Detection of the Helicobacter pylori infection among Coronavirus patients in Wasit province

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ABSTRACT

Helicobacter pylori (H.pylori) is normal flora in the stomach, it is opportunistic bacteria that can cause an infection for the stomach may be lead to diseases such as gastritis. About two-thirds of the world's population is H.pylori infection, where it is more common in developing countries and over (80%) of infected individuals are asymptomatic .some of recent studies highlight on there are correction between H.pylori infection and Gastrointestinal Symptoms in COVID-19 patients This study was aimed to Prevalence of Helicobacter pylori infection among COVID-19 patients in Wasit province. This study was conducted on 120 patients with COVID-19 Infection and gastrointestinal symptoms, their age 22 to 65 years, in period extended from September 2021 to June 2022 in Zahra Hospital; AL- Karma teaching 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 and the result confirmed by PCR .

The result has been showed H. pylori were prevalence 76 (63.3%), 73(60.8), 80(66.6 %) samples using SAT, Cag-IgG and PCR respectively. The highest rate of H. pylori infection was discovered among COVID-19 patients with the age group 55 – 65 years, with the significant differences between them ($p < 0.05$).the highest prevalence of H. pylori infection was founded in O blood.

Keywords: Helicobacter pylori, gastrointestinal symptoms, COVID-19.

Introduction

Two-thirds of the world's population thought infected with H.pylori, where it is more common in developing countries and over (80%) of infected individuals are asymptomatic.(1). According to few studies in Iraq, accurate information about the prevalence of this bacterium is not yet available. However, one study indicated that the prevalence of bacterium was 11.3-71.3% (2). *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] Subsequently, these symptoms can become more severity with existence of H. pylori that play an important role in increase in the expression of ACE2 receptors in the GI tract, and directly linked to the infection progression and promoting immunological dysregulation through its virulent factors (5).As a results, the initial discovery of H. pylori has become a crucial to the diagnosis and selection of the appropriate course of treatment.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, 120 patients with COVID-19 Infection and gastrointestinal symptoms, their age 25 to 65 years, in period extended from September 2021 to June 2022 in in Zahra Hospital; AL-Karma teaching 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 and the result confirmed by PCR .

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.

Extraction of DNA

The *16SrRNA* gene was used as housekeeping gene in this study to confirm the present of *H.pylori*, which appeared as band at (470bp), Extraction of genomics DNA was done and 120 Saliva samples were testede for the presenc of genomics DNA .Deoxyriboses nucleics acid of *H.pylori*. was extractd by pickings singl of colony usings sterl loop and suspndede in to (300 µl) of lysis bufer [10 mM Trise, 1mMEDTA (pH=8),1% SDS, 100 mM NaCl, 2% Twen 80], 300 µl phenols-chlorforms (1:1); it was shakens for 5 minute and centrifugede at (1000 rpm).Dry DNA pellets was re-suspendeds in 100 TE bufer and storede at -20°C until use (11).

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 of *Helicobacter Pylori*

Accurate diagnosis of *H.pylori* infection is a crucial part in the effective management of many gastroduodenal diseases. non-invasive diagnostic tests are available for the detection of *H.pylori* and each test has its usefulness and limitations in different clinical situations, Although none can be considered as a single gold standard in clinical practice, several techniques have been developed to give the more reliable results (8). Each of the methods for confirmation of *H.pylori* infection has a characteristic sensitivity and specificity which may influence an investigation's outcome. Selecting particular methods for the diagnosis depends on access to the methodologies and/or being cost-effective

1.Stool Antigen Test (SAT)

120 stool samples were collected from patients with COVID-19 Infection and gastrointestinal symptoms and tested by ELISA, the result revealed 76 (63.3%), of specimens were positive as shown in Table (1), The sensitivity & specificity was found to be (95.1 %), (86.3 %), respectively.

Table (1): Detection of *H.pylori* by ELISA in stool samples

Type of sample	Positive samples	Negative samples	Total
Stool	76 (63.3%)	44(36.7%)	120(100%)

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 (7), who used non-invasive techniques including stool antigen test (SAT) to confirm the *H.pylori* infection, 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 *et al.* (8) who reported much lower percentage (11.3%) from Kurdistan region, Iraq by using SAT method. The present study was recorded higher percentage than other Arabian study by by (9) from Egypt who stated 64.6% occurrence rate using SAT method Global study as (10). in Indonesia observe that the sensitivity of SAT was 88.8%.

2. Antibody-Based Tests

Due to the small amount of bacteria that colonizes the stomach, Thus, several indirect tests, including antibody based tests such as serology test have been developed to diagnose *H. pylori* infection (11). It was found that, the total number of sera samples (120) were tested by ELISA, there are 73(60.8), of specimens were positive by IgG antibody as shown in Table (2). The sensitivity & specificity was found to be (92.4 %) (84.6 %), respectively.

Table (2): Detection of *H.pylori* by ELISA in Serum samples

Type of sample	Positive samples	Negative samples	Total
Serum	**73 (60.8%)**	**47(39.2%)**	**120(100%)**

The specificity of current result was 60.8% which is less than the serological part of an iraqi study done (11) who found that, the specificity was 84.2%. Another study done by Atkinson and Braden (12) they found that the specificity of serological test was 79-90% which also higher than the current study. Amgalanbaatar *et al*. (13) found that a sensitivity was 82%, their result is being lower than current observed This method has a sensitivity and specificity of 76–84% and 79–90%, respectively according to (14) The ability of this test to detect active infections depends on the patient's age, clinical conditions of infection, the choice of the antigen

used for antibody preparation in ELISA kit, and the prevalence of infection (15).

3. Detection of *H.pylori* by16S ribosomal RNA gene

The *16SrRNA* gene was used as housekeeping gene in this study to confirm the present of *H.pylori*, which appeared as band at (470bp), the result found 80(66.6 %) of samples were positive as shown in Figure (1) and Table(3). The sensitivity & specificity was found to be (100%) (95.6 %), respectively.

Table (3): Detection of *H. pylori* by PCR in Saliva **samples**

Type of sample	Positive samples	Negative samples	Total
Saliva	80 (66.6%)	40(39.2%)	120(100%)

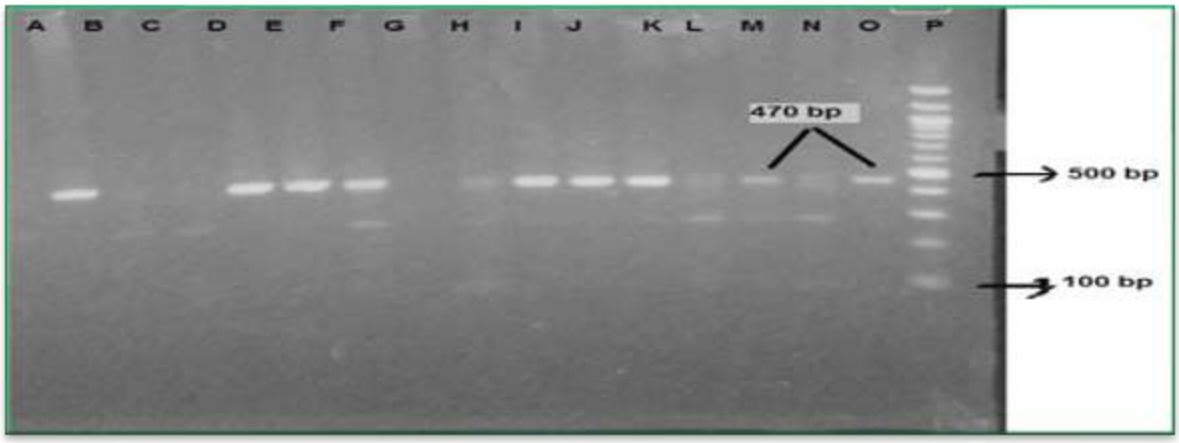


Figure (4-1): Agarose gel (2%) stained with RedSafe dye with 75V electrophoresis for detection of *16S rRNA* gene as a PCR product. Lane A,D,E,F,I,J,KM and O shows PCR product. Lane B,C,Land O shows no PCR product. P: DNA ladder (100 bp step), G : Negative control. (70V for 2hr)

The result revealed here was related to result obtained by (16) who found that 60.5% of the samples were positive for the presence of *H.pylori by* the amplification of the *16S rRNA* gene. This gene is present in all bacteria, at the same time, it comprises nucleotide sequences that are specific to a given bacterial genus (17). Findings from Nigeria based on *16SrRNA*, found only 52.38 % of gastritis samples were PCR positive for *H.pylori* (18). *16S rRNA* gene amplification method was found useful for bacterial identification and phylogeny (19). Several properties of the *16SrRNA* gene make it the "ultimate molecular chronometer", the most common housekeeping genetic marker, and hence, a useful target for clinical identification and phylogeny(20).

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 4: distribution of *H. pylori* infection among COVID-19 Patients based on age.

Age (years)	Total No.	Percentage (%)
25 -35	30	25
40-50	39	32.5
55-65	51	42.5
Total	120	100
X^2	9.65	
Calculated P value	0.008(S)	

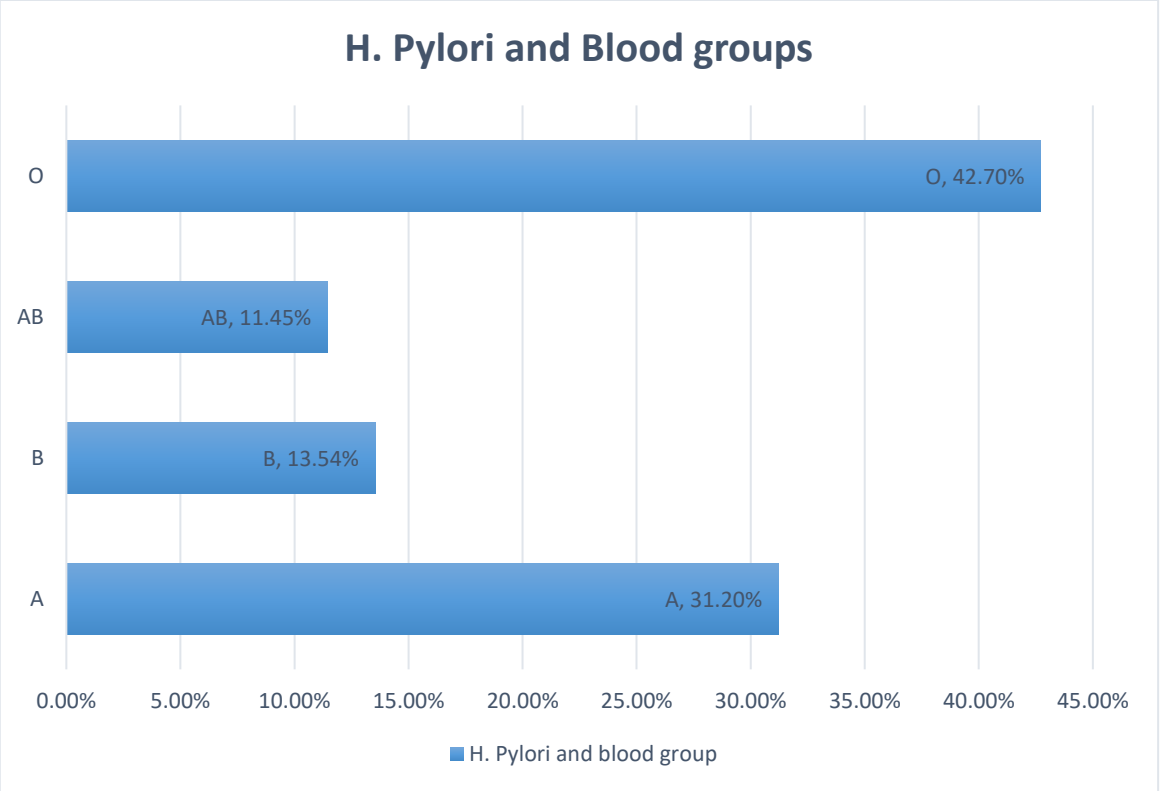
It was found that the infection percentage in age (55-65 y) more than other groups and that may be due to that, individuals in this age group have more chance to exposure to infection during their work. These results come in

agreement with a number of studies such as El-Shenawy *et al.*[21] 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*. (22).

Distribution of Patients According to Blood Groups

Among the COVID -19 infection patients , the highest H. pylori infection was founded in O blood group (43.7%), followed by A (31.2%), B (13.54%), and AB (11.45%). Figure (2) .

Figure (2): *H. pylori* and Blood groups



This result was similar to other studies that manifesting the higher sensitivity of O blood group to *H. pylori* infection (23).While the present results opposed with some studies which explained that The O blood group did not act as a risk factor for H. pylori infection (24).

Conclusion

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 55 – 65 years, with the significant differences between them ($p < 0.05$).

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