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 esophagogastrroduodenoscopy (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


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
Application of PCR and ELISA for Investigation the Helicobacter Pylori

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

**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.

**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).

**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), 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) stated a lower occurrence (11.3%) from Kurdistan, Iraq using SAT. Another study by 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 (65.8%).

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. 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.

A study done by by Khalifehgholi et al. (2013) 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
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%.

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


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