The Relation Between *Helicobacter Pylori* Infection and Symptoms of COVID-19

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ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*) represent one of the most prevalent pathogens in humans, which affecting more than half of the population. Infected people usually remain a symptomatic, but, approximately 30% of individuals may develop gastro-duodenal disease with symptoms. In COVID -19 patients, the most prevalent GI symptoms include nausea, vomiting, abdominal pain and diarrhea, which can occur in up to 50% of the patients ,Due to the presence of *H. pylori*, which is directly linked to the progression of the infection.

Purpose: This study was aimed to detect *H. pylori* in COVID-19 infection patients with gastrointestinal symptoms by using non-invasive techniques (Immunochromatography rapid tests), study the sensitivity, specificity for each test,

Methods: this study was conducted on 105 patients, their age 18 to 85 years, 65 patients with COVID-19 Infection and gastrointestinal symptoms after diagnosed by PCR in period extended from November 2021 to May 2022 in Al-Zahraa Hospital. And COVID-19 Isolation center. The stool and blood samples of COVID -19 Infection have been collected, Non-invasive test (SAT and Cag-IgG) were evaluated in all patients, CTK.biotech and Assure® *H. pylori* IgG Rapid tests were used.

Results: *H. pylori* were detected in 79 (75.2%), 73(69.5), samples using SAT, Cag-IgG respectively. With the sensitivity and specificity, (95.1%), (86.3%), (92.4%), (84.6%) respectively. The highest rate of *H. pylori* infection was discovered among COVID-19 patients with the age group 41 – 60 years, with the significant differences between them ($p < 0.05$)

Keywords: *Helicobacte r pylori*, gastrointestinal symptoms, COVID-19, Non-invasive tests

Introduction

Helicobacter pylori (*H. pylori*) represent one of the most prevalent pathogens in humans, which affecting more than half of the population. This bacterium is usually acquired during childhood to persist for many years if remain untreated. [1, 2]

Infected people usually remain a symptomatic, but, approximately 30% of individuals may develop gastro-duodenal disease with symptoms [3]. In COVID -19 patients, the most prevalent GI symptoms include nausea, vomiting, abdominal pain and diarrhea, which can occur in up to 50% of the patients [4], Due

to the presence of *H. pylori*, which is directly linked to the progression of the infection and promotes immunological dysregulation through its virulent factors these may subsequently become more severe [5]. A few studies conducted in Iraq indicate that more investigation is required to ascertain the bacterium `s exact prevalence. According to the most recent research by Majeed *and colleagues* [6], the prevalence of *Helicobacter. Pylori* was 11.3-71.3%. [6].

Materials and Methods

Sample Collection

Totally, 105 patients attended to, Al-Zahraa Teaching Hospitals, COVID-19 Isolation center and Private Lab, were subjected to this study that carried out from November (2021) to March (2022). All study populations were selected after their diagnosing by specialized physicians and PCR test to be infected with COVID-19 and having gastrointestinal abnormal symptoms. Non-invasive test (SAT and Cag-IgG) were evaluated in all patients

Blood collection

In gel and clot activator tube, about 3 ml of venous blood were collected from each patients .each tube was labeled by patients ID stickers with patients name, number, age and date of collection, the tubes were centrifuged and serum aspirated into several Eppendorf tubes (150µ) and stored at -20ºC until be tested [7].

Stool collection

In a sterile container, about 100-200 mg of stool were collected, if samples were not tested immediately. All stool samples were stored at 2-8 ◦ C or at room temperature up to 37ºC for 10 days. For longer storage, the extracted specimens were frozen at -20ºC [8].

Sample Examination

Stool antigen test (SAT)

This test was done by immune-chromatographic assay using HPAg rapid test for qualitative detection of HPAg in fecal specimen. The procedure was performed according to manufactures instructions [9]. Briefly, stool sample was collected into a disposable container, and shacked vigorously to ensure a homogenous liquid suspension. In case of watery feces, the plastic dropper was filled with the specimen; while in solid fecal samples, random specimen was collected using the collection stick in 2 -5 different sites .

The stool collection device was hold vertically and the cap was twist off and about two drops (70-85 µl) was dispensed of the solution in to the sample well of the cassette and the solution was not over load. Results were read at 10 minute; if only the C line developed, the result will considered negative and the test indicated no detectable *H. pylori* antigen in the specimen. Positive result appeared when the both C and T lines developed.

Detection of CagA-IgG

This test was done by *H. pylori* rapid test as an immunochromato-graphic assay for qualitative detection of *H. pylori* CagA- IgG Ab in human blood specimen, which constitute an antibody-antigen complexes with immobilized *H. pylori* antigens on the membrane. The bound antibody- antigen complexes can be detected subsequently by antihuman IgG conjugated to colloidal gold.

The test kit is having three bands. The first band is the control line that contains protein A that binds to human IgG and the anti-human IgG colloidal gold conjugate and act as an indicator for proper sample addition. The second band is the CIM line contains antibodies against recombinant current infection marker (CIM) which is indicative of current infection. The third band is the test line that contains an antigen of *H. pylori* [10]. Serum samples were tested and interpreted according to the manufacturer's instructions.

Statistical Analysis

SPSS, Version 27, was used for Statistical analysis, Chi-square and 0.05 alpha-level were used to elevate distinctions between groups.

Results & Discussion

Diagnosis Using Stool Antigen Test

Among the non-invasive tests, the stool antigen test (SAT)the result were distributed 21/40 (52.5%) in gastrointestinal disease patients and 58/65 (89.2%) in patients with COVID – 19 infection with gastrointestinal symptoms as

shown in Table.1, the sensitivity and specificity of this test was high as shown in (Table.2). The sensitivity & specificity was found to be (95.1 %), (86.3 %), respectively.

Table 1: Diagnosis of *H. pylori* using stool antigen test.

Patients type	Positive	Negative	Percentage
gastrointestinal disease	21	19	52.5%
COVID–19 infection	58	7	89.2%
Total	79	26	75.2%
X^2	17.93		
Calculated P value	0(S)		

S: Significant difference at P<0.05

Table 2: The sensitivity & specificity of the SAT.

True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)
79	19	03	04

Sensitivity = TP / (TP + FN) ×100 = 95.1 %
Specificity = TN / (TN + FP) × 100 = 86.3 %
Accuracy = (TP +TN) / (TP +TN +FP+FN) * 100 = 93.3%

When comparison current result with other Iraqi results of different studies it was found that, the present result was higher than other Iraqi study done by Hussein *et al*[11], who used non-invasive techniques including (SAT) to confirm the presence of *H.pylori*. The positive results was (67%), with 95% and 91.2% sensitivity and specificity respectively. Also the current result was much higher than that obtained by Al-Mashhadany *and colleagues,* [12] who reported significantly lower percentage (11.3%) in the Kurdish region, Iraq, using SAT test. The present study was recorded higher percentage than Galal *and colleagues* [13], study from Egypt, which used the SAT approach, started with a 64.6% occurrence rate. Global study as Miftahussurur and Yamaoka, [14] in Indonesia observe that the sensitivity of SAT was 88.8%. On contrast Negash *et al.* [15] recorded low positive result which was only (45.8%) for *H.pylori* SAT by ELISA.

The variation in results could be attributed to differences in socioeconomic standing, academic level, dietary practices, and health states of the studied regions. The SAT method, on the other hand, does not require fasting, and recently some variants of it are now commercially available that are not affected by PPIs. Moreover, numerous studies have demonstrated the efficacy of this method in diagnosing infected patients from treating patients, as well as its efficacy in evaluating *H.pylori* infection eradication [16].

Diagnosis Using Serological Tests.

The result revealed 73 /105 (69.5%) of specimens were positive to Assure® *H. pylori* IgG Rapid Test, the result were distributed 19/40 (47.5 %) in gastrointestinal disease patients and 54/65 (83.07 %) in COVID-19 infection according to (Table.4),both the specificity and sensitivity of this test was shown in Table.5 . The sensitivity & specificity was found to be (92.4 %) (84.6 %), respectively.

Table 4: Diagnosis of *H. pylori* Using Assure® *H. pylori* IgG Rapid test.

Patients type	Positive	Negative	Percentage
gastrointestinal disease	19	21	47.5%
COVID–19 infection	54	11	83.07%
Total	73	32	69.5%
X^2	14.79		
Calculated P value	0(S)		

S: Significant difference at P<0.05

Table 5: The sensitivity & specificity of the *H. pylori* IgG Rapid Test.

True Positive (TP)	True Negative (TN)	False Positive (FP)	False Negative (FN)
73	22	04	06

Sensitivity = TP / (TP+FN) ×100 = 92.4 %
Specificity = TN / (TN +FP) × 100 = 84.6 %
Accuracy = (TP +TN) / (TP +TN +FP+FN)*100 = 90.4 %

When comparison current result with other Iraqi results of different studies it was found that , the Seroprevalence of IgG antibodies in the present result was higher (69.5 %) than other study in different cities of Iraq, Such as in Erbil city , Al- Mashhadany *et al* .[17] which found that , the prevalence of *H. pylori* was (36.9%), While In Baghdad city , Al- Mossawei *et al* .[18] was found that 80% of those tested were positive for *H. pylori* IgG Ab rapid test with sensitivity and specificity (89.36 % and 85.64 %) . In Kurdistan region / Sulaimani city, the study by Al -Windi *et al*. [19] who used non – invasive ELISA technique which less than the present results (32.3%). On contrast other Arabian study, the present study was recorded higher percentage than Saudi Arabia (28%) Hanafi and Mohamed, [20]. On the other hand, lower rates were observed from China (18.6%) Shu *and colleagues,* [21] Australia (21.5%) Abdul Rahim *and colleagues,* [22], and Malaysia (30.4%) Sasidharan *and colleagues,* [23].

The *H. pylori* antibodies rapid test is a quick and simple test used to monitor serum antibodies of *H. pylori* infection which may indicate current or previous infection, different strain of *H. pylori* and host genetic variations are expected to result in different levels of IgG antibodies [24]. As a results, variations in results may be due to variation in educational attainment, dietary preferences, socioeconomic status and hygienic conditions of studied regions. The specificity, sensitivity and accuracy of each test was shown in (Table.7)

Table 7: The outcomes of each non – invasive *H. pylori* diagnostic tests

Technique	*Helicobacter Pylori* diagnosis n (%)					
	Positive	Negative	Specificity	Sensitivity	Accuracy (%)	Total (%)
SAT*	79(75.2%)	26 (24.7%)	86.3%	95.1%	93.3 %	105
Serology CagA-IgG	73(69.5%)	32(30.47%)	84.6%	92.4%	90.4 %	

Distribution of Patients According to Age

The age of the patients who enrolled in this study ranged from 18 to 85 year, and they were divided into five age groups. The result of present study clarified that the age group 41-60 had the highest infection (44.7%%) followed by the age group 61-85 represented (31.4%) while the lowest infection (23.8%) found in 18-40 age group as showed in (Table.8) with significant differences (P<0.05).

Table 8: Distribution of *H. pylori* infection among gastrointestinal disease & COVID-19 Patients based on age.

Age (years)	Total No.	Percentage (%)
18 -40	25	23.8
41-60	47	44.7
61-85	33	31.4
Total	105	100
X^2	9.65	
Calculated P value	0.008(S)	

It was found that the infection percentage in age (41-60 y) more than other groups and that may be due to that, individuals in this age group have more chance to exposure to infection during their work. These results come in agreement with a number of studies such as El-Shenawy *et al*.[25] who found that *H.pylori* infection increases with age, and can be found more in the population aged >_ 43 years 71.7% (43/60), on the other hand, 28.3% (17/60) were < 43 years. In Iraqi universities Hussein *et al*. [11] found that *H. pylori* infection was higher among students aged (24–30) years than those aged 18–20 year and student with age 21–23 year. Previous epidemiologic studies on *H.pylori* have shown a high prevalence (35%–67%) among adults in Saudi Arabia. Furthermore, the rate increases with age and is higher in female than male individuals, Abdoh *et al*. [26]. In contrast with Hedayati and Salavati. [27] Who found that there is no significant correlation statistically between *H. pylori* infection and patients' age, according to this research, there is no connection between age and *H.pylori* colonization. Phattharaphon *et al.* [28] another study done by Idris *et al*. [29] revealed that there was no statistically significant association between the incidence of *H.pylori* infection and age.

Conclusions

In the present work the following conclusions, based on the aforementioned results, the findings indicate that *Helicobacter Pylori* infection can be regarded as a contributing influence for progressive the gastrointestinal symptoms in COVID – 19 infection and the infection of *Helicobacter pylori* was discovered among COVID-19 patients among the age group 41 – 60 years, with the significant differences between them ($p < 0.05$).

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