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Evaluation of Two Housekeeping Gene in Diagnosis of Helicobacter Pylori and Compare It to another Routine Test

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ABSTRACT
Helicobacter pylori consider as one of the major components of the stomach microbiome, this bacterium is prevalent worldwide and more than half of the world populations are infected with it. Various methods have been developed for the diagnosis and detection of infection with *H. pylori* by using molecular and routine techniques with different levels of specificity and sensitivity. to evaluate and compare two diagnostic methods for the detection of H. pylori bacteria in Iraqi patients, A total of 105 patients and 20 individuals as control with age ranged 17-85 years who attended the gastroenterology and hepatology tertiary center, Baghdad – Iraq, they diagnosed through physicians according to endoscopic findings. Furtherly Biopsy samples from different places were collected by gastroenterologists from each patient, used for histopathological and genetic detection of two Housekeeping gene (16SrRNA and amiA) by PCR technique. Peripheral blood (3 ml) was also withdrawn from patients, then each blood placed into gel tube for separate serum and measure the IgG antibody by ELISA technique. Result: Histological examination' show that 78(74.2%) were positive for H.pylori , while the detection of *H. pylori* by using molecular method has shown that 69 (65.7%) of the patients were positive for H. pylori by using 16SrRNA gene and only 47 (44.7%) where positive for amiA gene. By ELISA 92 (87.6%) of patients were positive for IgG antibody. Conclusion: There is no test that we can considered it as golden standard method for the diagnosis' of H.pylori, the combination of more than test is a good choice to achieve the most reliable result.
Keywords: *Helicobacter pylori, 16S rRNA gene, amiA gene, ELISA.*

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) is a human gastric pathogen that usually found in the mucous lining of stomach, it is Gram-negative, microaerophilic bacterium that infects more than 50% of the world's population and could result in gastrointestinal diseases [1]. *H.pylori* plays a crucial role in pathogenesis of various illnesses of the digestive system, such a peptic ulcer, gastricadenocarcinoma and chronic gastritis, *H. pylori* is a well-known risk factor for gastric cancer [10]. Different diagnostic strategies developed for the detection of H.pylori infection with both of highly sensitivityand specificity for precise diagnosis of infection with *H.pylori* in clinical practice. The tests used for Diagnosis are usually divided into invasive (endoscopic-based) such as molecular, histopathological examination. Non-invasive diagnostic method such as serological test that based on the presence of bacterial antibodies [7]. Even though a lot of diagnostic methods are available for now, every test has its limitations, advantages and disadvantages. To pick out one of the methods, could be Relies on the accessibility and availability of tests, laboratories degree, clinical circumstances of the patients and possibility ratio of positivity and negative tests on different clinical circumstance [2].

Helicobacter pylori contain numerous of housekeeping genes that could be useful for the genetic diagnosis of bacteria, *16S rRNA* gene is the most common housekeeping genetic marker, amplification method of *16S rRNA* gene found to be valuable for the identification and phylogeny of the bacteria[11]. Another housekeeping gene N-Acetylmuramoyl-l-Alanyl Amidase (AmiA) has

a chief role in composition and structure of *H. pylori* Peptidoglycan [12].

Table 1. Description of the studied patients.

Patients characters (n=105)	Number (%)
Sex	
Males	51 (48.57%)
Females	54 (51.42%)
Age (years)	17-85 (Mean 47)
Signs and symptoms	
Dyspepsia	31 (29.5%)
Vomiting	18 (17.1%)
Bloating	10 (9.5%)
Weight loss	17 (16.1%)
Loss of appetite	15 (14.2%)
Dysphagia	12 (11.4%)
Melena	2 (1.9%)

2. SUBJECTS, MATERIALS AND METHODS

In a total of 105 patients' (51 males and 54 females) aged 17 to 85 years, they diagnosed through physicians according to' endoscopic findings, complaining' from clinical manifestations of dyspepsia or burning, vomiting, bloating, weight' loss, loss of appetite, dysphagia' and melena were enrolled in this study which was carried out November 2020- Julay 2021 (Table 1).

In addition,' to control group included twenty apparently healthy individuals 9 males' and 11 females; their age matched the patients' group, the participants have no any' gastrointestinal diseases. biopsy samples were collected' by gastroenterologists from 105 patient, four' gastric biopsies were taken from each patient' who underwent upper gastroduodenal endoscopy' in the gastroenterology and hepatology tertiary' center, Baghdad - Iraq.

Three biopsies placed' in formalin for histological examination. The last biopsy has been' placed in 1 ml of normal saline and' preserved in -20 ºC for molecular analysis, and also blood samples' for serology test were collected from each' patient.

2.1. Processing of Samples for PCR Assay

2.1.1. Extraction of DNA From Biopsy Specimens

Each biopsy collected was frozen (-20 c). These frozen biopsies' thawed, genomic DNA was then extracted directly from tissue using (Quick Genomic) DNA extraction' kit according to the instructions of manufacturers. The concentration and the purity of DNA were measured by Nano drop.

Table 2. Name and Sequence of Primers

Name of Primer	Sequence.	Reference s
16SrRNA	5’-TTGGAGGGCTTAGTCTCT-3’ 5'-AAGATTGGCTCCACTTCACA -3’	Karam et al., 2019
amiA	F- 5’-GTTTTAGACGCTGGGCATGG-3’ R-5’-CCATCAGCAATGCCCTTAGC-3’	

2.1.2. PCR Amplification Analysis

Detection of H. pylori by two' of Housekeeping Gene Using specific" Primers for 16S rRNA and amiA Genes' for confirmation the presence of H. pylori DNA in collected samples.

Optimization' of PCR for both' genes (16S rRNA' and amiA) were done separately" by using different specific sets of primer. 1 µl of each primer, 4µl DNA' sample, and 12.5 µl OneTaq master' mix (NEB-England) and complete' to the final' volume 25µl by using free nuclease water.

The source of all primers' used in this study was IDT® (Belgium). The name and sequence' are given in Table 2. Monoplex PCR reaction for for *16S rRNA* and amiA genes using OneTaq (NEB®) mastermix are shown in Table 3.

2.2. Evaluation of H. pylori Immunoglobulin G (IgG) by ELISA

Serum isolated from 105 blood samples through high-speed centrifuge 6000 rpm' for 5 mints, H. pylori antibodies was detection by ELISA using Accubind kit from USA [3].

Table 3. Monoplex PCR conditions for *16S rRNA* and amiA genes

Cycle No.	Stage	Temperature	Time
1	Initial Denaturation	94 ºC	1 mins
35x	Denaturation Annealing for 16SrRNA Annealing for amiA Extension	94 ºC 57 ºC 54 ºC 70 ºC	30 sec. 45 sec. 45 sec.
1	Final Extension	70 ºC	5 mins.

3. RESULT AND DISCUSSION

The result of present' study clarified that the invasive method by histological test revealed that 78/105 (74.2 %) of patients were positive and distributed by different percentage' according to histopathological findings. Histology plays vital role in detecting *H. pylori* and it also furnished more information about the grade of inflamation and related disease, such as atrophy gastritis (AG), gastric cancer and intes tinal metaplasia (IM). Same study done by Karam *et al.* [18] found that (54.7%) of samples were' positive by histological test to H. pylori.

Whereas for housekeeping genes 16SrRNA and amiA the result" was 69/105(65.7%), 47/105 (44.7) respectively, 16S ribosomal RNA' (16SrRNA) gene is could be found in all bacteria, in the same time, it consist of nucleotide sequences that are specific for a given bacterial' genus [13]. the amplification method of 16S rRNA gene found to be useful for the bacterial identification and phylogeny [11]. Numerous features of 16S rRNA gene which make it the "ultimate molecular chronometer" [14], the commonest genetic marker of housekeeping gene, and therefore, a suitable goal for clinically identification and phylogeny [15]. These characteristics comprise the following: Main, it is found in all bacteria; therefore, it is a universal target' for bacterial identification [16]. Additional, the function of this gene hasn't altered through a long time, so random changes of sequence is more likely to reveal the evolutionary modification of microbes (phylogeny) from selected changes which could modify the function of molecules [14]. Lastly, the 16S rRNA gene is big enough, about 1,500 bp, for informatics purposes [15][16].

Another housekeeping' gene N-Acetylmuramoyl-l-Alanyl Amidase (AmiA) which has a major role in the structure and composition' of H.pylori Peptidoglycan [12], The result by using this housekeeping was (47/105).

The outcome of using' housekeeping genes may due to many factors' such as the site of biopsy wasn't contain the' bacteria, technical factors or others.

several indirect antibody-based tests, such as the serological test which developed for the diagnose of *H. pylori* infection, Because of the small number of bacteria which inhabit the stomach. [17].

In our study, the serological' test 92/105 (87.6%) of specimens were positive by IgG antibody, there are many advantages that, serological tests reliability canot affected by gastric atrophy, ulcer bleeding, and antibiotics, that may lead to false negative' results in other tests.

Serological tests not a dependable to evaluate eradication therapy because the levels of antibody could remain in blood for long period even after eradication successfully [4].Because serological test can't differentiate active infection from previous exposure to H. pylori. So, before eradication therapy, other tests is required for further confirmation,

According to Thaker and Moon [3], The sensitivity and specificity of This method is76–84% and 79–90%, respectively. like these tests have a high negative predictive value (NPV), based on findings of many studies. The capacity of serological test to detect current infection is depending on many factors such as the infection clinical conditions, the selection of the antigen that used for antibody in ELISA kit, patient's age and the spread of infection [4]. Since serological testes LgG are less likely confounded by funnel of *H. pylori* by treatments, The serologic methods may be valuable, so this could be useful In patients who treated by colloidal ismuth, antibiotics, and PPIs, if it isn't possible to finish the mentioned medications[4]. Therefore, in particular, the serological method is the efficient diagnostic method specially in clinical states such a gastrointestinal bleeding, gastric carcinoma, MALT lymphoma, and atrophic gastritis. Another advantages offered by Serological testes such as broader accessibility, simplicity, cost-effectiveness and therefore are generally used in most of H. pylori epidemiological studies [5][8].

As well, a serological tests is also found' to be very useful for the evaluation of *H. pylori* infection in children [8]. The accuracy of the serological tests is wouldn't affected by ulcer bleeding and' gastric atrophy, which may lead to false-negative results' in another invasive or non-invasive experiments, this can be consider as another advantage of the serological test [6].

With the development of *H.pylori* infection therapies, the diagnostic methods have also been' expanded. In spite of this, the standard methods applicable, especially in the population who are at risk, is still missing [2].

4. CONCLUSION

There is no test that we can considered it as golden standard method for the diagnosis' of H.pylori, the combination of more than test is a good choice to achieve the most reliable result.

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