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Detection of Helicobacter pylori by rapid urease test and PCR technique from gastric biopsies

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ABSTRACT

This study aims to assess the invasive methods [endoscopy and rapid urease test] and noninvasive method [PCR technique] in the detection of H. pylori. The patients were referred to the gastroenterology clinic of Azadi Teaching Hospital and private Kirkuk clinic for upper and lower and bronchoscopy fiberoptic GIT endoscopy in Kirkuk city. The present study had carried out from November 2019 to February 2021. The number of the patient's group was 120. The patients were aged 3 to 75years. Selected eligible patients subjected to examination by invasive methods (endoscopy and rapid urease test) and non- invasive method using PCR technique. Biopsies had taken from 120 patients, 50(55.6%) showed positive for rapid urease test (RUT) in the antrum, and 25(83.3%) were positive in proximal stomach negative in the antrum in the same patients. Fifty antrum biopsies positive of *H. pylori* for detection virulence genes by using PCR technique. Gastric biopsy taken from multiple gastric areas is more accurate than taking only the antrum region, especially patients who underwent eradication therapy to the *H. pylori* infection. There is a significant association between PCR methods and the detection of *H. pylori* from the gastric biopsy.

1. INTRODUCTION

Four decades ago, Robin Warren and Barry Marshall definitively identified *Helicobacter pylori* by culturing an organism from gastric biopsy specimens that had been visualized for almost a century by pathologists [1]. In 1994, *H. pylori* were recognized as a type I carcinogen, and now it is considered the most common etiologic agent of infection-related cancers, which represent 5.5% of the global cancer burden. In 2005, Marshall and Warren were awarded the Nobel Prize of medicine for their seminal discovery of this bacterium and its role in peptic ulcer disease [2].

Helicobacter pylori is a Gram-negative bacterial pathogen that selectively colonizes the gastric epithelium. The bacterium is urease, catalase, and oxidase- positive is spiral-shaped and possesses 3 to 5 polar flagella that are used for motility. In addition, the majority of *H. pylori* strains express virulence factors that have evolved to affect host cell signaling pathways. Among many unique characteristics of *H. pylori*, one of the most remarkable is its capacity to persist for decades in the harsh gastric environment due to the inability of the host to eliminate the infection. Unlike other viruses and bacteria, *H. pylori* have evolved the ability to

colonize the highly acidic environment found within the stomach by metabolizing urea to ammonia via urease, which generates a neutral environment enveloping the bacterium [3].

The most important feature for *H. pylori* is the production of urease enzyme in invasive areas. The urease enzyme can be identified with a rapid urea test for gastric biopsy. The *H. pylori* can produce other enzymes such as oxidase and catalase enzyme also acid phosphatase, DNase, leucinarylamidase, Naphthol, and ASB enzyme [4].

The *H. pylori* organism is oxidase-positive and catalase-positive but negative for hippurat test, nitrate reduction, and Indol test, also not grow in media containing NaCl (b1% - 3.5%)5.

1.1 Genome structure

The genomes of two strains have been completely sequenced: *H. pylori* 26695 and J99. Both were sequenced using a random shotgun approach from libraries of cloned chromosomal fragments of ~2.5kb. The 26695 genomes were 24kb larger than the J99, but both of the genomes had GC% of 39%. Both genomes had similar average lengths of coding sequences, coding density, and the bias of initiation codons. The origin of replication of the genome J99 was not identifiable [6].

The genome of *Helicobacter pylori* strain "26695" is circular and contains 1,667,867 base pairs, and strain "J99" contains 1,643,831 base pairs. The chromosome of the organism contains genes that encode the urease gene cluster, cytotoxins in the membrane, and the cag pathogenicity island. In 1989, the *CagA* gene was found and identified as the marker strain of the risk of peptic ulcers and gastric cancer. The *CagA* pathogenicity island recognizes the type IV secretion system, in which *CagA* proteins are moved to the host cells [7]. The DNA content of *H. pylori* has GC range of 35-38% which categorized itself to the *Campylobacter* species. However, the comparisons of the *16S ribosomal RNA* showed that *H. pylori* were different from *Campylobacter* but similar to Wolinella succinogenes which its GC range was 42-49%. *H. pylori* were placed into its own genus, Helicobacter after the analysis of the ultra-structure, fatty acid composition, and biochemical tests which proved different for *H. pylori* and W. succinogenes [8].

The analysis of *H. pylori* sequences specifies the diversity and the development of the organism. The genome contains sequences that encode for the membrane proteins. For example, the F1F0 ATP synthase complex, various oxidoreductases such as cytochrome o, and some transporters [9]. Sequences show that *H. pylori* contain well-developed systems for motility, scavenging iron, and DNA restriction and modification. *Helicobacter pylori* are capable of uptake DNA from other *H. pylori*. Due to the uncertainty of the strain linkages, recombination occurs because of the repetitive DNA sequences, which allows high-frequency deletion and duplication and mismatch in-between the strands. Lack of mismatch repairing can increase in the frequency of random variation but it can also convert the gene which can bring down the diversity of the organism [7].

1.2 Acute gastritis

The acute phase of infection is scarce and largely comes from reports of subjects who deliberately or inadvertently ingested *H. pylori* or underwent procedures with contaminated material. Recently, a human challenge model for *H. pylori* infection was introduced; it allowed controlled studies of the acute phase of infection with deliberate infection of healthy volunteers with a well-characterized laboratory strain of *H. pylori* [10]. Together, these reports showed that the acute phase of colonization with *H. pylori* may be associated with transient nonspecific dyspeptic symptoms, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa, or pangastritis. This phase is

often associated with hypochlorhydria, which can last for months. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs [11].

1.3 Chronic gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis. This correlation results from the counteractive effects of acid on bacterial growth versus those of bacterial growth and associated mucosal inflammation on acid secretion and regulation. This interaction is crucial in the determination of outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori*, in particular, colonizes the gastric antrum, where few acid-secretory parietal cells are present. This colonization pattern is associated with antrum-predominant gastritis. Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria. Subjects in whom acid secretion is impaired, due to whatever mechanism, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis. The reduction in acid secretion can be due to a loss of parietal cells as a result of atrophic gastritis, but it can also occur when acid-secretory capacity is intact but parietal cell function is inhibited by vagotomy or acid-suppressive drugs, in particular, proton pump inhibitors (PPIs) [12].

1.4 Peptic ulcer

Peptic ulcer disease (PUD) refers to a break in the mucosal lining of the stomach or duodenum and occasionally the lower part of the esophagus [13]. There are five major complications of PUD; bleeding, perforation, penetration, obstruction and malignancy [14].

PUP occurs in 2% - 10% of patients with ulcer disease, it is the second most frequent complication after bleeding. Gastroduodenal perforation, with leakage of alimentary contents into the peritoneal cavity, is a common surgical emergency associated with morbidity and mortality in 50% and 30% of cases respectively [15]. PUP present as acute abdominal emergency conditions, with localized or generalized peritonitis and a high risk for further development of sepsis and death [16].

1.5 Gastric cancer

The strain of *H. pylori* a person is exposed to may influence the risk of developing gastric cancer. Strains of *H. pylori* that produce high levels of two proteins, vacuolating toxin A (*VacA*) and the cytotoxin-associated gene A (*CagA*), appear to cause greater tissue damage than those that produce lower levels or that lack those genes completely [17]. These proteins are directly toxic to cells lining the stomach and signal strongly to the immune system that an invasion is underway. As a result of the bacterial presence, neutrophils and macrophages set up residence in the tissue to fight the bacteria assault [18].

Gastritis caused by *H. pylori* is accompanied by inflammation, characterized by infiltration of neutrophils and macrophages to the gastric epithelium, which favors the accumulation of pro-inflammatory cytokines and reactive oxygen species/reactive nitrogen species (ROS/RNS). The substantial presence of ROS/RNS causes DNA damage including 8-oxo-2'-deoxyguanosine (8-OHdG) [19]. If the infecting *H. pylori* carry the cytotoxic *cagA* gene (present in about 60% of Western isolates and a higher percentage of Asian isolates), they can increase the level of 8-OHdG in gastric cells by 8-fold, while if the *H. pylori* do not carry the *cagA* gene, the increase in 8-OHdG is about 4-fold [20]. In addition to the oxidative DNA damage 8-OHdG, *H. pylori* infection causes other characteristic DNA damages including DNA double-strand breaks [21].

Helicobacter pylori also cause many epigenetic alterations linked to cancer development. These epigenetic

alterations are due to *H. pylori*-induced methylation of CpG sites in promoters of genes, and *H. pylori*-induced altered expression of multiple microRNAs [22].

1.6 Diagnosis of H. pylori

1.6.1 Endoscopy

A conventional endoscopic exam is usually performed to diagnose *H. pylori*- associated diseases, such as peptic ulcer diseases, atrophic gastritis, MALT lymphoma, and gastric cancer. Endoscopy is also an instrument routinely used to obtain specimens, usually gastric mucosa from biopsy, for further studies on other invasive tests, including rapid urease test, histology, culture, and molecular methods. The antrum is a preferential biopsy site for detecting *H. pylori* infection in most circumstances, but corpus biopsy from a greater curve is suggested for patients with antral atrophy or intestinal metaplasia to avoid false-negative results [23]. The uneven distribution of *H. pylori* in the stomach in different clinical settings inevitably leads to sampling errors in biopsy-based examinations and several attempts have been made for real-time diagnosis of *H. pylori* infection during endoscopic examination [24].

Most gastric mucosal features, such as redness, mucosal swelling, or nodular change, from conventional endoscopy, are not specific enough for a diagnosis of *H. pylori* infection and provide limited value in the accurate diagnosis [25]. Although careful close-up observation of the gastric mucosa pattern with standard endoscopy may increase the diagnostic accuracy, it may be time-consuming and not provide better results than other invasive tests [26].

1.7 Rapid urease test

Rapid urease test (RUT) is the most useful invasive test for the diagnosis of *H. pylori* infection because it is inexpensive, rapid, easy to perform, highly specific, and widely available. Based on the activity of the *H. pylori* urease enzyme, the presence of *H. pylori* in the biopsy specimen converts the urea test reagent to ammonia, leading to an increase in the pH and a color change on the pH monitor. Several commercial urease tests including gel-based tests (CLO test, HpFast), paper-based tests (PyloriTek, ProntoDry), and liquid-based tests (UFT300, EndoscHp) are available now, and different commercial RUTs have different reaction times to provide results. CLO test usually takes 24 hours to obtain an accurate result, whereas PyloriTek takes 1 hour and UFT 300 takes 5 minutes to provide more rapid results [27]. Reading the urease tests earlier than recommended time may lead to false-negative results [28]. In addition to the designs of commercial kits, the density of bacteria present in the biopsy specimen also affects the reaction time and diagnostic accuracy of RUT, while a minimum of 10000 organisms are usually required for a positive RUT result. Other factors influencing the diagnostic accuracy of the urease tests include H2-receptor antagonists, PPI, bismuth compounds, antibiotics, achlorhydria, and the presence of blood, all of which increase the possibility of false-negative results. Furthermore, formalin contamination of biopsy specimens also decreases the sensitivity of RUTs [29], [30].

In general, the commercial rapid urease tests have specificity above 95%-100% and sensitivity above 85%-95%. Increasing the number of gastric antral biopsies could increase the sensitivity of RUTs and dual biopsy specimens from gastric corpus and antrum are preferred than only antrum biopsy specimens as additional corpus biopsy increase the diagnostic accuracy and avoid sampling bias due to uneven distribution of *H. pylori* in the stomach. Moreover, combining antrum and corpus specimens before RUT, rather than separate specimens, also increased the sensitivity of RUT and accelerate the reaction time [28], [30]. Avoid medications that affect the urease activity and the density of bacteria is recommended before RUT to decrease false-negative results, such as 2week for PPI and 4week for antibiotics. Bleeding significantly decreases the sensitivity and specificity of RUTs and makes RUT become a more unreliable test than other tests in this

clinical condition [31].

1.8 Polymerase chain reaction

Polymerase chain reaction (PCR) to detect *H. pylori* infection, PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, stool, gastric juice, and variable specimens. PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting *H. pylori* in patients with bleeding. Several target genes including *UreA, UreB, glmM, UreC, 16S rRNA, 23S rRNA, HSP60, CacA*, and *VacA* genes, had been used for detection of *H. pylori*, and using two different conserved target genes can increase the specificity, which in turn avoids false-positive result, especially for samples other than gastric biopsy specimens. The other advantages of PCR, including fewer bacteria required in the sample, faster results, and no need for special processing supplies or transportation [32], [33].

Detection of virulence factors by PCR helps to evaluate the genetic variation within virulence factors of *H. pylori* and gives more information to understand the clinical discrepancies between patients infected with different strains of *H. pylori*. Several studies showed the presence of virulence factors, such as *CagA* and *VacA* gene, are associated with more severe gastric inflammation and a higher prevalence of peptic ulcer disease and gastric cancer [34- 36]. Duodenal ulcer promoter gene A (*DupA*) was also proposed to be associated with *H. pylori* induced ulcer formation, but inconsistent results which were suspected to be caused by primer mismatches were reported by previous studies. A newly designed RT-PCR with a specific primer designed based on an alignment of all 221 *DupA* gene sequences was introduced recently to improve the detection rate of the *DupA* gene. This method increased the detection rate to 64.2%, whether the commonly used PCRs had a detection rate between 29.9% to 37.8%. The authors pointed out that PCR design had a great influence on the detection of virulence factor and the detection of specific *DupA* allele was not the same as detection of actual *DupA* gene [37].

PCR is also helpful to detect *H. pylori* in environmental samples for epidemiological studies. A high prevalence on *H. pylori* detected in drinking water samples by PCR provided more information of *H. pylori* transmission through drinking water [38]. A higher detection rate of *H. pylori* contamination in un-washed vegetables suggested accurate washing of vegetables decreased *H. pylori* contamination [39]. PCR had also been used to detect genotyping of *H. pylori* in vegetables and high similarity in the genotyping pattern of *H. pylori* among vegetable samples and human specimens suggested that vegetable may be the sources of the bacteria [40].

2. MATERIAS AND METHODS

2.1 Biopsy samples

Endoscopy Olympus were performed (120) patients under intravenous sedation was used midazolam, or local sedation orally was used xylocaine, and endoscopic findings recorded. Biopsies were taken by Dr. Summer Saad Abd Al-Husain, a gastroenterologist in Azadi Teaching Hospital. Four biopsies specimens from the antrum and two biopsies from the proximal stomach (body and fundus), by biopsy forceps (Olympus FB13k), were sterilized by autoclaving.

Four biopsies specimens from the antrum and proximal stomach were processed in the urea broth test and quick test for *H. pylori*, also two biopsy specimens were placed in the Eppendorf tube add 125 µl of DNA/RNA shield were stored at a temperature of less than -20ºC for molecular analysis.

2.2 Rapid Urease Test (RUT)

2.2.1 Urea Rapid Broth

This broth contains the PH indicator phenol red. If gastric *Helicobacter* from present, *Helicobacter* urease breaks down the urea; with the release of ammonia, arise in PH and a color change from yellow to pink within 10 minutes. If color, not changes incubate the urea broth at 37ºC for 24hours when a color change occurs given a positive result.

The test prepares from dissolved these ingredients

- Yeast extract (0.10gm)
- Na2HPO4 (0.095 gm)
- KH2PO4 (0.091 gm)
- Phenol red (0.01 gm)
- Urea (20 gm)
- Distill water (1000 ml)

Finally, sterilization by autoclave at 120ºC for 90 minutes and then put in a plain tube 5ml in each tube [41].

2.2.2 Helicobacter pylori quick test

The Helicobacter pylori quick test kit is based on a biochemical reaction to qualitatively determine H. pylori urease activity directly from biopsy specimens, using kits from Biohit Health Care, Finland, catalog No. REF 602015.

2.3 Principle

The quick test developed for detection of *Helicobacter pylori* infection in stomach is based on the activity of the urease enzyme in biopsy specimen. The biopsy specimen taken from the stomach is tested immediately. The development of the color in the test gel informs whether urease enzyme is present in the biopsy sample or not. In strong *H. pylori* colonization (high density the reaction time is 2 minutes, in low density *H. pylori* colonization the reaction can take up to 30 minutes. The assay proceeds according to the following reaction:

$[NH_2]_2CO + 2H_2O + H \rightarrow 2NH + HCO_3$

Helicobacter pylori produce urease, which degrades urea to ammonia [NH4].

The ammonia formed is detected by an indicator color present in the gel.

2.4 The primers used in PCR technique

The primers were lyophilized, they dissolved in the free ddH_2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH_2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).

1- The specific primer UreA of gene

Primer	Sequence	Tm (ºC)	GC (%)	Product size
Forward	5'-TGATGGGACCAACTCGTAACCGT- 3'	60.2		244 base pair
Reverse	5'- CGCAATGTCTAAGCGTTTGCCGAA- 3’	60.4	50	

2- The specific primer UreB of gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-ATGCCTTTGTCATAAGCCGCTTGG- 3'	60.2	50	645 base pair
Reverse	5'-AGTAGCCCGGTAGAACACAACATCCT-3'	61.2	50	

3- The specific primer VacA of gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-TGGGTAATGTGTGGATGGGC - 3'	60.03	55	977 base pair
Reverse	5'- ATTGATGCGCGATTGACTGC- 3'	59.97	50	

4- The specific primer 16s RNA of gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair
Reverse	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1	

3. Results

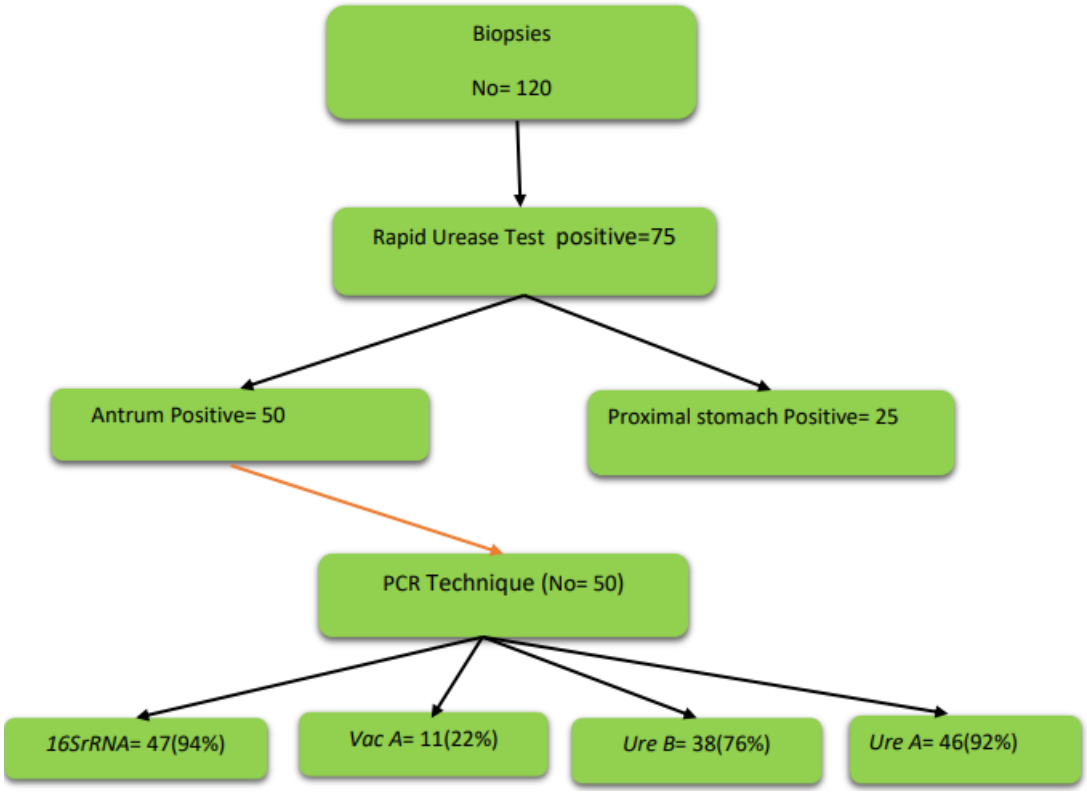

Figure (1) Flow chart of the study with summarized results

3.1 Rapid urease test (RUT)

Biopsies had taken from 120(65.6%) patients, 50(55.6%) showed positive for rapid urease test (RUT) in the antrum, and 25(83.3%) were positive in proximal stomach negative in the antrum in the same patients. Statistically, there was a highly significant difference between the areas of the stomach in the rapid urease test (p= 0.003), as seen in the table (1). *Helicobacter pylori* status evaluated by RUT had reported in 75(60%), the percentage was 50(40%), and 25(20%) out of 125 patients, as shown in Figure (2).

Table (1) Result of rapid urease test

Result	Positive (%)	Negative (%)	Total (%)
Distal stomach (Antrum)	50(55.6%)	40(44.4%)	90(100%)
Proximal stomach (funds and body)	25(83.3%)	5(16.7%)	30(100%)
Total % (n= 120)	75(62.5%)	45(37.5%)	120(100%)
	Chi-Square = 8.955 P-Value = 0.003		

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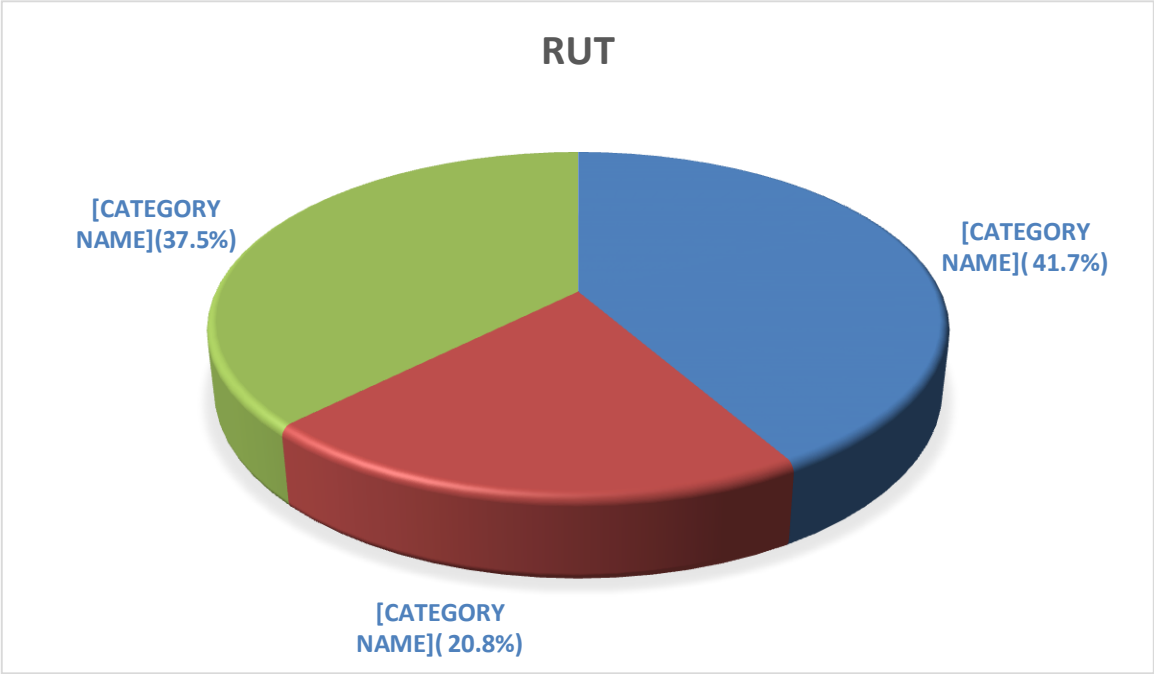


RUT= rapid urease test

Figure (2) Association *Helicobacter pylori* in gastric biopsy by RUT

3.2 Endoscopic diagnosis

With positive rapid urease test for antrum was 50(41.7%) to the *Helicobacter pylori* showed endoscopic findings was a higher prevalence 0f chronic gastritis were 13(26%), followed by gastric ulcer 12(24%), gastric cancer 10(20%), acute gastritis 8 (16%), and duodenal ulcer 7(14%) as seen in figure (3).

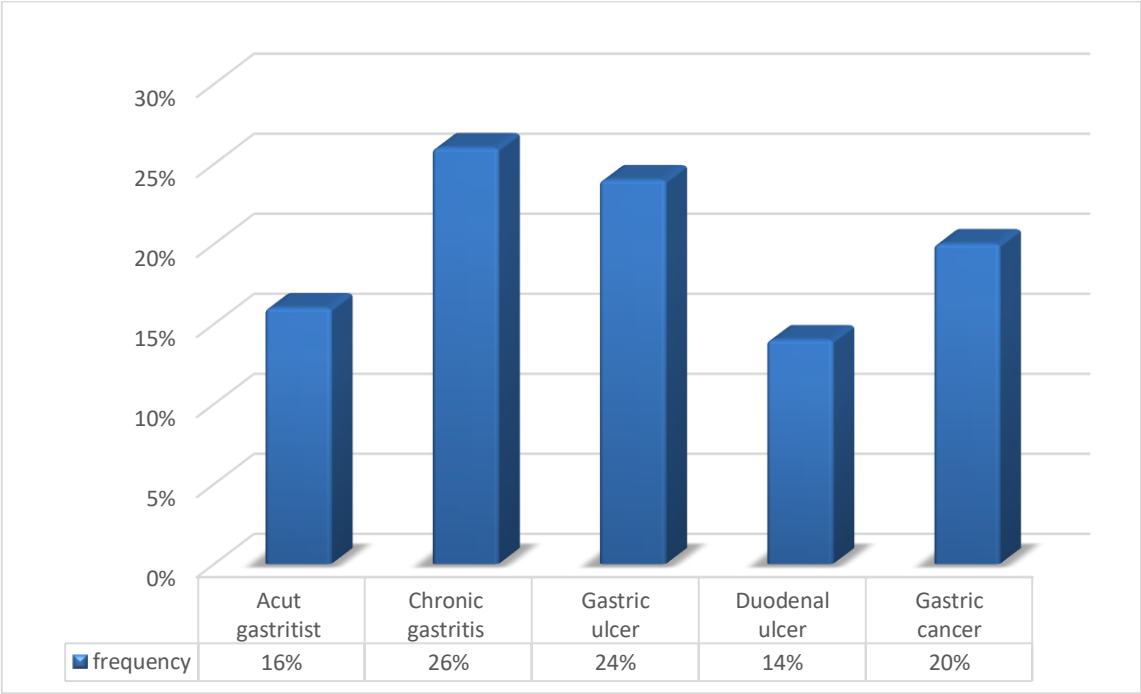


Figure (3) Relation between rapid urease test and endoscopic diagnosis

3.3 Prevalence of H. pylori genes (Ure A, Ure B, Vac A, and 16SrRNA) detected by using PCR technique

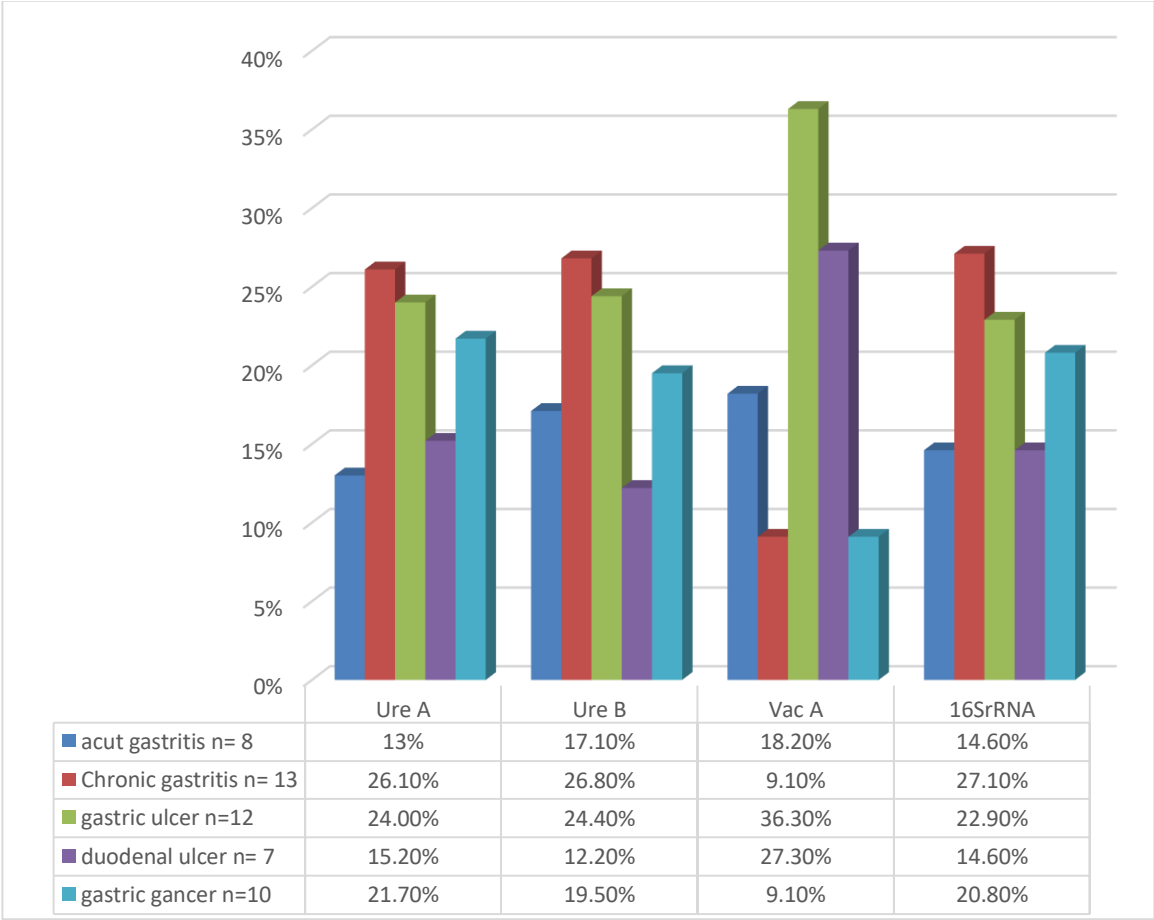
Out of the 50 antrum biopsies screened were positive for *Helicobacter pylori* DNA, four types of virulence genes had been detected by PCR and the statistical analysis between the PCR technique showed a highly significant difference (p= 0.00004). The *16SrRNA* showed a high percentage of 47(94%), *Ure A* 46(92%), *Ure B* 38(76%), and *Vac A* 11(22%) as shown in Table (2).

Table (2) Results of four examined genes using PCR technique

PCR results	*Ure A*	*Ure B*	*Vac A*	*16SrRNA*
Positive	46(92%)	38(76%)	11(22%)	47(94%)
Negative	4(8%)	12(24%)	39(78%)	3(6%)
Total	50(100%)	50(100%)	50(100%)	50(100%)
	Chi-Square = 90.614 P-Value = 0.00004			

3.4 Relation between PCR examined genes of and gastric diseases

VagA gene showed a high percentage to gastric ulcer 4(36.3%), while *16SrRNA* gene were found in 13(27.1%) of chronic gastritis followed by *UreB* gene 11(26.8%), and *UreA* gene 12(26.1%), as seen in figure (4).



	Ure A	Ure B	Vac A	16SrRNA
acut gastritis n= 8	13%	17.10%	18.20%	14.60%
Chronic gastritis n= 13	26.10%	26.80%	9.10%	27.10%
gastric ulcer n=12	24.00%	24.40%	36.30%	22.90%
duodenal ulcer n= 7	15.20%	12.20%	27.30%	14.60%
gastric gancer n=10	21.70%	19.50%	9.10%	20.80%

4. Discussion

4.1 Rapid urease test and endoscopic finding

In the current study, two kinds of rapid urease tests were used manual RUT and manufactured RUT kit. Their purpose had thoroughly investigated the presence of *H. pylori* in gastric biopsies. The results were more accurate in the manual RUT, the reason has attributed may be due to the difference in the ingredients between them also lack the salts in the manufactured kit.

Helicobacter pylori have detected in 60% of patients tested by RUT. 40% of patients had a positive RUT taken in antrum in comparison 0f 20% taken from the proximal stomach. There was a highly significant association between *H. pylori* localization and *H. pylori* colonization degree from the distal and proximal stomach. The biopsies gave positive results in the RUT taken from proximal stomach 83.3% among the patients in this group. The RUT is an efficient way to determine the presence or absence of *H. pylori* infection. Requires endoscopy accuracy markedly affected by PPI, bismuth, antibiotic use resulting in high false negative rate. Proximal migration out of antrum and patchy topography of *H. pylori* can result in false negatives [42]. The present study in agreement with [43] demonstrated 78.3% had taken from fundic mucosa. However, the sensitivity of the RUT test increases if had taken biopsies from both the corpus and antrum. Thus, a positive RUT result indicates the presence of *H. pylori* and makes it possible to prescribe treatment, but a negative result does not allow excluding *H. pylori* therefore it is recommended to confirm the diagnosis with an additional method [44]. Another study from Thailand who detection rats of RUT were 28.3% at antrum and 34.3% at the body in patients who have been recently taking PPI, the *H pylori* detection rate from a combination of RUT at antrum and body [45]. From turkey found *H. pylori* positivity was 49.7% in the corpus, while 57.9% in the antrum, and there was no significant association between *H. pylori* localization and *H. pylori* colonization degree. The pathogenesis and mechanism of *H. pylori* related diseases in the proximal stomach have not been explained [46].

On the other hand, During the endoscopy, many pathological conditions had diagnoses such as gastritis, gastric ulcer, gastric cancer, and duodenal ulcer. The most prevalent chronic gastritis demonstrated 26% of the total cases showed the positive results of RUT in the antrum.

Helicobacter pylori-associated gastritis showed lesser curvature predominant endoscopic atrophy in the corpus mucosa. Characterizes endoscopic atrophic mucosa as having a pale color, increased vessel visibility, low mucosal height, and green color in the purple corpus mucosa. Endoscopic atrophic mucosa and histological atrophic gastritis according to patients' age. Atrophic gastritis extended upward from the antrum to the corpus, along the lesser curvature, and laterally from the lesser curvature to the anterior and posterior wall over time, but not much in the greater curvature. Later, *H. pylori* was established as a definite pathogen of chronic atrophic gastritis [47]. Chronic gastritis had diagnosed in case of loss of superficial capillary network or focal lesions not matching the definition of other focal lesions or cancer. [48] from Germany demonstrated chronic gastritis of 73% by endoscopy findings. Hussein [49] demonstrated Antral gastritis was a commonly endoscopic finding (66%) in Iraq, and [50] from Nepal showed 60% of chronic gastritis from endoscopic gastric biopsies were positive *H. pylori* by RUT. However, another study showed antral ulcer and erosion as the most available endoscopic findings [51]. Prevalence of *H. pylori* colonization is high in chronic gastritis and there is a parallel increase in the severity of gastritis with an increase in the severity of *H. pylori* load [50].

4.2 PCR technique and Helicobacter pylori

The current study had highlighted that molecular tests were performed not only from an antral biopsy but had taken from multiple gastric biopsies. It was well- known that *H. pylori* could show a patchy distribution in the stomach, and the more biopsies had performed, the higher the diagnostic accuracy [52].

Data revealed that the overall prevalence of the *16SrRNA* gene was 94%, *Ure A* gene 92%, and *Ure B* gene Showed 76%. Regarding distributions of *16SrRNA*, *Ure A*, and *Ure B* genes with endoscopic findings were 27.1%, 26.1%, and 26.8% of chronic gastritis, respectively. The prevalence of a *Vac A* gene was 22% by PCR, and concerning the endoscopic finding, it was more prevalent in peptic ulcers 63.6%. According to the statistical analysis, they were highly significant.

The priority of the study was the identity of *H. pylori* by a noninvasive PCR assay using gastric biopsies samples and determining the most prevalent virulent gene for *H. pylori* infection and relation to gastric diseases. [53] from Saudi Arabia found a prevalence of *H. pylori* by using the PCR method was 46.5% among gastritis. The study was in agreement with the study conducted by [54] from Indonesia *16S rRNA* gene has been shown better sensitivity than other target genes for *H. pylori* detection, evaluated the sensitivity of several genes for *H. pylori* detection including, the *16S rRNA*, *23S rRNA*, and *UreA*. The sensitivity of the three genes was 55%, 41%, and 43%, respectively. Another study in Iraq had used *16SrRNA* and the *flagella* gene for detection of *H. pylori* showed the *flagella* gene protein more accurately than the *16SrRNA* gene. This result was logical because the Flagellar protein gene was normal and considering that flagella are the main structure for *H. pylor* [55].

The current study conflict with other studies, [56] who found the prevalence of the *VacA* gene is more sensitive to gastric cancer. Also, analysis of all combined genotypes of the *Vac A* alleles may play a significant role in determining *H. pylori*-related clinical outcomes. [57] from Iran showed that *H. pylori* in gastrointestinal disorders were more prevalent with *CagA* and *VacA* virulence factors. PCR for the diagnosis of *H. pylori* is an accurate and specific method, providing a fast and safe diagnosis [58]. Current molecular diagnostic methods employ various technologies for targeting a sensitive gene in the presence or absence of *H. pylori*. These methods have several limitations, such as the false-positive result of loop-mediated isothermal amplification and the costly device for endpoint analysis of the amplified products (electrophoresis, fluorescence) [59].

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