done elsewhere, we found no association between *H. pylori* infection and ABO blood groups. This study also showed that serological methods (EIA and IB) can be used as diagnostic tools to determine the status of *H. pylori* infection and for epidemiological studies, because they are generally simple, reproducible, inexpensive, and can be done on stored serum samples. However, the performance of these serological methods varies considerably depending on the methods, antigen used and type of populations studied. Another limitation is, use of serology in assessing the eradication of *H. pylori* after treatment is very limited, because antibody levels decrease decreases slowly in the serum. In addition, the inclusion of patients receiving antibiotics for other infections or who have been treated for *H. pylori* infection in the past may affect the diagnostic accuracy of serology.

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