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Molecular Methods for Detecting Bacteria Causing Gastric Ulcers and Gastritis in Nineveh Governorate

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ABSTRACT

Helicobacter pylori is one of the most widespread bacteria in human communities worldwide and is primarily associated with gastrointestinal diseases such as chronic gastritis, peptic ulcers or even gastric cancer because it contains a number of virulence factors. The purpose of this study was to find 16S rRNA in stomach biopsies from patients with gastritis in Mosul and uncover some of the virulence genes of *H. pylori*. The study was carried out in hospitals in Mosul, Iraq, from June 2023 to April 2024. Fifty samples were collected from 50 individuals, ages 14 to 85. During endoscopy, the gastroenterologist, a specialist physician, collected gastric biopsy samples from the corpus stomach. PCR was performed on the gastric biopsies. Fifty gastritis-afflicted samples are included in this investigation. The highest frequency was found in patients aged 26–40 and 41–60, while the lowest frequency was found in patients aged 61–85. PCR was used to find *H. Pylori.* The study discovered that 50 (100%) of the gastrointestinal patients had positive 16SrRNA results. Furthermore, this study discovered that 44 (88%) of the cagA gene was negative and 6 (12%) positive. The high frequency of these factors in gastritis patients' biopsies is concerning and necessitates ongoing monitoring, considering the significant role that the studied virulence factors play in the pathophysiology of *H. pylori*.

Keywords: *Helicobacter pylori*, cagA,16SrRNA, PCR, Biopsy.

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الطرق الجزئية للكشف عن البكتيريا المسببة لقرحة المعدة والتهاب المعدة في محافظة نينوى

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الملخص

تعتبر الجرثومة الملوية البوابية من أكثر أنواع البكتيريا انتشارا في المجتمعات البشرية على مستوى العالم، وترتبط بشكل أساسي بأمراض الجهاز الهضمي مثل التهاب المعدة المزمن أو قرحة المعدة أو حتى سرطان المعدة لأنها تحتوي على عدد من عوامل الضراوة. هدفت هذه الدراسة إلى الكشف عن بعض جينات الضراوة لجرثومة الملوية البوابية وتحديد 16SrRNA في خزعات المعدة لمرضى التهاب المعدة في الموصل. أجريت الدراسة بين يونيو 2023 وأبريل 2024 في مستشفيات مدينة الموصل بالعراق. تم أخذ ما مجموعه 50 عينة تتراوح أعمارهم بين 14 و 85 سنة (50 مريضا). أخذ الطبيب المختص (أخصائي أمراض الجهاز الهضمي) عينات خزعة المعدة من تجويف المعدة والجسم أثناء التنظير الطبيعي. تم استخدام خزعات المعدة لتقنية تفاعل البوليميراز المتسلسل. الدراسة الحالية تشمل 50 عينة تعاني من التهاب المعدة. كان لدى المرضى حسب الفئات العمرية 26–40 و41–60 أعلى معدل تكرار، في حين كان لدى 61–85 أقل معدل تكرار. تم الكشف عن بكتريا الملوية البوابية بواسطة PCR. وفقًا لنتيجة 16SrRNA ، وجد البحث أن مرضى المعدة الإيجابيين كانوا 50 (100٪). بالإضافة إلى ذلك، وجد هذا البحث أن جين cagA كان 6 (12٪) إيجابيًا و44 (88٪) سلبيًا. إن ارتفاع معدل هذه العوامل في خزعات مرضى التهاب المعدة أمر مقلق ويستلزم المراقبة المستمرة، مع الأخذ في الاعتبار الدور المهم الذي تلعبه عوامل الضراوة المدروسة في الفسيولوجيا المرضية لـ H. pylori.

INTRODUCTION

Helicobacter pylori is a pathogen linked to disorders of the stomach and duodenum, Formerly referred to as Campylobacter pylori ,This spiral bacterium, which is This spiral bacteria is a microaerophilic, Gram-negative bacillus that moves similarly to a corkscrew with the aid of polar flagella [1]. it was found Many thousands of years ago [2, 3]. According to earlier research and reports, this bacterium affects over 50% of people worldwide in developed nations, with a higher prevalence of up to 80% in developing nations. Furthermore, there are variations in the infection rate by gender and age. [4]. Even though it is very common, only a small portion of people exhibit symptoms[5]. Although they can get stomach cancer, only 3% of patients have an *H. pylori* infection, though that number can rise to higher levels[6]. If left untreated, an infection with this bacterium can persist for a lifetime [7]. 16S rRNA and cagA are two of the most aggressive virulence indicators expressed for *H. pylori* and are likely the most researched. This cagA gene gives the bacteria the capacity to alter the host's cell metabolism, and its existence is linked to the emergence of stomach cancer and ulcers. One of the most virulent factors that has been suggested as a potential marker to differentiate between different strains of *H. pylori* is the cytotoxic associated gene A (*cagA*). Among the many virulence factors present in *H. pylori* bacteria, the *cagA* gene encodes a 120–145 kDa protein that is contained inside the ~40 kb pathogenicity island (cag PAIs). It is believed that bacterial adhesion is necessary for *Helicobacter* to colonize the stomach epithelium[8]. Because these toxins promote the release of pro-inflammatory mediators, including cellular attractants like IL-8,

which is a substance generated by the body's lymphocytes and promotes gastritis, they are associated with the development of stomach cancer. This inflammation leads to the formation of gastric ulcers and peptic atrophy, which is the prelude to stomach cancer [9] There are numerous invasive and noninvasive diagnostic techniques that are capable of identifying an *H. pylori* infection. Non-invasive detection techniques include stool antigen measurement, serological testing, and the urea breath test (UBT) [10]. Diagnostic procedures that involve endoscopic examination and the collection of gastric pylori biopsy samples are considered invasive. These tests consist of rapid urease testing, PCR, culture testing, and histological examination [11]. PCR has been used extensively to diagnose *H. pylori* from gastric biopsy specimens, saliva, feces, gastric juice, and varied specimens since it was first used to detect infection[12].

The purpose of this investigation was to detect the prevalence of *H. pylori* by determining16S rRNA and identifying virulence genes (cagA) in gastric biopsies using Polymerase Chain Reaction (PCR) Technique.

MATERIALS AND METHODS

Between June 2023 and April 2024, the study was conducted in the Microbiology Department and the Endoscopy and Gastroenterology Unit of Nineveh Governorate hospitals. The patients' consent was obtained before the biopsy was conducted.

Samples Collection:

This study was conducted on 50 patients (26 females/24 males, aged 14-85 years) with gastrointestinal diseases who underwent endoscopy by a surgeon specialized in the gastrointestinal and colon endoscopy department at the hospital. During endoscopy, the gastroenterologist (specialist) used endoscopic forceps to remove the gastric biopsy samples from the stomach's corpus. When transporting biopsies, we use sterile tubes containing normal sterile saline. PCR was performed on gastric biopsies.

Molecular assay

DNA was extracted from biopsy specimens using a kit (Geneaid, Sengapure), and Primers unique to the *CagA* gene and the *H. pylori* 16S rRNA gene were used for PCR amplification [13].

Table 1: The name, sequence and product size of primers

Target gene	Primer pair (5′-3′)	Size(bp)	Tm
16sr RNA	27F : 5'-AGAGTTTGATCMTGGCTCAG-3' 1522R : 5'-AAGGAGGTGATCCARCCGCA-3'	1500	56
Cag A	F: 5'-TAACGCTGTCGCTTCATACG-3' R: 5'-AGGGATAGGGGGTTGTATGG-3'	355	58.1

Table 2: combination of the particular gene interaction for diagnosis

Components	Concentration
Taq PCR PreMix	10µl
Forward primer	10 picomols/µl (0.5 µl)
Reverse primer	10 picomols/µl (0.5 µl)
DNA	3µl
Distill water	6 µl
Final volume	20µ

Initial denturation at 95 °C for 6 minutes, followed by 35 cycles at Anealing temperatures (58.1°C) for cagA (56 °C) for16srRNA, and its PCR cycler conditions are used. Initial denaturation (95 °C for 3 minutes), denaturation (95 °C for 15 seconds), annealing (50 °C for 30 seconds, extension at 72 °C for 30 seconds), and final hold (10 °C for 1 minute) .After staining with RedSafe Nucleic Acid Staining (1 mg/L) and visualising under UV light, 5 µL of ampl icon was electrophoresed in 1.5%agarose gels for (45 min) at 70 V in 1X TBE buffer.

RESULTS AND DISCUSSION

According to our findings, *H. pylori* raises the risk of gastric cancer and is substantially linked to gastric diseases, including gastritis. These outcomes were anticipated given that this bacteria may cause a severe inflammatory response in the human stomach epithelium.

Both sexes were included in the study, The percentage of Female was (26)52%, while the percentage of Male was (24) 48%. Based on their age ranges, patients between the ages of 14 and 85 were divided into five groups. Based on the age-group-specific distribution of gastric patients from 26-40 and 41-60 year had the highest frequency, and hose aged 14-25 and 61-90, who had the lowest. These results are consistent with the findings of the researchers [14] who enrolled (79 women and 41 men) out of a total of 120 patients infected with *H. pylori* bacteria in their study. Due to lifestyle, changes in female hormones, and women's visits to the doctor.A Study in Iraq indicated that high Spread of *H.pylori* occurs in patient with age group 26-34 and 45-54 (24%) years old compared to younger age (12%) [15].

Identification of the *H. pylori* 16SrRNA gene

The 16S rRNA gene has been extensively utilized for bacterial species identification through sequence analysis. comprise several functionally different regions. Some of them are distinguished by highly conserved sequences, meaning that they are shared by a large number of bacteria. Highly variable sequences, or nucleic acid sequences unique to a species or genus, are found in other locations. Figure (1) shows that 50 (100%) of the 50 biopsy samples had positive PCR findings for the 1500 bp domain of the 16S rRNA gene.

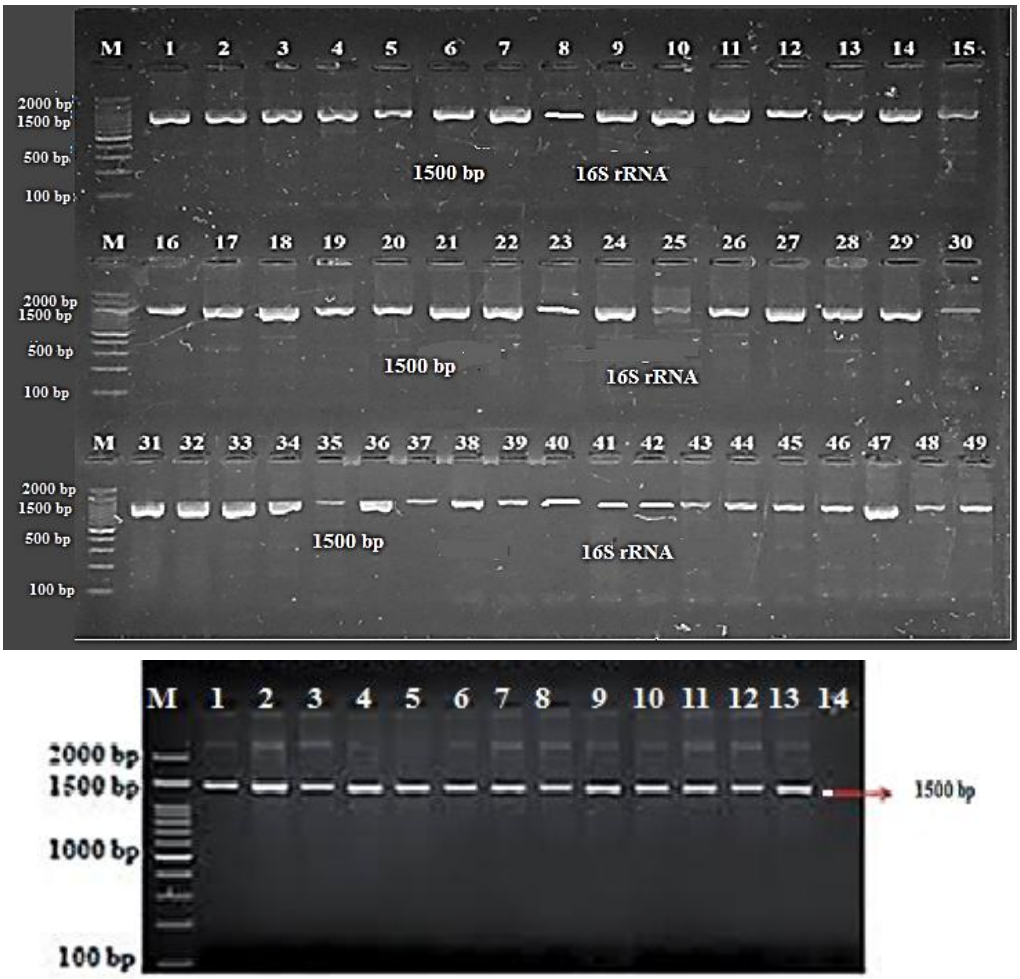

Fig. 1: Endoscopy biopsy amplified *H. pylori*16SrRNAgene(1500bp) PCR results that show that *H. pylori*16SrRNA is positive(1-50). M :Scale Marker(100bp) It was electrophoresis on 1.5% agarose at 72 volts per centimeter. One x TBE buffer for one and a half hours

The 16S rRNA gene possesses the most common housekeeping genetic marker, making it a good target for phylogenetic study and clinical identification, Using molecular methods like PCR, it is possible to correctly establish the presence of infection as well as evaluations of the diversity, pathogenicity, and resistance patterns of these bacteria. The findings were 100% positive. These results are consistent with a study conducted by researchers [16] who discovered that 87% and 100% of samples tested positive for the 16S rRNA gene [17, 18]. 16S rRNA is one of the particular targets used to confirm an *H. pylori* infection. A positive *H. pylori*-specific DNA amplification can be regarded as concrete proof of the pathogen's presence[19].

Detection of cytotoxin associated gene A (Cag A)

Genetic detection results revealed that 12% (6/50) of *H. pylori* isolates carried the *cagA* gene, as shown in gel electrophoresis Figure (2). The shiny bands indicate some positive results of the *cagA* gene with amplified size 355 bp when compared to M:Scale Marker (100 bp).

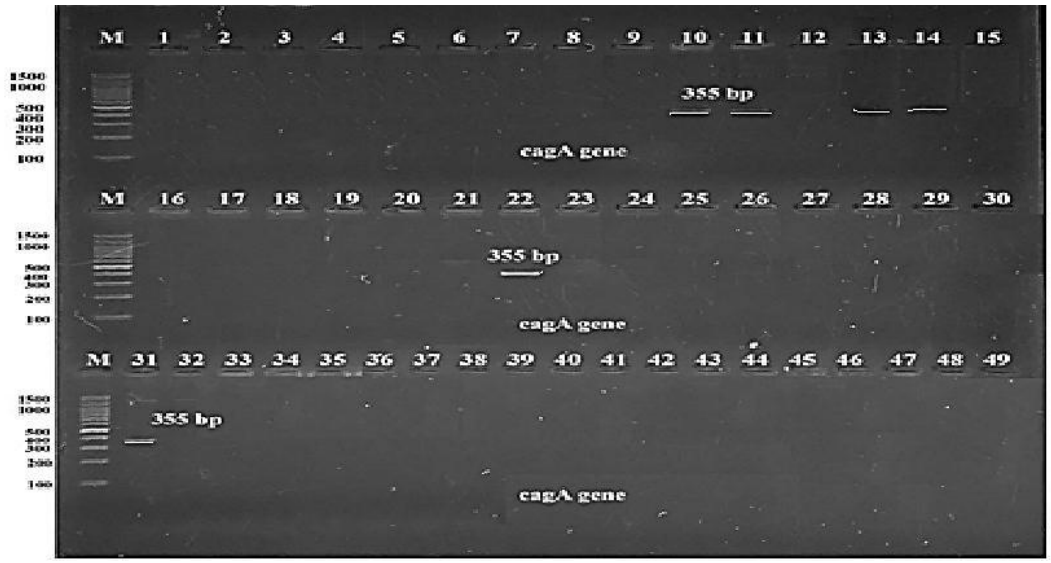

Fig. 2: Endoscopy biopsy amplified *H. pylori cagA* gene (amplified size 355 bp) PCR results that show that 10, 11, 13, 14, 22, and 31 indicate positive results. M: Scale Marker (100 bp) , It was electrophoresis on 1.5% agarose at 72 volts per centimeter. One x TBE buffer for one and a half hours

According to our findings, people with the cagA gene who have a history of gastric epithelium damage are at an increased risk of developing gastric cancer. A study conducted in India found that the cagA rate was 66.6% [20] and a study by [21]found that the prevalence rate was high at 100%. Geographical, social, and environmental factors, as well as sampling techniques, location, and sample size, can all have an impact on how widely these strains propagate [22]. The gene Cag A encodes Cag A, a crucial virulence component of *H. pylori*. Numerous studies have demonstrated that, in Western nations, infection with bacteria that carry the Cag A gene is linked to chronic gastritis, a high incidence of stomach cancer, and gastric ulcers. However, in Asian countries, the association between positive strains of the gene and clinical disease is unclear because Most strains of *H. pylori* are Cag A positive [23] It was demonstrated that in certain situations, such as chronic gastritis, the incidence of stomach cancer rises and falls varies from one place to another [24].

CONCLUSION

This study examined the relationship between peptic ulcer disease and the *H. pylori* genes. We may thus draw the conclusion that differences in the cagA genotype and 16S rRNA can be utilized as predictive markers in clinical isolates of *H. pylori* to pinpoint a particular strain as the source of cancer

or gastritis. It may be concluded that host genes and diet have a major role in the development of gastric cancer and other infectious diseases, in addition to *H. pylori* infectious disorders.

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