



## DETECTION OF HELICOBACTER PYLORI INFECTION IN PATIENTS WITH COVID-19 INFECTION AND GASTROINTESTINAL SYMPTOMS

Warkaa Mohammed Khadim

Department of Biology, College of Science, University of Wasit, Kut, Iraq

Email: warkaamoh87@gmail.com

Alaa Ali Matrood

Department of Biology, College of Science, University of Wasit, Kut, Iraq

Khairi Jameel Al-Ruaby

Department of Biology, College of Science, University of Wasit, Kut, Iraq

### 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:** *Helicobacter 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 (150 $\mu$ ) and stored at -20°C 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 37°C for 10 days. For longer storage, the extracted specimens were frozen at -20°C [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 shaken 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 <sup>2</sup>	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 =  $TP / (TP + FN) \times 100 = 95.1 \%$

Specificity =  $TN / (TN + FP) \times 100 = 86.3 \%$

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%
$\chi^2$	14.79		
Calculated P value	o(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

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) \times 100 = 92.4 \%$$

$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP}) \times 100 = 84.6 \%$$

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{TN} + \text{FP} + \text{FN}) \times 100 = 90.4 \%$$

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	<i>Helicobacter Pylori</i> 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 <sup>2</sup>	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  $\geq$  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 ( $p < 0.05$ ).

## References

1. Carroll IM; Ahmed N; Beesley SM; Khan AA; Ghousunnissa S; and Morain CA. (2004). Microevolution between paired antral and paired antrum and corpus *H. Pylori* isolates recovered from individual patients. *J. Med Microbiol*, 53, 669-77.
2. Parente JM; da Silva BB; Palha-Dias MP; Zaterka S; Nishimura NF; and Zeitune JM. (2006). *H. Pylori* infection in children of low and high socioeconomic status in northeastern Brazil. *Am. Trop. Med. Hyg.*, 75(3), 509-12.
3. Kayali S, Manfredi M, Gaiani F, Bianchi L, Bizzarri B, Leandro G, Di Mario F, De' Angelis GL.(2018).*Helicobacter pylori*, transmission routes and recurrence of infection: state of the art. *Acta Biomed.* Dec 17; 89 (8-S):72-76. Doi: 10.23750/abm.v89i8-S.7947.





4. Kopel J, Perisetti A, Gajendran M, et al. (2020). Clinical insights into the gastrointestinal manifestations of COVID-19. *Dig Dis Sci*; 65:1932–9.
5. Balamtekin N, Artuk C, Arslan M, Gülşen M. (2021). The effect of helicobacter pylori on the presentation and clinical course of coronavirus disease 2019 infection. *J Pediatr Gastroenterol Nutr.* 72(4):511-3.
6. Majeed PD, Khoshnaw KJ. (2020). Seroprevalence of Helicobacter pylori infection among patients with gastroduodenal disorders in Erbil city. *Diyala J Med.* 18(1):91–101.
7. Chow, T.P. (2001). Assure™ Helicobacter pylori rapid test for the accurate detection of Helicobacter pylori infection. NUS-ICMR-JSPS International Symposium on Helicobacter and Viral Hepatitis, p. 87.
8. Pelerito, A., Oleastro, M., Lopes, A. I., Ramalho, P., Cabral, J., & Monteiro, L. (2006). Evaluation of rapid test Assure® Helicobacter pylori for diagnosis of H. pylori in pediatric population. *Journal of microbiological methods*, 66(2), 331-335.
9. Hussein R.A.; Al-Ouqaili M.T. and Majeed Y.H. (2021). Detection of Helicobacter Pylori infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. *PLoS ONE*, 16(8): e0256393.
10. Al-Mashhadany, D. A. and S. M. Mayass. (2018). Prevalence of Helicobacter pylori in Human in Dhamar governorate/Yemen. *J. Med. Pharm. Sci. Issue.* 1(2): 1-18.
11. Galal YS, Ghobrial CM, Labib JR, Abou-Zekri ME. (2019). Helicobacter pylori among asymptomatic Egyptian Children: Prevalence, risk factors, and effect on growth. *J Egypt Public Health Assoc.* 94(1):1-8.
12. Miftahussurur, M. and Y. Yamaoka. 2016. Diagnostic Methods of Helicobacter pylori infection for epidemiological studies: Critical importance of indirect test validation. *Biomed Res. Int.* 24: 1-14.
13. Sokpon M.; Salihoun M.; Lahlou L. et al. (2016). Predictors of Helicobacter pylori ( Hp ) infection in chronic gastritis: about a Moroccan study. *J Afr Hépatol Gastroentérol.* 10(4), 203–207.
14. Odigie AO, Adewole AJ, Eknwe AA. (2020). Prevalence and factors associated with Helicobacter pylori infection among treatment naïve dyspeptic adults in University of Benin City, Nigeria. *African Journal of Clinical and Experimental Microbiology.* 21(2), 97-105.
15. Lawson–Ananissoh L.M.; Bouglouga O.; Bagny A. et al. (2015). Epidemiological profile of peptic ulcers at the Lomé campus hospital and university center (Togo). *J Afr Hépatol Gastroentérol*, 9(3), 99–103.





16. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, et al. (2020). Helicobacter pylori virulence factors-mechanisms of bacterial pathogenicity in the gastric microenvironment. *Cells*. 10(1):27.
17. Al-Mashhadany DA. (2020). Epidemiology of Helicobacter pylori among human at Erbil governorate/Kurdistan region /Iraq. *Researchgate.net* Vol.9, Issue 11,435-447.
18. M.T. Al-Mossawei, W. H. Rzooqi and S. Abdulrazzaq. 2016. Detection of Helicobacter pylori IgG and IgM antibodies in Iraqi dyspeptic patients. *J. Biotechnol. Res. Center*. 10(1): 5-9.
19. Al-Windi, A., Hussain, A.H. and Salih, N. (2013). Seroprevalence of anti-Helicobacter pylori antibodies in population of Sulaimani governorate/Kurdistan Region/Iraq. *Journal of Zankoy Sulaimani- Part A (JZS-A)*.15(3):175-185.
20. Hanafi M and Mohamed A M. (2013). Helicobacter pylori infection: Seroprevalence and predictors among healthy individuals in Al Madinah, Saudi Arabia. *Journal of the Egyptian Public Health Association* 88(1), 40-45.
21. Shu X, Ping M, Yin G, Jiang M. (2017). Investigation of Helicobacter pylori infection among symptomatic children in Hangzhou from 2007 to 2014: a retrospective study with 12,796 cases. *Peer J* 5, e2937.
22. Abdel Rahim NR, Benson J, Grocke K, Vather D, Zimmerman J, and Moody T. (2017). Prevalence of Helicobacter pylori infection in newly arrived refugees attending the Migrant Health Service, South Australia. *Wiley Online Library. Helicobacter* 22(2), e12360.
23. Sasidharan S, Ghayethry B, Ravichandran M, Latha YL. (2012). Prevalence of Helicobacter pylori among patients referred for endoscopy: Gender and ethnic differences in Kedah, Malaysia. *Asian Pacific Journal of Tropical Disease* 2(1), 55-59.
24. T. B. Abadi, (2018). Diagnosis of Helicobacter pylori using invasive and noninvasive approaches. *Journal of pathogens. Hindawi.com*.
25. El-Shenawy A.; Diab M.; Shemis M.; El-Ghannam M.; Salem D.; Abdelnasser M. and Saber M. (2017). Detection of Helicobacter pylori vacA, cagA and iceA1 virulence genes associated with gastric diseases in Egyptian patients. *Egyptian Journal of Medical Human Genetics*, 18(4), 365-371.
26. Abdoh Q.; Kharraz L.; Ayoub K. et al. (2018). Helicobacter pylori resistance to antibiotics at the An-Najah National University Hospital: a cross-sectional study. *Lancet*, 391(2), 32-6.



27. Hedayati, M. A., & Salavati, S. (2021). Transcriptional profile of *Helicobacter pylori* virulence genes in patients with gastritis and gastric cancer. *Canadian Journal of Infectious Diseases and Medical Microbiology*.
28. Phattharaphon W.; Chariya C.; Banchob S.; Wises N. and Kiaticchai F.(2018). Detection and genotyping of *Helicobacter pylori* in saliva versus stool samples from asymptomatic individuals in Northeastern Thailand reveals intra-host tissue-specific *H.pylori* subtypes. *BMC Microbiology*, 18, 10.
29. Idris A.B.; Hassan H.G.; Ali M.A.S.; Eltaher S.M.; Idris L.B.; Altayb H.N. and Hassan M.A. (2020). Molecular phylogenetic analysis of 16S rRNA sequences identified two lineages of *helicobacter pylori* strains detected from different regions in Sudan suggestive of differential evolution. *International Journal of Microbiology*.

