PREVALENCE OF HELICOBACTER PYLORI INFECTION USING FECAL ANTIGEN AND SEROLOGY AND ITS RELATION TO IRON DEFICIENCY ANEMIA

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ABSTRACT

Background

Helicobacter pylori is an important pathogen, that colonize the mucus layer of epithelial mucus of the stomach in approximately 50% of humans worldwide and can be diagnosed by both invasive and noninvasive methods.

Objectives

to compare the diagnostic accuracy of noninvasive tests such as serology and stool antigen test in symptomatic patients and observing the relation of these tests to hematological parameter.

Materials and Methods

One hundred and fifteen blood and stool samples were taken from patients with dyspeptic symptoms from primary health care center of Shahid baxtyar clinic in Sulaimani (Iraq) in a period from June to December 2015, and analyzed for detection of *Helicobacter pylori* infection by different methods.

Results

The most attending group that have *Helicobacter pylori* infection were adult female with age group 26-35 years. Different percentage was recorded for each diagnostic maethod, 76.52% for rapid test, 62.60% for stool Ag, 67.82% for IgM and 86.96 % was recorded for IgG ELISA test. Serum ferritn was assessed for all patients and it was observed that 78 (67.82%) showed low serum ferritin level that includes all cases of positive stool Antigen.

Conclusion

H. pylori can be detected by all non invasive methods with predominent of serodiagnostic test and most of positive cases had iron deficiency anemia with low serum ferritin level.

Keywords: *Helicobacter pylori, Noninvasive methods, ELISA test, Stool Ag.*

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection occur worldwide, with high geographic variability and it's the most important risk factor that has been associated with the development of gastric cancers in human populations [1, 2].

The infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families. The main source of transmission is the mother within families, and acquired in early childhood and persists for life [3]. According to a traditional classification; *H. pylori* infection can be diagnosed by both non invasive tests such as *H. pylori* antigen in stool specimen, urea breath test, serology, and invasive methods such as histology which require endoscopy and biopsy specimens [4]. However these procedures are invasive and expensive and may suffer from sampling error thus non invasive methods such as serology and stool antigen avoid the costs, and discomfort of endoscopy [5] .

H. pylori can create an autoimmune response against parietal cells of the stomach, these cells are responsible for the production of the hydrochloric acid that helps to break down food, damage to these cells can cause a drop in the stomach acid levels and food may not be broken-down properly as a consequence, the iron may not be absorbed effectively from the gut [6].

The mechanisms by which *H. pylori* can cause iron deficiency anemia are still unclear. One hypothesis clarify that chronic infection leads to atrophy of the gastric glands and reduction of gastric hydrochloric and ascorbic acid secretion [7]. *H. pylori* absorbs iron from lactoferrin via a specific lactoferrin binding protein that is expressed by the bacteria and its levels in the gastric mucosa have been shown to be significantly higher in *H. pylori*-positive patients with iron deficiency anemia [8].

MATERIAL AND METHODS

This cross sectional study carried out on 115 patients attending primary health care center of Shahid baxtyar clinic in Sulaimani (Iraq) in a period from June to December 2015.

Participants were eligible of any age group experienced one or more dyspeptic symptoms such as post-prandial fullness, early statiety, epigastric pain and epigastric burning for at least one or three days per week, in the last three months of their participation in the study [9].

All possible causes of acute and chronic abdominal pain were excluded from the study by taking full history and examination and bleeding history from adult females also taken, abdominal ultrasound and with blood investigation for hematological parameter and liver function test were all recorded.

Stool examination were requested from all patients for analysis of stool Ag for *H.pylori* and fecal occult blood according to manufacture recommendation (plasmatic laboratory product UK) .

Ten milliliter of blood were obtained from all participants and 2 ml of blood separated and put in EDTA tube for complete blood count and pictures and the remaining 8 ml of blood were centrifuged and the serum separated for further analysis of *H. pylori* and ferritin level by using Immulite kit (chemiluminescent immunoassay system, UK), level of 28-365 ng/ml was regarded normal for males and 5-148 ng/ml for females.

H. pylori status was analyzed by rapid immune chromatography test (Plasmatic chromatography product, UK) and ELISA test for IgG, and IgM antibodies in the serum by using Monobind Inc USA Accu Bind ELISA, level of <20 U/ml regarded as negative for IgG and <40 U/ml for IgM.

RESULTS

The participants in our study were 115 patients with predominance of female to male 104 / 11 , all age groups were included in the study (Table 1) with a range between 5-55 years.

The most attending group in this study was adult female with age group 26-35 years and small attendance was recorded among 5-15 and 46-55 years and male were the less complainer, statistically this age distribution is significant (p <0.05).

Stool were requested from all patients for stool Ag for *H. pylori* and fecal occult blood and the data revealed that stool for *H. pylori* antigen were positive in 72 (62.6%) patients, while occult blood were detected in 32 patients (Table 2). Statistically the relation of stool Ag test to stool occult blood was significant (p value < 0.05).

Blood were analyzed for complete blood count and blood film for all participants and the data revealed that 61 (53.04%) patients had low hemoglobin level with microcytic hypochromic type of anemia and 98.36 % out of them had positive stool antigen for *H. pylori* (Table 3) statistically this relation is significant.

Serum sample were separated from whole blood and assessed for ferritin level in all patients and the results showed that low serum ferritin were observed in 78 cases including all 61 cases of low hemoglobin level and all 72 cases of positive stool Ag for *H. pylori* (Table 4).

Rapid chromatography test were applied with ELISA (IgM, IgG) test for all the participants serum; the result showed that 88 cases were positive by rapid test and all 88 cases were positive for IgG , while IgM were positive in 78 patients and IgG were detected totally among 100 cases (Figure 1) .

Table 1. Age distribution of participants.

Age group (years)	Female	Male	Total
5-15	6	1	7
16-25	32	4	36
26-35	40	4	44
36-45	20	1	21
46-55	6	1	7
Total	104	11	115

T-test-2.707, p value : 0.0133

Table 2. Relation of fecal occult blood and stool Ag for *H. pylori*.

Stool Ag	Occult blood positive	Occult blood negative	Total
Positive	30	42	72
Negative	2	41	43
Total	32	83	115

Chi square test is 18.367 ; P value : 0.000018

Table 3. Relation of stool Ag to iron deficiency anemia.

Hemoglobin	Stool Ag positive	Stool Ag negative	Total
Low Hb level	60	1	61
Normal Hb level	12	42	54
Total	72	43	115

Chi squre test 70.929 p valu = 0.00001

Table 4. Relation of all *H. pylori* test with serum ferritin level

Serum ferritin level	Non- invasice H. pylori test			
	Rapid test	Stool Ag	ELISA IgM	ELISA IgG
Low	70	71	65	74
Normal	18	1	8	26
Total	88	72	78	100

The chisequre test =21.671, p value =0.000077

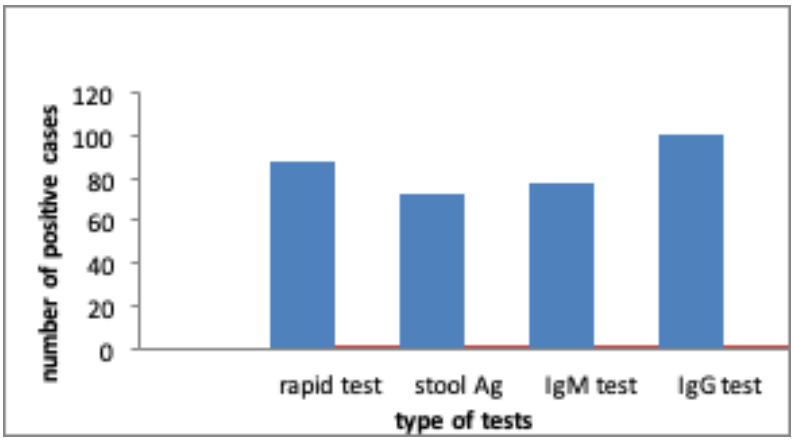

Figure 1. Percentage of noninvasive diagnostic methods for *H. pylori* infection.

DISCUSSION

Different non invasive methods were used for diagnosis of *H. pylori* infection and they are cheap test and recommended, whenever urea breath test was not available [10]. Stool antigen for *H. pylori* were detected in 72 (62.60 %) cases among all attending patients that reflect true infection and this test compared to one of the serodiagnostic technique such as IgM level by ELISA test that reflect acute true infection. It was proved that stool antigen for *H. pylori* is a highly reliable diagnostic method before extending its use in the general healthy population. Many studies have compared the accuracy of this test with that of an invasive test in symptomatic patients and good record were observed [11].

There will be a high probability of positive serology among population as non invasive methods for diagnosis, the prevalence of 86.95% was recorded by IgG Elisa test and 76.52% by rapid test that reflecting previous infection (chronic infection), these antibodies may remain positive for years after successful eradication of *H. pylori* [12], although the best noninvasive methods are urea breath test but its application is somewhat difficult in children and its coasty that made serum antibodies advantages of simplicity, low cost, and utility for epidemiological studies and screening programs [13].

There are other studies that recording high level of infection possibly due to life habits and improper sanitary condition in these areas [14, 15]. Others studies reviewing several researches from Iran, 5 studies from the Kingdom of Saudi Arabia, 4 studies from Egypt, 2 from the United Arab Emirates and one study from Libya, Oman, Tunisia, and Lebanon; all recording different prevalence rate of *H. pylori* infection by different laboratory tests that indicate high prevalence of infection in developing countries [16].

One of the parameter which was tested in this study is serum ferritin level which is the major storage protein in the body for iron and the most powerful test for diagnosis of iron deficiency anemia without inflammation [17], and it was found that low serum ferritin level were recorded in all age group with positive *H. pylori* infection although the causes in female may be related to menstrual blood loss but no history of bleeding among patients were recorded.

Concomitant inflammation can greatly affect the level of serum ferritin, as the level of ferritin for iron-deficiency without concomitant inflammation varies from 12–15 ng/ml and with concomitant inflammation to more than 50 ng/mL [18].

Earlier studies suggest an association between *H. pylori* induced gastritis and iron deficiency anemia and several epidemiologic studies have shown that seropositive persons for *H. pylori* infections have a significant lower serum ferritin level [19] and eradication of *H. pylori* infection in iron-deficient anemic patients was reversing the iron deficiency status to normal level children and adults [20].

Blood loss due to gastric lesions and reduced iron absorption due to an elevated pH of gastric juice have been attributed to causes anemia in *H. pylori* infection that should be proved by invasive methods such as endoscopy [21]. There are several studies performed in children with decreased iron absorption in *H.pylori* infection [22, 23] and other observing patients with seropositive *H. pylori* infection had significant low serum level of ferritin compare to normal healthy population [24], but there are data in which failed to show the association between low level of serum ferritin and chronic *H. pylori* infection inspit of chronicity of infection [25, 26].

Other test which was used in this study is fecal occult blood which is found in 32 (27.82%) cases among all participants and 93.75% out of this were positive for stool antigen test, although occult blood it's an indicator of lowers gastrointestinal bleeding rather than *H. pylori* infection [27].

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