RESEARCH ARTICLE

Application of PCR and ELISA for Investigation the *Helicobacter pylori* in Stool Specimens of Infected Patients

Saja A Al-Jumaili[1*], Amina N Althwani[1], Ahmed Dawood Ahmed[2], Ahmad Abd alraheem Ibrahem Dahy[3], Zina Najim[1], Nawal M Al-Khalidi[4]

[1]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.*
[2]*Bilad Alrafidain University Collage, Iraq.*
[3]*Mustansiriyah University, Iraq.*
[4]*Gastroenterology and Hepatology Teaching Centre, Ministry of Health, Iraq.*

Received: 12th October, 2023; Revised: 28th November, 2023; Accepted: 05th December, 2023; Available Online: 25th June, 2024

ABSTRACT

Background: Infection with *Helicobacter pylori* is a global health issue, and non-invasive and accurate testing is a promising diagnostic method. Because *H. pylori* antibodies collapse quite gradually even after effective treatment. So, the tests for identifying the antibody of *H. pylori* are missing specificity and sensitivity. Instead, the tests of stool antigen for *H. pylori* are described as an alternative method because they have more reliability and simplicity.

Aim: The study was aimed to assess the performance of enzyme-linked immunosorbent assays (ELISA), for the detection of *H. pylori* antigen in stool and compare it to molecular tests by using two housekeeping genes.

Methods: Stool samples were collected from patients (85) who attended the Gastroenterology and Hepatology Teaching Center in Baghdad, Iraq, and underwent esophagogastroduodenoscopy (EGD) for biopsy in a period extended from November 2020 to July 2021. PCR and ELISA were used to Investigate *H. pylori* in stool specimens of infected patients.

Results: The result revealed that 74/85 (87.88%) of specimens were positive by ELISA, whereas 49/85 (57.6%) were positive for the 16 SrRNA gene related to *Helicobacter* genus and 35/85 (41.1%) were positive for amiA gene related to *H. pylori* species, and that means the ELISA test is more accurate than PCR analysis for detection of *H. pylori* bacteria in stool specimen.

Conclusion: Detection *H. pylori* Ag using the ELISA test was superior to the molecular test. Therefore, the ELISA test might replace other methods and could be used for the detection of active *H. pylori* infection before the inauguration of treatment between dyspeptic patients.

Keywords: *Helicobacter pylori*, 16S rRNA gene , AmiA gene, ELISA , Stool antigen.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.68

How to cite this article: Al-Jumaili SA, Althwani AN, Ahmed AD, Dahy AAAI, Najim Z, Al-Khalidi NM. Application of PCR and ELISA for Investigation the *Helicobacter pylori* in Stool Specimens of Infected Patients. International Journal of Drug Delivery Technology. 2024;14(2):1055-1058.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Helicobacter pylori is the cause of most public bacterial infections in humans.[1] It has been involved in the pathogenesis of gastrointestinal diseases, for instance, peptic ulcer, gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma.[2,3] According to a meta-analysis that occurred between (1970-2016) of 184 studies found that infection with *H. pylori* in (62) countries at different periods was expected to infect 4.4 billion people in 2015, it was concluded that more than 50% of the world's population was infected with *H. pylori*.[4]

A lot of methods were developed for the diagnosis of infection with *H. pylori*. Most of these methods are highly sensitive and specificity for accurate diagnosis in clinical practice. The tests of diagnosis divided into invasive (endoscopic-based) examination and non-invasive, such as the *H. pylori* stool-antigen (HpSA) test, it could be the best alternative method for detection of active bacterial infection.[5,6]

H. pylori have many of housekeeping genes that could be helpful for the genetic diagnosis of the bacteria, and most common housekeeping genetic marker for bacterial identification and phylogeny is *16S rRNA* gene.[7]

**Author for Correspondence: sajoo94@yahoo.com*

N-acetylmuramoyl-l-Alanyl amidase (*amiA*) is another housekeeping gene, it has a crucial role in the structure and arrangement of *H. pylori* peptidoglycan.[8]

MATERIAL AND METHOD

Totally 85 patients (45 females and 40 males) age ranged from 17 to 85 years, who diagnosed through doctors according to endoscopic findings, suffering from clinical manifestations of dyspepsia or burning, bloating, vomiting, loss of appetite, weight loss, dysphagia and melena were joined in this study which was carried out during November 2020- July 2021. control group included apparently healthy individuals 11 females and nine males; their age corresponded to the patients group, the members of this group haven't any gastrointestinal diseases.

Stool samples were collected from 85 patients, each of them underwent upper gastro duodenal endoscopy in the gastroenterology and hepatology teaching center, Baghdad - Iraq and from individuals of control group.

The stool samples were divided into two parts, the first used in molecular detection and the second used for ELISA.

Estimation of *H. pylori* Antigen by ELISA

H. pylori antigen was detected by ELISA using a Sunlong kit depending on the instructions of the company.

Detection of *H. pylori* by PCR Assay

Extraction of DNA from stool samples

Each stool sample was frozen at (-20°C), then thawed and genomic DNA was extracted directly according to the instructions of the manufacturer's DNA extraction kit (Quick Genomic). The concentration and purity of DNA measured by Nano drop.[9]

Amplification analysis of PCR

Specific primers for housekeeping genes were used for the detection of *H. pylori* to confirm the presence of bacterial DNA in collected samples. These genes are *16S rRNA* and *amiA*, the source of all primers used here was IDT (Belgium). The name and sequence are mentioned in Table 1.

Both genes (*16S rRNA* and *amiA*) were optimized in PCR separately using different and specific sets of primer. About 4 µL of DNA sample, 1-µL of each primer, 12.5 µL OneTaq master mix (NEB-England) and then complete to the final volume to 25 µL using free nuclease water (Table 2).

Table 1: Primers name and sequence

Primers name	*Sequence*	*References*
16SrRNA	5'- TTGGAGGGCTTAGTCTCT-3' 5'- AAGATTGGCTCCACTTCACA -3'	11
amiA	F- 5'-GTTTTAGACGCTGGGCATGG-3' R-5'-CCATCAGCAATGCCCTTAGC-3'	12

Table 2: Monoplex PCR conditions for *16S rRNA* and *amiA* genes

No. of cycle	*Stage*	*Temperature (ºC)*	*Time*
1	Initial denaturation	94	1 minute
35x	Denaturation	94	30 seconds
	annealing for	57	45 seconds
	16SrRNA	54	45 seconds
	Annealing for *amiA* extension	70	1 minute
1	Final extension	70	5 minutes

RESULT AND DISCUSSION

Detection the infection of *H. pylori* by non-invasive methods are increasingly exploited during last years, particularly using the ELISA tests to detect HpSA. This study shown that, there are 76/85 (89.4%) of specimens were positive for *H. pylori* by SAT and the sensitivity of this test reached to 96.1%. These results are higher than other local studies done by Hussein *et al.* (2021),[10] who obtained (67%) of their cases using non-invasive techniques, including SAT, with 91.2 and 95% specificity and sensitivity, respectively, which is close to the sensitivity we obtain. And of Al-Mashadany *et al.* (2018) [13] stated a lower occurrence (11.3%) from Kurdistan, Iraq using SAT. Another study by[14] from Egypt also indicated a lower percentage (64.6%) using SAT method. Also, the current is much higher than the result obtained by Markos *et al*. (2018) where the positive percentage by the SD H. pylori Ag ELISA was only (45.8%).

The *H. pylori* Ag ELISA tests was considered of the best value when compared with *H. pylori* Ab because the titter of *H. pylori* antibodies fall slowly even after effective treatment. Hence, detecting *H. pylori* antibodies has a slight of specificity and sensitivity and applying antibody tests might then influence to overusing of drugs which could have a harmful economic effect, amplified danger of drug resistance, and exposure to needless drug adverse effects.

The detection of *H. pylori* by non-invasive methods may lead to revolutionizing the administration of *H. pylori*-infected patients by adopting eradication treatment with no need for endoscopy.[15]

On the other hand, HpSA test are described as an alternative test for many other methods because of their reliability and simplicity and now is has been available in the market as an optional technique. Nevertheless, in the study area, the comparative performance of HpSA test for discovering the presence of the bacteria in clinical specimens is not assessed.

These non-invasive methods have an important advantage including that discomfort and we can avoid risk of invasive endoscopy. The authorities have approved on the non-invasive test (stool antigen test), because this test distinguishes patients with active infection from those with only the previous H. pylori infection.[16]

A study done by by Khalifehgholi *et al*. (2013) [17] has reported that SAT is promising as a primary diagnostic method and it is helpful for the follow-up after treatment for the progression of bacterial infection.

The outcome of the molecular detection of *H. pylori* using stool samples showed 49(57.6%) of the patients were positive for this test by using 16SrRNA gene, which appeared at (400

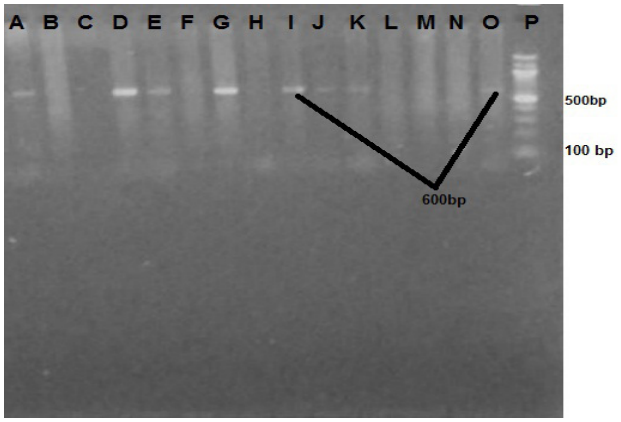

Figure 1: Agarose gel (1.8%) stained with RedSafe dye with 75V electrophoresis for detection of *16S rRNA* gene as a PCR product. Lane A,D,E,F,I,J,KM and O shows PCR product. Lane B,C,Land O shows no PCR product. P: DNA ladder (100 bp step), G : Negative control.

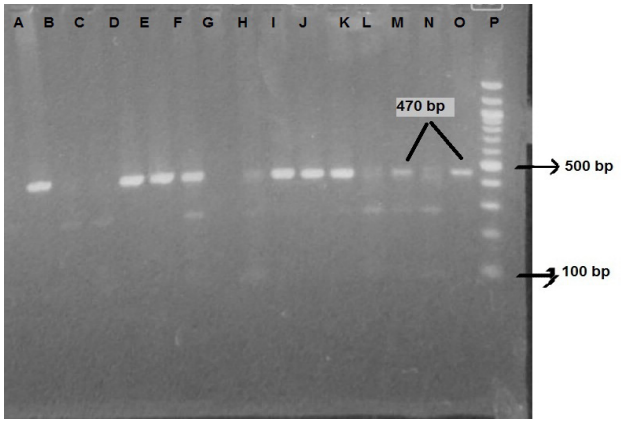

Figure 2: Agarose gel (1.8%) stained with RedSafe dye with 75V electrophoresis for detection of *amiA* gene as a PCR product. Lane A,D,E,G,I,J,K and O shows PCR product. Lane B,F,H,L,M and N shows no PCR product. P: DNA ladder (100 bp step), C : Negative control

bp) that confirmed 49 isolates belonged to Helicobacter genus as shown in Figure 1.

Up to our knowledge the molecular investigation of HPSA was rarely performed and it was found that few studies about this field. Nigeria- a pilot study done by Smith *et al*. (2012),[18] found that (39.2%) of patients were positive for *H. pylori* by PCR, through amplification of 16S rRNA gene. Their results were lower than the percentage found in this study.

While for *amiA* gene, only 35 (41.1%) were positive for this gene, which appeared at (600 bp) which confirmed 35 isolates belonged to the *Helicobacter* species *pylori* genus, as shown in Figure 2.

There is highly significance of using housekeeping genes in stool samples, as shown in Table 3. The molecular detection of stool Ag by PCR, this done by using *H. pylori*-specific primers, in the long run may also be valuable, especially when UBT cannot be convenient for infants or very young children and patients with certain neurological disorders are being screened. The method makes it easy and convenient for sample collection and it even can be collected at home.

Table 3: Detection of *H. pylori* by using two of the housekeeping gene in stool samples

Gene name	*Positive samples*	*Negative samples*	*Percentage of positive*
16SrRNA	49	36	57.4%
amiA	35	50	41.17%
Chi-Square *p-value*©	---	---	5.398 * (0.0266)

** ($p \leq 0.01$).

Detection of *H. pylori* infection by stool-PCR may be valuable method, but the success rates for the detection of bacterial DNA in feces were reported as vary from 25 to 100%.[19,20] The variability of the percentage may due to that there were inhibitors such as complex polysaccharides and *H. pylori* degradation in the gastrointestinal tract and also its presence in low concentration in stools.[21,22]

Using fast and rather inexpensive non-invasive tests such as SAT could be used as a powerful primary screening tool because most medical centers cannot afford costly equipment such as PCR.

REFERENCES

1. Karam DS, Al-Thwaini AN, Khalaf IA, Askar BA. Designing of molecular tool for the detection of helicobacter pylori in Iraqi patients using multiplex PCR technique. Annals of Tropical Medicine & Public Health, 2019: 22 (9).
2. Ghose C. Perez-Perez GI., Torres VJ, Crosatti M, Nomura A, Peek RW, Jr., et al. Serological assays for identification of human gastric colonization by Helicobacter pylori strains expressing VacA m1 or m2. *Clin Vaccine Immunol.* 2007;14(4):442–50.
3. Burucoa C. and Axon A. Epidemiology of *Helicobacter pylori* infection.Helicobacter. 2017, 22: 1.
4. Pichon M. and Burucoa C. Impact of the gastro-intestinal bacterial microbiome on *Helicobacter*-associated diseases. *Healthcare (Basel)*, 2019:7(1), 34.
5. Hooi JK, Lai WY, Ng WK, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology, 2017;153:420-429.
6. Maxime P., Benoit P., Thierry B., Chloé P., Vincent C., Ginette F., Alexandre C., et al. Diagnostic Accuracy of a Noninvasive Test for Detection of Helicobacter pylori and Resistance to Clarithromycin in Stool by the Amplidiag H. pylori_ClariR Real-Time PCR Assay. Journal of Clinical Microbiology, 2020: 58(4).
7. Markos N., Afework K., Bemnet A., Gizachew Y. and Beyene M. (Evaluation of SD BIOLINE H. pylori Ag rapid test against double ELISA with SD H. pylori Ag ELISA and EZ-STEP H. pylori Ag ELISA tests). BMC Clinical Pathology, (2018): 18(4).
8. Catherine C., Chantal E., Nadine P., Jean-Claude R., Abdelkader N. and Ivo G. B. Role of the N-Acetylmuramoyl-l-Alanyl Amidase, AmiA, of Helicobacter pylori in Peptidoglycan Metabolism, Daughter Cell Separation, and Virulence *MICROBIAL DRUG RESISTANCE* , 2016; (22) 6.
9. Vaira, L. Gatta, C. Ricci et al., "A comparison amongst three rapid urease tests to diagnose Helicobacter pylori infection in 375 consecutive dyspeptic," Internal and Emergency Medicine, 2010; 5, 1: 41–47.
10. Hassan HG, et. al. Molecular Phylogenetic Analysis of 16S

rRNA Sequences Identified Two Lineages of Helicobacter pylori Strains Detected from Different Regions in Sudan Suggestive of Differential Evolution. Hindawi International Journal of Microbiology, 2020; 8825718, (12). 31-34

11. Falsafi T, Paria L, Ilnaz B, Sadegh M; Zahra L. Evaluation of an Iranian Home *Helicobacter pylori* Stool Antigen ELISA Kit) Jundishapur J Microbiol. 2014; 7(6): e10629.
12. Hussein RA, Al-Ouqaili MTS, Majeed YH. Detection of Helicobacter Pylori infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq. A validation study. 2021: 16(8).
13. Stella I.S., Muinah A.F., Olufunmilayo A. L., Elizabeth A., Peter O.,Fatimah B.A., Charles A.O, Chimere A.A. and Monica C. (Application of stool-PCR for the diagnosis of Helicobacter pylori from stool in Nigeria- a pilot study). SpringerPlus, 2012: 1(78).20-24
14. Kabir S. Detection of Helicobacter pylori in faeces by culture, PCR and enzyme immunoassay. J Med Microbiol, 2001: 50, 1021–1029.
15. Delgado S, Suarez A, Otero L, Mayo B. Variation of microbiological and biochemical parameters in the faeces of two healthy people over a 15 day period. Eur J Nutr. 2004; 43:375–380.
16. van Tongeren SP, Slaets JP, Harmsen HJ, Welling GW. Fecal microbiota composition and frailty. Appl Environ Microbiol. 2005;71:6438–6442
17. Krogfelt KA, Lehours P, Megraud F. Diagnosis of Helico-bacter pylori Infection. Helicobacter. 2005;10, 1:5–13.
18. Sambrook J, Russel DW. Molecular Cloning. 3rd ed. NY, USA: Cold Spring Harbor Laboratory Press; 2001.
19. Galal YS, Ghobrial CM, Labib JR, Abou-Zekri ME. *Helicobacter pylori* among symptomatic Egyptian children: prevalence, risk factors, and effect on growth. *J Egypt Public Health Assoc*. 2019;94(1):1–8.
20. Khalifehgholi M, Shamsipour F, Ajhdarkosh H, Daryani NE, Pourmand MR, Hosseini M, et al.. Comparison of five diagnostic methods for *Helicobacter pylori*. *Iran J Microbiol*. 2013;5(4):396.
21. Al-Mashhadany DA. Application of stool antigen test for monitoring *Helicobacter pylori* among human in Erbil governorate, Kurdistan region/Iraq. *Int J Pharm Pharm Sci*. 2018;10(11):49–53.