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ASSOCIATION OF HELICOBACTER PYLORI INFECTION WITH THE MOST COMMON AFFECTED AGE: A STATISTICAL STUDY

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Abstract

Helicobacter pylori (H. pylori) is a bacterium that causes infections, and the most prevalent symptom is upper abdominal pain. However, the age group of people who are most susceptible to *H.pylori* infection is still a matter of debate. In this study, we aim to analyse and report the results of *H. pylori* tests for patients amongst the Iraqi population. The descriptive and retrospective studies involve 224 Iraqi patients of both genders, aged between 18-67 years who gave oral consent and displayed upper abdominal pain. All patients were interviewed and clinically examined before being tested with a qualitative serological test and stool examination. The population of the study consisted of 114 males and 125 females with mean age 25 and 26 years, respectively. The results showed that there were no negative serum tests for both genders: the peak age of infection was between 30-39 years and 50 patients showed positive for both tests. Results showed that the middle-aged population is significantly the more affected group according to serological tests which were positive in all tested patients. Moreover, the study also showed that the antigen detection of *H. pylori* by IgG, IgM and IgA antibodies using the Rapid Test strips is the most trustable biomarker and is recommended as the initial test for any work involving *H. pylori*.

INTRODUCTION

Helicobacter pylori (previously known as *Campylobacter pylori*) is a well-known Gram-negative and microaerophilic bacterium that infects the gastric and duodenal mucosa. Its helical shape facilitates the penetration of the mucosal lining of the stomach causing infection [1]. *H. pylori* has been identified for the first time in 1982 by two Australian doctors, Barry Marshall and Robin Warren, in a patient suffering from gastric ulcers [2]. *H. pylori* in the stomach is linked to mucosa-associated lymphoid tissue and diffuse large B-cell lymphoma [3]. Many studies worldwide are focusing on the pathophysiology of *H. pylori* in peptic ulcer and other gastrointestinal diseases, including gastric lymphoma. Indigestion and upper abdominal pain are the most common symptoms of *H. pylori*. *H. pylori* approximately affects half of the world's population, with increasing infection especially in children and adolescents. Interestingly, *H. pylori* is more common among Hispanics, Asians and Arabs [3,4,6]. Once the patient is infected, *H. pylori* can adapt to the strong acidic medium of the stomach,

enabling it to survive in the patent indefinitely, resulting in chronic infections.

H. pylori infection is a widespread disease and presents in different ways in both genders and varies in all ages, suggesting that its manifestation could be age-dependent [5]. In Iraq, many researchers study *H. pylori* due to its wide occurrence in the populations and neighbouring countries. For example, the percentage of infected people in Turkey was 69.0%, 49.7% in Kuwait, 25% in Jordan, 61.87% in Iran and 71.33 % in Saudi Arabia. However, within the Iraqi population, *H. pylori* infection incidence was 59.2% in Baghdad city, 54.5% in Basrah city and 61.32% in Mosul city have been reported [7,8]. *H. pylori* symptoms present in different ways from being mild to severe. Individuals may be asymptomatic or display only mild symptoms, but some infected individuals may suffer from abdominal discomfort, nausea, vomiting and bloating. Other symptoms include dark or tarry-like stools, upper abdominal pain, peptic ulcers, and vomiting that may include blood [9,10]. *H. pylori* was reported being present in patient's stool, breath and on their teeth. *H. pylori* can spread by faecal and by oral route from human to human via unwashed hands, close contact, especially within families in the same household, between kids and the elderly population in nursing homes [11,12].

Several studies have reported that *H. pylori* infection is associated with multiple factors: the internal factors such as gastric adenocarcinoma, diabetes mellitus type 2 and coronary heart diseases [13-15]; the external factors such as ABO blood group, age, gender, smoking, use of some anti-inflammatory drugs, alcohol consumption and salt intake [16-18]. This study sought to determine the relationship between *H. pylori* and the most commonly affected age in the Iraqi population at Al-Ramadi Teaching Hospital in Iraq.

Although esophagogastroduodenoscopy is present in Al-Ramadi Teaching Hospital, we focused on the *H. pylori* antigen detection in the blood and stool samples, as they are simple and easy tests to conduct and readily available in our laboratories.

MATERIALS AND METHODS

This descriptive and retrospective study included 224 orally consented Iraqi patients from both genders, aged 18-67 years who complained of upper abdominal pain. The examinations were carried out in multiple laboratories. All patients were interviewed and clinically examined before being tested with a qualitative serological test type *Onsite ™ H. pylori* type AB combo Rapid Test made by CE-CTK Biotech, Inc, and also by stool examination using the strips of *H. pylori* type Antigen Rapid Test named *OnSite™ H. pylori* Ag Rapid Test - Cassette (Faecal Specimen) made by CTK Biotech, Inc.

AB combo Blood Rapid Test is a sandwich lateral that is based on flow chromatographic immunoassay. It qualitatively detects specific antibodies (IgG, IgM and IgA) to indicate an active *H pylori* infection. It is suitable for human serum, plasma or whole blood. Another type of AB Combo Blood Rapid test can also be used for stool test. Typically, it can detect about 1 ng/ ml of polyrilysate antigen in stool specimens with 100 % sensitivity and 93.8 % specificity.

The tests have been carried out following a simple procedure based on a company's manufacture. A blood specimen was placed into a collection tube with no anticoagulants. For a serum sample, blood was allowed to clot, then it was centrifuged then serum was carefully withdrawn into a new pre-labelled tube. The refrigerated or frozen samples were then brought to room temperature and once serum was thawed, it was mixed well before performing the assay. About 30-45 µL of blood serum were placed into the well of the test cassette device with making sure there were no air bubbles. Immediately, about 35-50 µL of sample diluent, provided with the kit, were added onto the top of the sample with the bottle positioned vertically. Results were read at 15 minutes. Positive results usually can be obtained in as short as 1 minute and negative results can be confirmed after 20 minutes only.

For stool samples, two types of specimens were considered: Watery faecal specimens and Solid faecal specimens. For the Solid faecal specimens, a collection stick was replaced and tighten securely to close the stool collection device, provided with the kit, each containing sample extraction buffer. The stool collection device was then shacked vigorously to ensure a homogenous liquid suspension and kept for the assay test. For watery faecal specimens, two drops were dispensed into the stool collection device was then shacked vigorously to ensure a homogenous liquid suspension and also kept for the assay test. After preparing the samples, about 90 µL of ready thawed samples were placed into the sample well of the cassette. Results were recorded at 10 minutes. Positive results can be detected in as short as 1 minute and negative results must be confirmed after 15 minutes only. Data of both stools and serum were collected and analysed using SPSS software and Microsoft excel.

RESULTS AND DISCUSSION

Patients were 114 males and 125 females, and the mean age was 25 and 26 years, respectively. Overall, there were no negative serum tests for all patients of both genders. The peak age was between 30-39 years, but previous studies in Turkey showed the peak age was between 40 and 80 years [19]. Fifty patients showed positive tests for stools and serum for both genders as described in Tables 1 and 2. *Helicobacter pylori* prevalence is associated with various factors [13-18], some of which are internal and other externals. Age is a very important factor which differs among countries depending on the life expectancy. Other factors are based on lifestyle such as diabetes, smoking

Table 1. Serum and stool tests in relations to age for males who are infected with *H. pylori*.

Age (year)	Serum tests	Stools tests	Total tests
0-19	2	0	2
20-29	29	12	41
30-39	45	19	64
40-49	33	16	49
50-59	4	3	7
60+	1	1	2
Total	114	51	165

Table 2. Serum and stool tests in relation to age for females who are infected with *H. pylori*.

Age (year)	Serum tests	Stools tests	Total tests
0-19	2	0	2
20-29	2	14	16
30-39	36	25	61
40-49	49	6	55
50-59	27	1	28
60+	9	1	10
Total	125	47	172

and alcoholism. In this research, we studied the association of age with *H. pylori* infection in Iraqi patients. The samples were selected from many laboratories, giving a broader sample size with more variation in contrast to using only a single hospital in the study [26,27]. As we can see in Fig.1 A and B, the most affected age group who tested positive in serums for both genders is between 30-39 years. Similarly, males who tested positive in stools showed a similar percentage which was 59 ± 2 % while females who tested positive in stools showed 10 % increase in the total percentages of ages (as described in Fig. 2 A and B). Although there is a slight increase in the female stool sample, however, these results are agreed with established studies reported in Iraq and other countries [28,29]. There were no significant differences showed between both serum and stool tested for both genders, which are similar to many reported papers [30, 31]. An increase of male prevalence in *H. pylori* infection was shown in previous studies which may be due to many factors such as a larger sample size and country epidemiological difference of *H. pylori*, sanitation and environment clearance [32-34]. Furthermore, there is no significant variation between both genders for serological and stool tests (as described in Tables 3 and 4), which indicates the gender is an independent factor in *H. pylori* infections. Our study showed that both tests were positive at the same time for each patient, which was presented by 98 patients (51 males and 47 females). The findings indicate both rapid tests are reliable tests of *H. pylori* infection [35,36]. These may give the physician the confirmation of *H. pylori* infection in the absence of the Urea Breath Test or invasive Esophagogastroduodenoscopy, although those two tests would give an accurate diagnosis [37-39].

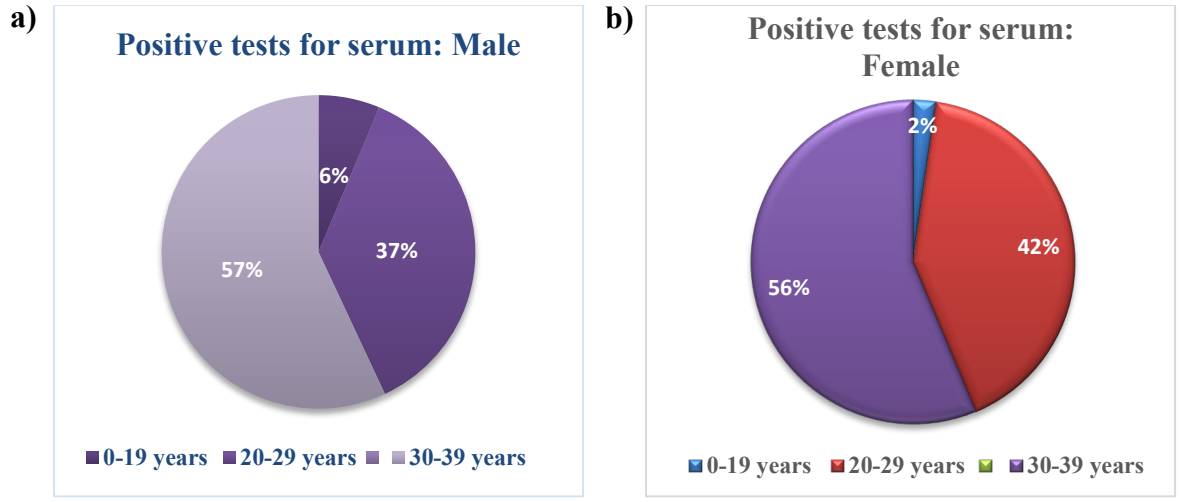

Figure 1. A pie chart shows the correlation between the positive serum tests and the age for the (Panel A) males and (Panel B) females who are infected with *H. pylori*.

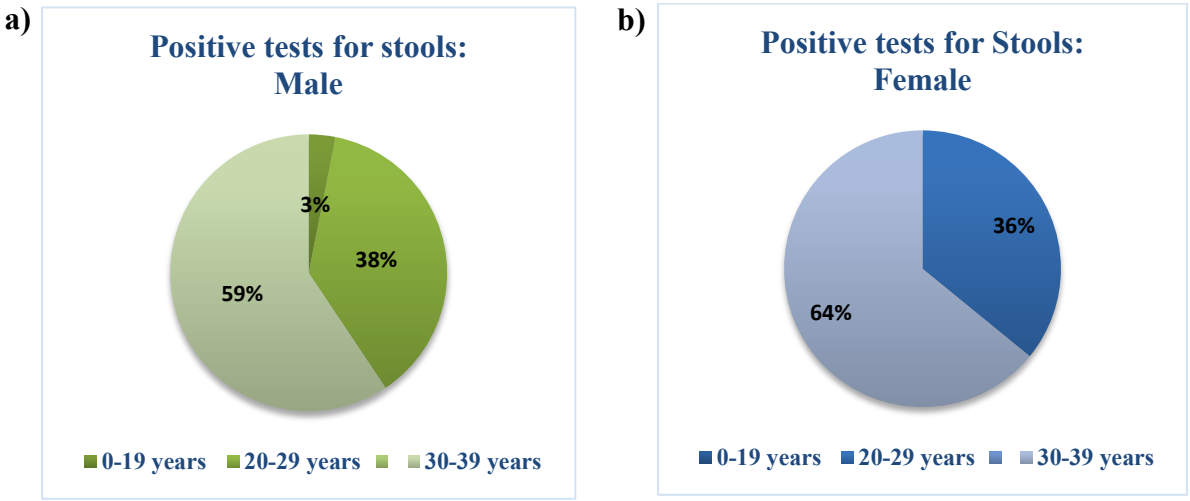

Figure 2. A pie chart shows the correlation between the positive stool tests and the age for the (Panel A) males and (Panel B) females who are infected with *H. pylori*.

Table 3. Descriptive statistical analysis between the serological test for both genders which reveals that there is no significant variation between both groups.

	Males sample	**Females samples**		**Total**
N	6	6		12
Sum	114	125		239
Mean	19	20.833		19.917
Mean square	3976	4515		8491
Std. Dev.	19.0263	19.549		18.41
Correlations	Sum of squares SS	df	MS	
Between both genders	10.083	1	10.08	
Within both genders	3720.83	10	372.08	
Total	3730.91	11		f=0.0271

Table 4. Descriptive statistical analysis between the stools tests for both genders which reveals that there is no significant variation between both groups.

	Males sample	**Females samples**		**Total**
N	6	6		12
Sum	51	47		98
Mean	8.5	7.833		8.167
Mean square	771	859		1630
Std. Dev.	8.215	9.907		8.68
Correlations	Sum of squares SS	df	MS	
Between both genders	1.222	1	1.333	
within both genders	828.33	10	82.833	
Total	829.66	11		f=0.0161

It is worth mentioning that there are many methods used for *H. pylori* diagnosis, but the best method is still controversial. Laboratory diagnosis of *H. pylori* infection is reported by both invasive and non-invasive tests: the invasive tests require esophagogastroduodenoscopy followed by a biopsy for either a histological examination or a culture, then a rapid urease test. Other tests, such as serological tests, are performed by urea breath test and require urine or blood. The detection of *H. pylori* antigen in the stools can be imprecise due to other factors that could affect the examination, such as a previous infection by *H. pylori*, and some drugs like proton pump inhibitors and salicylates [40-44]. Blood tests could also be affected rarely by other factors such as WBC, neutrophil or lymphocyte counts [45]. Also, stool tests could be affected occasionally by some factors such as stomach ulcers, which may cause no symptoms or upper abdomen pain [46]. Nevertheless, the serological *H. pylori* antigen detection by IgG, IgM and IgA antibodies can be stated as the most frequently test used for diagnosing *H. pylori* infection because of the simple collection and rapid results. Also, this test is not influenced by the use of antibiotics and proton pump inhibitors, thus it is a suitable method for quick and reliable evaluation for the patients [47,48].

CONCLUSION

H. pylori is associated with both internal factors such as gastric adenocarcinoma, diabetes mellitus type 2 and coronary heart diseases, and external factors such as ABO blood group, age, gender, smoking, use of some anti-inflammatory drugs and alcohol [13-18]. Our analysis showed that middle-aged people are significantly more vulnerable to *H. pylori* infection. This study included 114 males and 125 females with mean age 25 and 26 years, respectively, out of 224 samples. For both genders, there were no negative serum or stool tests, peak age was shown to be between 30-39 years and 50 patients had a positive for both serum and stool tests. We also showed that serological *H. pylori* antigen detection by IgG, IgM and IgA antibodies is the most frequently used test used by physicians for diagnosing *H. pylori* infection because of its simple collection and rapid result. The outputs of our research have led us to outline the following: the serological and stool tests are the best laboratory diagnostic tests of *H. pylori* infection in the absence of esophagogastroduodenoscopy. Also, a national survey of *H. pylori* infection would be required for best detection in both Iraqi hospitals and around the world. Last but not the least, an *H. pylori* Eradication Day should be declared by WHO.

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ETHICAL APPROVAL

All patients were examined according to the ethical standards of the responsible committee on human in the Ramadi city Institutions of Health, Ministry of Health. Informed consent was gained from all patients for their involvement in the study.

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