The association of *Helicobacter pylori* mucosal density with low Serum Ferritin

Nidhal Raoof Mahdi[1] *PhD*, **Nidhal abdul Mohaymen[2]***PhD*.

Abstract

Background: Although there are several methods to detect *Helicobacter pylori* infection, there is no simple validated test to quantify the density of infection, which is believed to play a major role in the pathogenesis of *H. pylori*-associated Gastritis and serum Ferritin level.

Objective: The aim of this study was to assess the association of low serum Ferritin level with the intensity of *H. pylori* infection.

Patients and Methods: Sixty four patients mean age of 34 years (14-66 years) who underwent upper gastrointestinal endoscopy because of gastrointestinal complaints, were studied. Patients were grouped as *H. pylori* positive group, *n*=47and *H. pylori* negative group, *n*=17.

A number of both invasive and non-invasive diagnostic tests were used for the diagnosis of *H. pylori* infection (Ultra Rapid Urease Test (URUT), slide impression smear test and *H.pylori* IgG ELISA Test).

Fasting serum Ferritin were determined using VIDAS Ferritin (Enzyme Linked Fluorescent Assay).

Results: Forty seven of the 64(73%) patients were *H.pylori* positive group. patients were classified according to the age group and gender. The rates of the *H.pylori* infection were higher in female age group 21-30 years. A total 16 of the 47 (34%) infected patients showed low serum Ferritin values with high rate in female with age group 21-30years. Twenty eight of the 47(60%) patient biopsies showed positive microscopic examination with slide impression smear test .Twenty seven of the 47(57%) infected patients showed seropositive results to anti-*H.pylori* IgG antibody and also positive with URUT,10 individuals of this group showed low serum Ferritin values. While ten of the47 (21%) infected patients showed seronegative results to anti-*H.pylori* IgG antibody but positive with URUT,5 individuals of this group showed low serum Ferritin values.

Conclusion: The possible relationship between mucosal *H.pylori* loads with low serum Ferritin level.

Keywords: *Helicobacter pylori infection,* serum Ferritin, anti-*H.pylori* IgG antibody ELISA test, Ultra Rapid Urease, Enzyme Linked Fluorescent Assay.

Introduction

Helicobacter pylori is a gram negative, curved, microaerophilic and motile organism with multiple polar flagella. It resides in the stomach of man and other primates, lining up the gastric mucus secreting cells. It is estimated that about 50% of all humans carry *H. pylori* in their stomach [1,2].

[1]Dept. Medical Microbiology. College of Veterinary Medicine, Baghdad University, [2]Dept. Medical Microbiology. College of Medicine, Al-Nahrain University,
Adress Correspondence to: Dr. Nidhal R. Mahdi. E-mail: nidhal_rauf@yahoo.com
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The prevalence of *Helicobacter pylori* infection in developing countries is about 70 to 90% and it is only 20–50% in developed countries [3]. The persistent infection induces a state of chronic gastric inflammation that frequently remains asymptomatic. In some patients, however, the infection causes disease, such as peptic or gastric ulceration, the development of a mucosa-associated lymphoid tissue lymphoma, or even gastric cancer [4]. It is not yet clear why only some people develop more severe forms of disease despite the high prevalence of *H. pylori*

in the human population. Certainly, host genetic factors play a role in determining the clinical outcome of the infection [5]. On the other hand, *H. pylori* virulence factors also play a role in pathogenesis, since virulent strains are associated with more aggressive tissue damage and an increased risk of a severe clinical outcome[6]. Finally, environmental factors such as nutrition are also thought to be important [7]. Epidemiologic studies have shown that the prevalence of *H. pylori* varies considerably with age [8] *H.pylori* needs to have at least four basic characteristics to be able to colonize and establish an infection in the gastric mucosa: urease, flagella, a particular shape, and adhesins. *H. pylori* is able to adhere to the surface and sites of epithelial cells and to the basement membrane of gastric epithelial cells [9]. When *H.pylori* is introduced in the stomach, a pH-neutral microenvironment around the bacteria is produced by exogenous shedding of urease, which converts urea to ammonia ions that neutralize the acidic gastric juice, and thereby enables *H. pylori* to survive and multiply in the stomach [10]. Thus, the disease outcome is determined by a combination of host, bacterial, and environmental factors.

The acute *H. pylori* infection that is dominated by abdominal pain and infiltration of polymorph nuclear leucocytes (PMNs) in the gastric mucosa only lasts for a few weeks [11-13]. Thereafter, it turns into an active chronic superficial gastritis with an increased recruitment of lymphocytes and other mononuclear leucocytes. In the humoral immune response to *H. pylori* infection, IgM antibodies to *H. pylori* are produced shortly after colonization whereas IgG antibodies to *H. pylori* seem to be delayed up to 3–6 months [14, 15]. Thus,

within a few weeks of the primary exposure to *H. pylori,* a true infection can be established.

The superficial gastritis may or may not evolve to atrophic gastritis, which later may lead to intestinal metaplasia, dysplasia, and gastric cancer [16]. As the inflammation progresses, the specific immune response becomes more dominating and even the PMNs lose their ability to recognize the specific *H. pylori* strain in the host as a foreigner [17].

The diagnostic methods available for detecting *H. pylori* infection include conventional PCR and real-time PCR [18, 19]. Rapid urease test is highly specific for *H. pylori* infection and is commonly used for the detection of *H. pylori* infection at endoscopy. It requires a high density of bacteria [20]. The sensitivity of urease test is reduced in patients who are taking proton pump inhibitors (PPI), antibiotics or bismuth compounds [21, 22]. Any antibiotic active against *H. pylori* will cause a reduction in the numbers of bacteria in the stomach [23].

Increase Iron Uptake and Utilization by Bacteria

Epidemiologic studies have shown that persons seropositive for *H. pylori* infection have a significantly lower serum ferritin level [24, 25, 26, 27, 28]. Although *H. pylori* infection is common, iron deficiency anemia does not develop in all infected patients. The ability to cause iron deficiency anemia does not appear to be related to the virulence of the organism because ferritin levels did not differ between patients infected with cytotoxin-associated gene A (CagA)-positive and CagA-negative strains of *H. pylori* [24]. It may be possible that other bacterial virulence factors or host factors are responsible for the development of iron deficiency anemia.

Several mechanisms have been hypothesized to explain the possible effect of *H. pylori* infection on iron stores. A more likely mechanism is decreased iron absorption from hypo- or achlorhydria resulting from chronic gastritis [29]. Gastric hydrochloric acid facilitates iron absorption by reducing non-heme iron from the ferric to ferrous form. Another important effect of *H. pylori* gastritis that may cause reduced iron absorption is a decrease in gastric juice ascorbic acid concentration. Ascorbic acid facilitates iron absorption by reducing iron to the ferrous form [30]. Ascorbic acid is secreted into gastric juice, and it has been shown that gastric juice ascorbic acid levels are significantly lower in *H. pylori* -infected vs. uninfected persons[31,32], another mechanism to explain decreased iron absorption associated with *H. pylori* infection is increased hepcidin production from hepatocytes in response to IL-6 production associated with *H. pylori* gastritis [33]. Another possible mechanism by which *H. pylori* could result in decreased availability of iron is sequestration of iron in lactoferrin in the gastric mucosa. *H. pylori* takes up iron from human lactoferrin through a receptor-mediated method [34, 35], and lactoferrin secretion in the gastric mucosa appears to be influenced by the *H. pylori* organism [36, 37].

Another hypothesized mechanism to explain an association between *H. pylori* infection and iron deficiency is uptake of iron by the *H. pylori* organism. Like many bacteria, *H. pylori* require iron as a growth factor, and it possesses a 19-kDa iron-binding protein resembling ferritin (Pfr), that may play a role in storage of excessive iron by the bacteria [38]. Acquisition and storage of iron in *H. pylori* are controlled by the ferric uptake regulator gene product (Fur), which regulates transcription of iron uptake genes and Pfr iron storage [39].

Scientists have long known of *H. pylori*, but only in the last 10 years it has been recognized as a potential health threat. It causes stomach ulcers and gastrointestinal cancer and may play a role in the incidence of many other diseases.

Materials and Methods
Patients:

A total of 64 patients (41females and 23 males), aged between 14 and 66 years, were screened for this study. Patients attended the Gastroenterology Unit at AL-Kadhimyia teaching hospital in Baghdad from 1rst April to October 2007 because of recurrent abdominal pain and other gastrointestinal complaints, such as vomiting. All subjects filled out a questionnaire with regard to their general health and were excluded if they had been previously treated for *H.pylori* infection. The study was approved by the ethics committee of the Hospital. After an overnight fast, each patient underwent esophagogastroduodenoscopy, during which four antral biopsies were taken from within 2 cm of the pylorus using sterilized biopsy forceps (Olympus 16K; Olympus Corp., Tokyo, Japan). Biopsy specimens for the urease test were taken before those used for histological examination to avoid contamination with formalin.

Ultra rapid urease test:

Each specimen was subjected to Ultra Rapid Urease test as mentioned by Berry V, Sagar V [40] but with some modification. Briefly the medium used for the test was urea broth. It consists of urea, phenol red indicator and distilled water. 10 gm of urea was dissolved in

80ml of distilled water and final volume was made up to 100ml. To it 0.002 gm of phenol red was added, pH was adjusted up to 6.4 to 6.8 by using dilute hydrochloric acid. The broth was sterilized by using 0.22*um* Millipore filter, and dispensed in aliquot (0.5-1 ml) into a capped polypropylene tubes. The biopsy specimen for the URUT was removed from the biopsy forceps with a sterile toothpick and placed immediately into the polypropylene tube. Particular care was taken not to shake the tube after placing the biopsy into it so that a rapid positive result could be achieved [41]. A positive test result was indicated when there was a color change in the medium surrounding the biopsy from yellow to magenta. The test tube was left at room temperature and examined at intervals over 24 h. Convenient times chosen were 1, 5, 10, 20, 30 min and 1, 2, 3 and 24 h after insertion of the biopsy specimen into the urease test reagent.

Presence of H.pylori in the impression smears:

Impression smear was performed from the positive and negative specimen in the URUT test; crushed between two sterilized glass slides; heat fixed; stained with 40% carbolfuchsin for 1 min and examined under an oil immersion lens for the presence of a helical or more strikingly curved bacteria (Figure 1)

Blood samples:

The basal blood samples for assays of IgG antibodies for *H.pylori* and serum Ferritin were drawn after an overnight fast. Class antibodies to *H.pylori* were determined using specific ELISA tests (*Helicobacter Pylori* IgG ELISA Test Kit Cat. No. 601 040.01, Biohit Plc, Helsinki, Finland) according to the Instructions of the manufacturer. Samples with an ELISA value of <34 EIU (EIU=enzyme Immune Units) were

considered negative, and samples with an ELISA value >42 EIU were considered positive. Samples with values between 34-42 EIU (Cut –off ~38 EIU) were considered as Borderline

Serum Ferritin was determined using VIDAS Ferritin (Enzyme Linked Fluorescent Assay Kit Cat.No.30 411, bioMerieux sa) according to the instructions of the manufacturer. Serum Ferritin values were considered as:

Iron deficiency = If concentrations are lower than 20 ng/ml in women and 30 ng/ml in men.

Inflammation = If concentrations are greater than 250 ng/ml in women and 350 ng/ml in men.

Histological evaluation of gastric biopsies

Two antrum biopsies were fixed in formalin and paraffin-embedded, and stained with hematoxylin and eosin; and subsequently evaluated by an experienced pathologist. The degree of inflammation present in the histological specimens was classified according to the updated Sydney system [42] (data not shown in this paper). A grading from absent, mild, moderate and severe was assigned for four histological variables: chronic inflammation (mononuclear cell infiltration),activity (polymorphonuclear neutrophil infiltration), glandular atrophy, and intestinal metaplasia.

Definition of H. pylori Infection

The gold standard for classifying a patient as being infected with *H. pylori* (in present study) was either detection the organism in the gastric biopsy by having the Ultra Rapid Urease test /or anti–*H. pylori* antibodies and histology results with or without visualizated by microscopic examination. Patients were considered uninfected with *H. pylori* when all tests were negative.
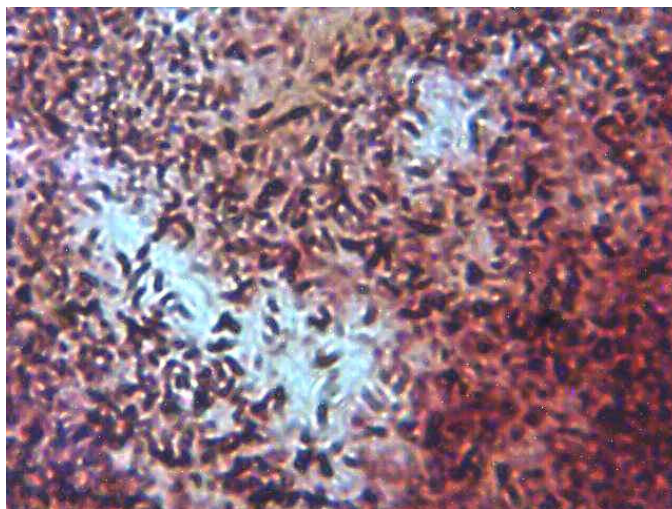
Figure 1: Antral gastric biopsy shows tufts of *H. pylori* a helical or more strikingly curved appearance and bluntly rounded ends

Results

According to the non-invasive and invasive diagnostic methods used in this study a total of 47of the 64(73%) patients were considered as *H.pylori* positive group, 37 of the 47 (80%)patients were positive with Ultra Rapid Urease test, 10 individual of this group(21%) were seronegative to *anti-H.pylori* IgG antibody,28 of the 47(60%) patients biopsies showed positive microscopic examination with impression smears and 37 of the 47(80%)patients were positive with EIA test for anti-*H.pylori* IgG antibody, 6 individual of this group(12%) showed negative results with Ultra Rapid Urease test(Table 1).

A total of 16 of the 47(34%) infected patients showed low serum Ferritin values. The results in (Figure 2) shown the percentage of low serum Ferritin in total patients among age group and gender, were found more commonly in female infected patients (15 of the 47. 32%) than male; and the rate of the *H.pylori* infection were higher in female age group of (21-30) years.

(Figure3) shown the percentage of low serum Ferritin in the infected patients when diagnosed with different methods according to the age group and gender, high rate of low serum Ferritin shown in female age group 21-30 years mainly when they were positive with Ultra Rapid Urease test.

Table 1: Prevalence (%) of *H. pylori* infected patients according to the noninvasive and invasive diagnostic used methods in this study.

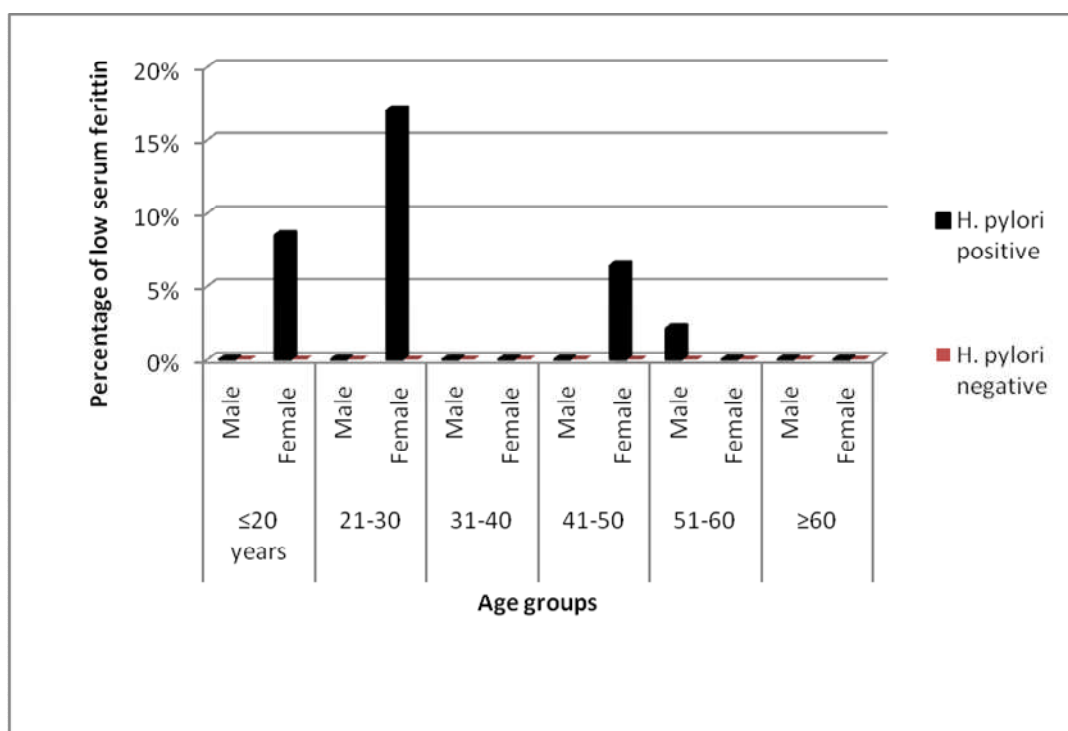| Methods used	*H pylori* Infected patients, *n* (%)
Ultra Rapid Urease test	37(80)
Positive Ultra Rapid Urease test with negative EIA test	10(21)
positive Ultra Rapid Urease test with positive EIA test	27(57)
positive impression smears	28(60)
EIA test	37(80)
Positive EIA test with negative Ultra Rapid Urease test	6(12)

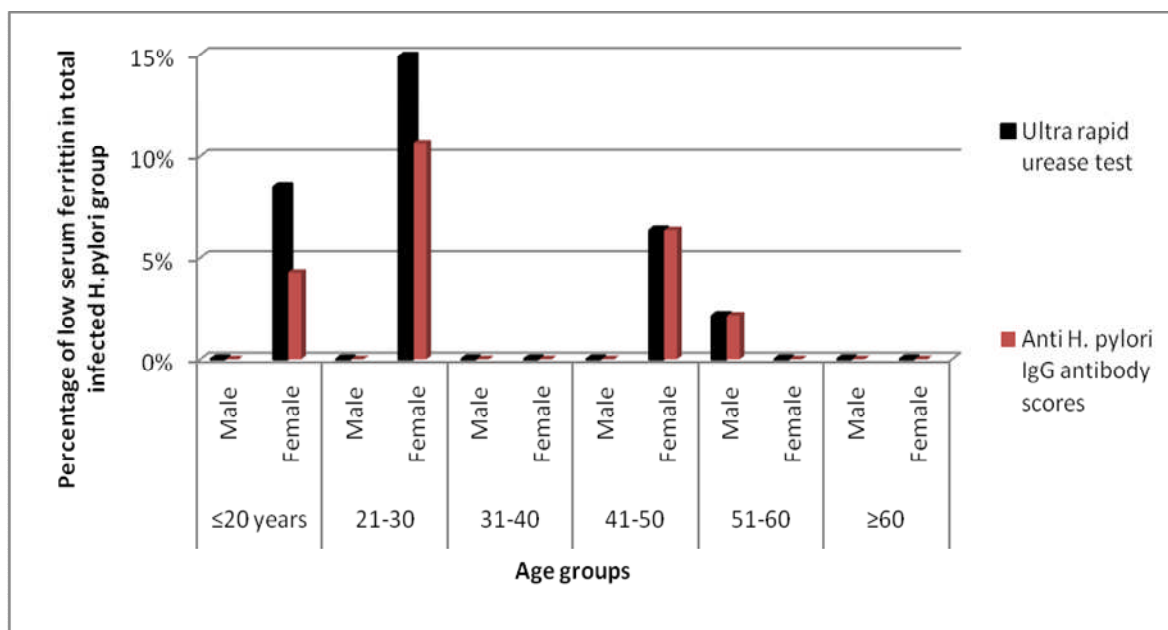**Figure 2: Percentage of low serum Ferritin in total patients among age group and gender**

Figure 3: percentage of serum Ferritin in total *H.pylori* infected patients among gender and age groups with different diagnostic used methods.

Discussion

Currently, there are a number of both invasive and non-invasive diagnostic tests available for the diagnosis of *H. pylori* infection; each has its limitation in clinical applications. Urease-based biopsy tests require endoscopy and are not reliable in cases where patients use proton pump inhibitors. Histological examination follows endoscopy and its accuracy is dependent on the stain selected and on the pathologist's skill. Serology is inexpensive but is not reliable in determining the presence of active infection, which is important for clinical interpretation and diagnosis.

The appearance of IgG antibodies to *H. pylori* is delayed following onset of the infection and may not appear for many months [43] such that the working definition of an acute *H. pylori* infection has been a positive test for active *H. pylori* infection (e.g., histology, culture, urea breath test (UBT), or stool antigen test)and a negative IgG serology [44,45],this

finding agrees with the present results as showed in (Table 1),that 10 of the 47(21%) *H.pylori* positive patients detected by URUT showed seronegative anti-*H.pylori* IgG. Also this results could be explained by Laine et al [46]. noted that sensitivity of all urease-based tests for detection of *H. pylori* is dependent upon the bacterial load in the stomach; Kobayashi *et al* [47]. used real- time PCR to estimate the total number of *H. pylori* genomes in biopsy samples and compared these with values obtained by UBT and showed a correlation between the results; other authors including Moshkowitz *et al* [48]. have reported that the intragastric bacterial density can assessed by urease activity. Moreover, the results in (Table 1) showed six individual from the37 who were positive with E I A test for anti-*H.pylori* IgG antibody, they showed negative results with URUT, this could be explained that the tissue biopsy sample contain a very low bacterial number, this finding agreed

with Karnes, et al. [49] that serologic tests may be positive in patients with gastric atrophy, in which the number of *H. pylori* organisms is so small as to be undetectable by biopsy methods.

Further, the presence of *H. pylori* was also diagnosed by slide impression smear test, (Figure 1) shown that the morphology of the *H.pylori* observed in biopsy specimens as a helical or more strikingly curved bacteria. This finding was in agreement the other study, found that *H.pylori* usually appears as a curved or straight rod in culture, whereas stained tissue biopsy samples usually reveal a helical or more strikingly curved appearance [50], also it demonstrates bluntly rounded ends [51].

In recent studies, a positive relation was detected between *H. pylori* infection and some micronutrient malnutrition in adults. Serum iron, vitamin B12, folate, vitamin A, and vitamin C levels were found to be low in the presence of *H. pylori* infection [52]. A strong association was found between *H. pylori* infection and iron deficiency [53]. However, the mechanisms by which *H. pylori* infection causes iron deficiency have not been well established. A plausible mechanism that may explain the development of iron deficiency in *H. pylori*–infected subjects might be the result of the pattern of gastritis and related effects on gastric physiology, affecting the normal process of iron absorption [54]. In the current study five of seronegative infected patients showed low serum Ferritin value. This could be explained that *H. pylori* may affect iron uptake and thus deplete iron stores in persons; this finding agree with Perez-Perez and Israel [49], reported that *H. pylori* may cause iron deficiency anemia by competing with the host for iron absorption. Iron is an essential growth factor for all bacteria, including *H. pylori*, which contains a system of iron-repressible outer membrane proteins that may be involved in iron uptake as well as a system for intracellular storage of iron that consists of the ferritin-like molecules Pfr and NapA [49].

Furthermore, the results in (Figures 2 and 3) showed that the percentage of low serum ferritin were found more commonly in female infected patients with age group of 21-30years . These results corresponding with the other studies that; an epidemiologic study of Australian women showed significantly lower ferritin levels in women with *H. pylori* infection compared to non-infected controls despite similar dietary iron intake [27], also Atherton *et al*. [55] they proposed that measurement of *H. pylori* density in gastric mucosa may be useful in determining the severity of infection and its influence on histologic changes and clinical outcomes.

In conclusion, the present results show that *H.pylori* positive results with URUT and slide impression smears test of the biopsy samples in the majority of infected patients indicates that, it has true a potential in aiding the diagnosis and management of patients with active *H. pylori* infection; as well as, the possible relationship between mucosal *H.pylori* loads with low serum Ferritin level.

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