SERO-PREVALENCE OF HELICOBACTER PYLORI INFECTION AMONG HEALTH BLOOD DONORS IN ADDIS ABABA, ETHIOPIA.

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ABSTRACT

Background: ***The discovery of Helicobacter pylori by Marshall and warren in 1982 revealed that, this organism has been implicated as the main etiological agent in the development of acute and chronic active gastritis, peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma in humans. According to recent epidemiological studies, more than half of the world's population is infected with Helicobacter pylori; a higher prevalence is reported in developing countries particularly in children and in early adult life. Thus, it is necessary to perform sero-prevalence study in order to have a clear understanding of the role played by H. pylori in the Ethiopian context, where peptic ulcer disease and gastric cancer are a major cause of morbidity and mortality respectively.***

Methods: ***A cross-sectional study was conducted in January 2001. Blood samples were collected from 150 adult healthy blood donors at blood bank unit Ethiopian Red Cross Society in Addis Ababa. The sera were obtained from blood by centrifugation and were examined for presence of IgG antibodies against Helicobacter pylori glycine extract antigen using the conventional enzyme immuo assay (EIA) method.***

Results: ***A total of 150 healthy blood donors were investigated for H. pylori serology. Blood donors aged between 15 to 34 years predominated among the study groups and represented 72.0% of all. Among the blood donors, 123(82.0%) were males and 27(18.0%) were females. Among these, 133(89.0%) of the sera were positive of Helicobacter pylori, whereas the remaining 9(6.0%) and 8(5.0%) of the sera were negative and borderline respectively.***

Conclusion: ***This study showed that, the prevalence of Helicobacter pylori infection among healthy blood donors of Addis Ababa was very high. Community based studies need to be conducted to confirm that this data reflects the actual situation of H. pylori infection in the general population. In addition to this, further epidemiological investigations should be performed in order to determine the sources, modes of transmission and the risk factors involved for Helicobacter pylori infection in the Ethiopian context.***

Key words: Blood donors, Helicobacter pylori, Enzyme Immuno Assay, Relative Antibody Activity, IgG.

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INTRODUCTION

The discovery *Helicobacter pylori* by Marshall and Warren (1) revealed that, this organism has been implicated as the main etiological agent in the development of acute and chronic active gastritis, peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma in humans (2). This newly discovered organism is a spiral shaped, gram-negative bacillus that is oxidase, catalase, and urease positive and grows slowly in culture. This organism was first named *Camplyobacter pyloridis* later changed to *Camplyobacter pylori* and today it is known as *Helicobacter pylori* (3). The genus *Helicobacter* has expanded during the last 20 years and consists of more than 18 species including non-gastric *Helicobacter* (4). T he ecological niche of *H. pylori* is the human stomach, where the organism establishes long-term colonization of the gastric mucosa (5).

The prevalence of *H. pylori* infection depends upon the age, the geographic location and the socio-economic status of the subjects studied. According to recent epidemiological studies, more than half of the world's population is infected with *H. pylori* ; a higher prevalence (more than 90%) is reported in developing countries particularly in children and in early adult life (6). A recent cross-sectional study in Ethiopia showed that infection with *H. pylori* is infected at the age of 60 years (8). Despite the high prevalence of infection, not all infected individuals develop disease. The mode of transmission of *H. pylori* infection is most likely a result of complex interactions among host, bacterial and environmental factors.

A variety of diagnostic methods (invasive and non-invasive) are now available to diagnose *H. pylori* infection. Invasive methods include detection of the bacteria in gastric biopsy specimens by rapid urease test, culture, immunohistochemistry, or by polymerase chain reaction (10). All these invasive methods require endoscopy. In contrast, the less invasive methods such as, ureas breath test, serology, and stool antigen test do not require endoscopy (1.1). The choice of the method used to diagnose of *H. Pylori* infection will depend, in most cases, on the clinical information sought, the local availability and the cost of individual tests.

In developing countries, infection with *Helicobacter pylori* appears to wide spread, and the prevention of this important cause of peptic ulcer disease and possibly even gastric cancer, should be a priority. However, in the absence of vaccine, prevention efforts must be based on informtions obtained from epidemiological studies. Among the epidemiological studies, prevalence study using enzyme immunoassay (EIA) method is preferred, because this method is relatively cheap and it is the most commonly used method for diagnosis of *H. pylori* infection in populations without having to take biopsies from the stomach for culture or histopathology. The sensitivities and specificities of EIA from various sources ranges from 86-100% and 76-98% respectively. Thus, diagnostic performance of properly evaluated EIA is comparable to that of biopsy based methods and urea breath test, EIA is also used to monitor eradication of H. pylori infection following therapy, especially with high pre-treatment antibody titres (12). The classic treatment of *H. pylori* infection consists of triple therapy; two antibiotics (amoxycillin/ tetracycline/ metronidazole and clarithromycin) and proton pump inhibitor. This triple therapy combination consistently eradicates *H. pylori* infection in Ethiopia (7, 14-18). This study was therefore undertaken to study the seroprevalence of *H. pylori* infection using EIA method, in order to provide additional epidemiological markers as a basis for future studies and to design strategies for

treatment and prevention of *Helicobacter pylori* infection.

MATERIALS AND METHODS

A cross-section study was performed to determine the seroprevalence of *Helicobacter pylori* infection among blood donors in Addis Ababa, Ethiopia.

Study Subjects:-A total of 150 informed and consented adult blood donors (15 or more years of age) from the blood bank unit of Ethiopian Red Cross Society were investigated for *Helicobacter pylori* during January 2001. Before donation of blood, the health status of all these blood donors was evaluated according to the criteria set by the Ethiopian Red Cross Society, All were found to be apparently healthy (personal communication).

Informations related demographic data (age, sex, address) were taken from each blood donor after interviewed by nurses. Finally, all the relevant data (demographic and laboratory data) were transferred to the questionnaire prepared for this study.

Collection, Handling and Transport of Specimens

Five to ten ml venous blood were collected from each blood donor for serology. The sera were obtained from the blood by centrifugation (3000 RPM for 10 minutes) and were transported immediately to the Bio-Medical Research Training Project Laboratory (BRTP), Faculty Medicine, Addis Ababa University, and kept at -20^0C until tested. In addition to this, blood grouping and Rh factor were determined using commercially prepared antisera by slide agglutination technique.

Enzyme Immuno Assay

A total of 150 sera collected from adult healthy blood donors were examined for the presence of IgG antibodies against *H. pylori* pooled antigens (CCUG, 17874) using EIA method, as described by Guruge et al. (19). Briefly microtiter plates (Maxisorp immunoplate NUNC, Rosklide, Denmark) were coated overnight with acid glycine released cell surface proteins from *H. pylori* reference strain CCUG 17874. The protein concentration was adjusted to 0.5 µg per well. Each serum was diluted 1/800 in washing buffer solution containing phosphate buffered saline (PBS) and Tween-20 and then tested in triplicate with one uncoated well as a negative control for each serum tested. The absorbance was read by using a microplate reader (Bio Rad) at 405nm and the EIA results were calculated according to the procedure described by Blomberg et al. (2) and expressed as relative antibody activity (RAA). RAA values >35 and <25 units are 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 paralled throughout the procedure as a quality control (provided from Lund University, Sweden).

All serum samples were analysed for IgG-EIA at Bio-Medical Research Training project Laboratory (BRTP), Faculty of Medicine, Addis Ababa University.

RESULTS

Study Subjects:-The age and sex distribution of adult healthy blood donors are shown in Figure 1. During the period of January 2001 a total of 150 healthy blood donors were investigated for *H. pylori* serology. Out of these, 108 (85 males and 23 females) (72%) were between the ages of 15 and 34 years; 25 (22 males and 3 females) (16.6%) were aged 35-44 years, whereas age group 45 years and above (16 males and 1 female) represented 11.4%.The overall male to female ratio was 4.5:1.

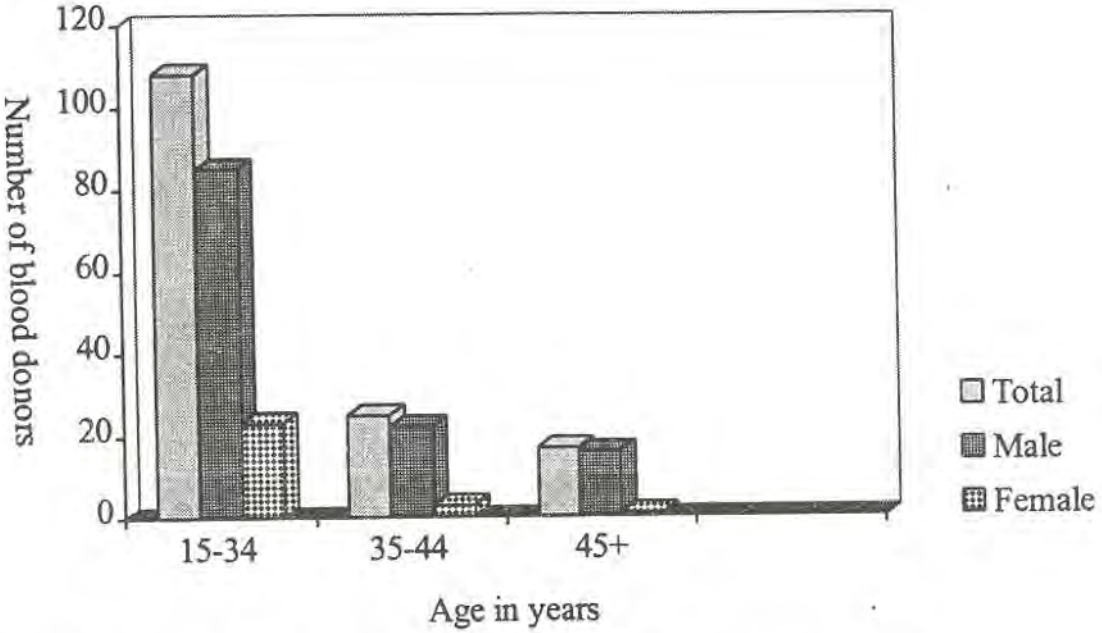

Figure 1. Age and sex distribution of blood donors investigated for pylori

Enzyme Immuno Assay (EIA)

The age and sex distribution of blood donors seropositive for *Helicobacter pylori* by EIA are shown in Figure 2. A total of 133(89.0%) sera were positive for *H. pylori*, whereas the remaining sera 9(6.9%) and 8(5.0%) were negative and borderline respectively. Among the 133 blood donors, which were positive for H. pylori, there were 109 males and 24 females (82.0% vs, 18.0%, P>0.05), resulting in an overall male to female ratio of 4.5:1. The peak rate seropositivity for *H. pylori* was observed between the age group of 15-34 years (90.0%) (Figure2).

The frequently observed blood group in this study was group O, 71(47.3%), followed by group A, 38(25.3%) group B, 36 (24.0%) and group AB, 5 (3.0) (Data not shown). Blood donors with blood group O had the highest seropositivity rate for *H. pylori* 61(40.6%) as compared from those who had another blood group pattern.

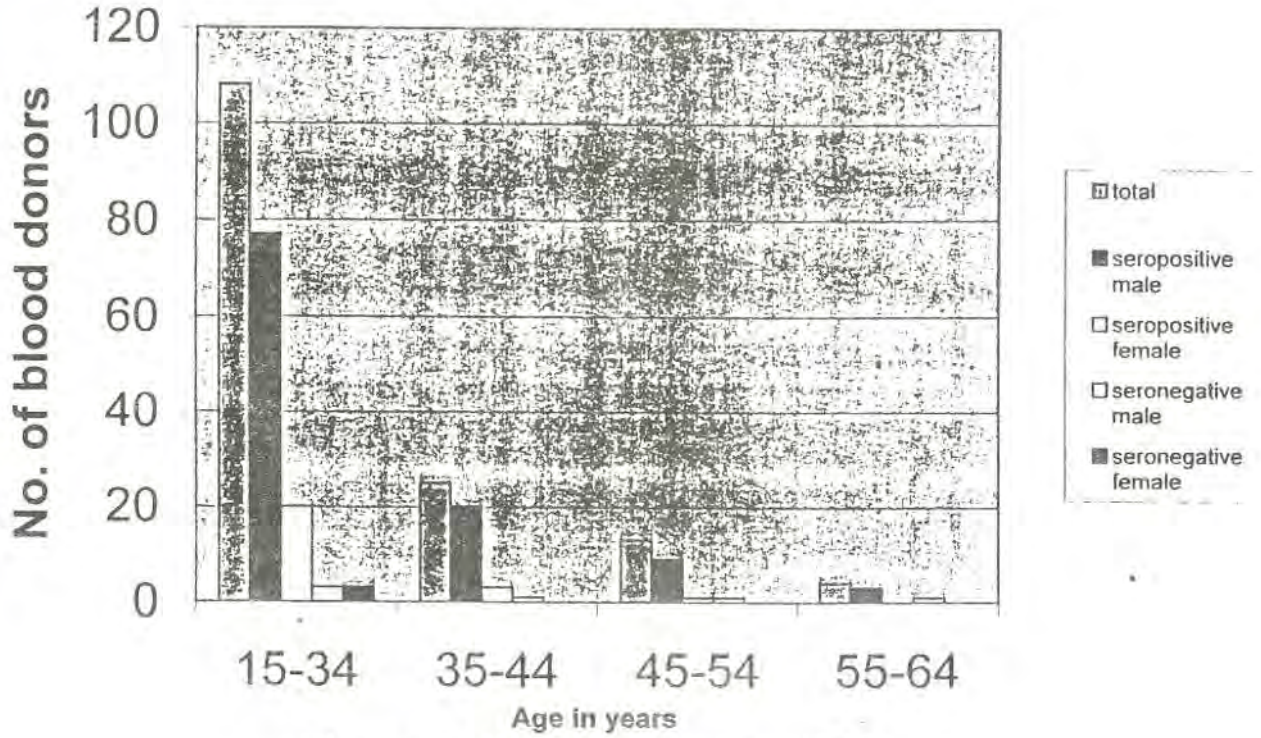

Figure 2. Seroprevalence of *H. pylori* infection among blood donors

DISCUSSION

Helicobacter pylori has a worldwide distribution and it is estimated that approximately 50% of the world's population is infected. Seroepidemiological studies have shown that there are two patterns of distribution of *Helicobacter pylori* infection. The first pattern is observed in developing countries where most children become infected before 4 years of age and the prevalence reaches almost 100% in adulthood. The second pattern is typical of developed countries, where only a few people are infected before 5 years of age and prevalence increases steadily with age (21).

All blood donors in the present study were from the blood bank unit of Ethiopia Red Cross Society. In this study the overall rate of seropositivity of H. pylori infection was 89.0%. The frequency of seropositivity for *H. pylori* infection in this study (89.0%) is similar to that reported in other studies performed in developing countries e.g. in Algeria, Ivory Coast, Thailand, Saudi Arabia, Chile, Peru, Southern, India, South Africa, with figures ranging from 13 to 70% in the 0-20 years-old age group and from 70-94% in those over 30 years old (21). However there are reports from different parts of the world with lower rate of seropositivity from England France, Scandinavia, Italy, Belgium and USA with ranges 5-15% in children and 20-65% in adults (21). A recent cross sectional study in Ethiopia also showed that, the sharpest rise in HP seroprevalence was found in the age range 2-4 years, reading almost 100% in the 4 years olds (7), whereas a study conducted in South Africa showed that, the highest HP seroprevalence was found in the age range 21-40 years (94.0%), reaching 100% in the 41 years olds and above (22). In this study, it was also observed that blood donors with blood group O had the highest

seropositivity rate (40.6%) for *H. pylori*. As suggested by Boren et al. (23), there is clinical relevance fro H. pylori attachment mediated by the fucosylated blood group antigens as individuals of blood group O phenotype run a 1.5-2.5 fold increased risk of developing peptic ulcer disease as compared from those who had another blood group pattern. However in this study it is difficult to conclude that those who had blood group O showed the highest seropositivity for *H. pylori*, because this blood group represents the highest proportion of the sampled population.

A possible explanation for the high seropositivity of *H. pylori* infection in our study as compared with reports from some of the different countries mentioned earlier may be related to socioeconomic factors. Poor living conditions in child hood with low hygiene levels have been reported as predictors of *H. pylori* infection. It is also well known that, acquisition of H. pylori infection is associated with several risk factors such as microbial and host factors. Among the host factors, HLA genotypes, ABO blood group antigens and host gastric acid physiology play an important role in the acquisition of H. pylori.

Eventhough serological tests like EIA are generally accepted for large epidemiological surveys, there are limitations, which can affect specificity of the test. One of the limitation of this method is, cross-reacting antibodies which minght five false positive reactions. A high specificity can be obtained by performing western blot, which is also a confirmatory test. In addition, this method helps to detect IgG antibodies against cagA and vacA antigens expressed by H. pylori, which play an important role in the pathogenesis of peptic ulcer disease and gastric malignancy.

In conclusion, our study showed that, the prevalence of Helicobacter pylori (HP) infection among healthy blood donors of Addis Ababa is very high. To confirm whether this data reflects the actual situation of *H. pylori* infection in the general population, community based studies need to be conducted using different diagnostic methods such as rapid urease test, culture, histology, serology and stool antigen test. In addition to this, the prevalence of H. pylori in symptomatic individuals should also be investigated in order to know the role played by H. pylori in the causation of peptic ulcer disease or malignancy in the Ethiopian context.

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