ORIGINAL ARTICLE

Molecular Study of CagA gene in *Helicobacter pylori* Isolated from Gastritis

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ABSTRACT

Background: *H. pylori* infection is strongly related with chronic gastritis of the stomach, which causes impairment in gastric acid and secretion of pepsin, and linked to male absorption of food-vitamin B12. the most important virulence genes accompanying stomach and intestine disease are (cag A *and* vac A).
Aim: To evaluate the rate of gastric ulcer infection with *H. pylori* and its detect CagA gene in H.pylori isolated from gastritis..
Methods: A total of 200 biopsy patients (130males and 70 females) aged from (5- 65) years were collected from Baqubah teaching hospitals and Al-Batool teaching hospital. They were suffering from gastric upset and attended to endoscopic unit of department of medicine. Questioners including, sex, age, smoking, presence of cancer, and biopsies of gastric tissue were collected from the corpus or the ant rum or corpus and ant rum of the patient's stomach. Three biopsies were taken from each patient. Histopathologic study, gram staining, and rapid urease test working for each patients. Serology Test Serum Enzyme-Linked Immunosorbent Assay Testing (ELISA). Molecular test including Extraction of genomic DNA from tissue biopsy.
Results: The result showed that age group(45-60) was the most age group of people with bacteria *H. pylori* as the percentage (29.10%). From 200 biopsy samples, 106(96.4%) patients gave positive PCR results of the 515 bp domain for 16SrRNA gene and also in control group 3(3.3%) gave positive result , PCR products for 411bp of UreA when compared to the molecular ladder(2000-100). The virulence genes of bacteria *H. pylori* were detected in all samples of patients who had positive results in diagnostic detection using 16sRNA gene (109 patients from 200 studies group), according to that the virulence gene were identified for *H. pylori* in biopsies contain this bacteria, where the UreA gene was detected , it is one of the most important virulence factors of this bacteria, which the size 411bp. This gene was identified in(81) from patients infected with this bacteria(109) and percentage (73.60%). The statistical analysis is high significant (p 0.001),
Conclusion: *H. pylori* infection is strongly related with chronic gastritis of the stomach. The presence of this genes related with chronic gastritis and stomach ulcer.
Keywords: Gastric ulcer, H. pylori, Cag A gene, Stomach ulcer, Duodenal ulcer.

INTRODUCTION

Helicobacter pylori is a gram negative bacteria and spiral-shaped, that colonizes the stomach mucoid lining[1]**.** It is characterized by polymorphism phenomenon it may appear in the form of cocci or bacillary form, it is the main cause of stomach and duodenal ulcers, these disease have become common in recent times due to the spread this type of bacteria ,specially between families and close area, it is highly pathogenic ,affect more than half of the world population[2]. The incidence of these bacteria is due to the virulence genes that are carried by particular genetic patterns of this bacteria it is the most important virulence genes accompanying stomach and intestine disease are (cag A *and* vac A)[3]. To avoid the acidic condition in the gastric lumen, this bacteria have developed an antibiotic resistant to the stomach acid of the microbial through colonization in a very deep place of gastric lactation and secretion of the urease which analyses urea located in the medium to ammonia which have the effect of the acidic acid in the stomach lining which enables them to stay in the human stomach lifelong if not treated with antibiotics and peptoon pump[4]. This bacteria causes many diseases such as Chronic gastritis, Gastric ulcers, duodenal ulcers, Gastric and doudenal cancer and Mucosa-associated lymphoid-tissue (lymphoma)[5]. The highest rates of infection are associate with low socioeconomic status, family size, local crowding , low level of education, poor sanitation and water supplies[6]. Diagnosis *H. pylori* infection can be made by use several invasive or non-invasive techniques. Invasive diagnostic assays include: rapid urease test, histological examination, PCR and culture[7]. These bacteria are characterized as being fastidious organism, they appear very poorly in the tissue, making it difficult to development and considered a slow growth microorganism and the proposes to be diagnosed directly from clinical models by using molecular techniques like (PCR) which is unique in its sensitivity and high specificity in diagnosis and accuracy in determining both the presence of infection and the genotype of these bacteria[8]. Detect CagA positive *H. pylori* was higher in patients with gastric cancer compare to those with chronic superficial gastritis and atrophic gastritis[4]. *H. pylori* infection is strongly related with chronic gastritis of the antrum of the stomach, which causes impairment in gastric acid and secretion of pepsin, and linked to malabsorption of food-vitamin B12. *H. pylori* can cause vitamin B12 deficiency. It is also a known contributor to gastritis ulcers and it can prevent to the stomach from being able to absorb the vitamin B12 you consume and leads to a deficiency of vitamin B12[9]. The main aims of this study are: To isolate *H. pylori* from patients suffering from gastric ulcer, to detect *H. pylori* using PCR, to determine the antibiotic resistance of *H. pylori*.

PATIENTS AND METHODS

A total of 200 biopsy patients (130males and 70 females) aged from (5- 65) years were collected from Baqubah teaching hospitals and Al-Batool teaching hospital. They were suffering from gastric upset and attended to endoscopic unit of department of medicine. Biopsies of gastric tissue were collected from the corpus or the ant rum or corpus and ant rum of the patient's stomach. Three biopsies were taken from each patient. Histopathologic study, gram staining, and rapid urease test working for each patients. Biopsies classified according to the performed test. That used for histopathology was transported to the histopathology unit with 10% buffered formalin for at least 24 h. That used for culture was transported to the culture unit with 2-2.5 mL-1 Tryptical Soy Broth (TSB). For RUT was tested immediately. Bacterial diagnosed based on[11]. Serology Test Serum Enzyme-Linked Immunosorbent Assay Testing (ELISA). Molecular test including Extraction of genomic DNA from tissue biopsy, 2Determination of DNA concentration, The concentration of extracted DNA was calculated by using Visible scanning spectrophotometer according to the method of[12].

The primers used in present study are show in the table.

Prime	Sequence 5'- 3'		Size (bp)	Source
16SrRNA	F	TGGCAATCAGCGTCAGGTAATG	515	NCBI Gene-Bank data base
	R	GCTAAGAGATCAGCCTATGTCC		
Cag A	F	TGATGGCGTGATGTTTGTTGA	1320	NCBI Gene-Bank data base
	R	TCTTGGAGGCGTTGGTGTATT		

Statistical analysis:- Data of study were analyzed by using Chi-square (X^2) test to compared between percentage. Odd ratio (OR) and relative risk (RR) were used to measure strength association between presence factor . (T) test to compared between two parameters and used (ANOVA) to compare between more than two parameters. So, we used (Spearman's rho and Pearson correlation) to detection type and strong relationship between parameters. A level of significance of α=0.05 was applied to test. (SPSS v.22, Excel 2013 and Graph pad prism v.6) programs used to analyze current data.

RESULTS

A total of 200 biopsy patients (127males and 73 females) aged from (10- ≥ 60) years were collected from Baqubah teaching hospitals. They were suffering from gastric upset and attended to endoscopic unit of department of medicine, class of patients are 60 patient (the first diagnosis showed symptoms of the disease) and the control class is 100 people(the first diagnosis is the absence of symptoms of the disease). Bacterial culture(biopsy samples), rapid urease enzyme test (biopsy samples) ,ELISA test (blood samples) and PCR(biopsy samples) are made for each patient. The result showed that age group(45-60) was the most age group of people with bacteria *H. pylori* as the percentage (29.10%). From 200 patients, 86(43.0%) patients were positive for RUT and 114 (57.0%) patients were negative for RUT shown the table 1 were statistically high significant(p value 0.001).

Table 1: Rapid urease test for biopsy samples according to study group.

Rapid urease test		Groups		Total
		Controls	Patients	
Positive	N%	0(0.0%)	86(78.2%)	86(43%)
Negative	N%	90(100.0%)	24(21.8%)	114(57%)

P value=0.001***
RR=0.00
OR=0.00
Sn=0.00%
Sp= 22%

This test used for detect *H. pylori* IgG antibodies in the serum among all 200 patients using one step test . Of the 200 studied patients,109 (54.5 %) positive for(RDT) and 91 (45.5 %) negative for (RDT), the difference significant is high(p 0.001), show the table (2).

Table 2: Rapid diagnostic test for detection *H. pylori* IgG antibodies.

Rapid diagnostic test		Groups		Total
		Controls	Patients	
Positive	N%	0(0.0%)	109(99.1%)	109(54.5%)
Negative	N%	90(100.0%)	1(0.9%)	91(45.5%)

Pvalue=0.001***
RR=0.00
OR=0.00
Sn=0.00%
Sp= 0.9%

Rapid diagnostic test within study groups .Three tissue biopsy were given positive results in bacteriology culture test from (110) tissue biopsies of the patient group with(3.6%). The percentage of samples negative for culture test was(96.4%), while no tissue biopsy was given a positive result from (90) for controls group. The statistically significant(p 0.009), as show in table(3).

Table 3: Results bacteriology culture test for biopsy tissue.

Bacteriology culture test		Groups		Total
		Controls	Patients	
Positive	N%	0(0.0%)	3(3.6%)	3)2%)
Negative	N%	90(100.0%)	107(96.4%)	197(98%)

Pvalue =0.09
RR=0.00 OR=0.16 Sp.=0.0
Sn.=0.97

Culture	
Positive	3(3.60%)
Negative	107(96.40%)

Statistics: 0.001***

The colonies were identified as *H. pylori* depending on the shape of the colonies, catalase test, urease test and oxidase test. After culture the samples on skirrows medium and period of incubation 7-14 day, under condition suitable for the development of these bacteria, there is little oxygen by use gas generation kit, four isolates were obtained for these bacteria . The colonies were small to middle in size, rounded and creamy in color, All *H. pylori* isolates were identified by Gram's staining and biochemical tests. The bacteria were observed as Gram negative, spiral or curved

in shape. In the present study, the positive result of histopathology test from 110 patients was 10(9.0%), the percentage of negative result was (91.0%), table (4).

Table 4:Histopathology test results with studies group.

Histopathology test		Groups		Total
		Controls	Patients	
Positive	N%	0(0.0%)	10(9.0%)	10(5.0%)
Negative	N%	90(100.0%)	100(91.0%)	190(95.0%)

P value 0.003**
RR=0.00 OR=0.05 Sp.=0.0
Sn.=0.90

Histopathology	
Positive	10(9%)
Negative	100(91%)

Statistics: 0.001***

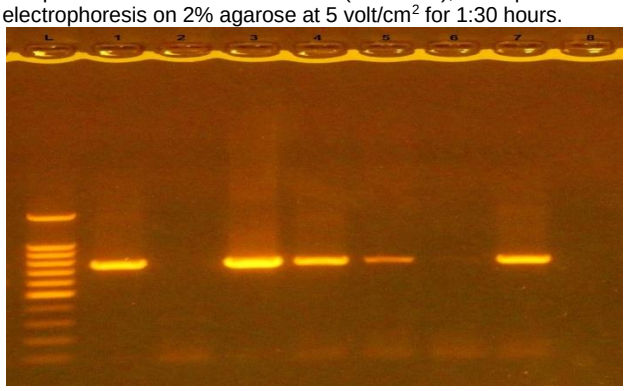
The genomic DNA was extracted directly from 200 biopsy samples of studies group and used also for PCR to determine *H. pylori* infection. Whole DNA extracted from gastric biopsies directly were subjected to 2% agarose gel electrophoresis. From 200 biopsy samples, 106(96.4%) patients gave positive PCR results of the 515 bp domain for 16SrRNA gene and also in control group 3(3.3%) gave positive result , as shown in table (5). The PCR products were analyzed using 2% agarose gel electrophoresis.

Table 5: the percentage of 16sRNA according to study group.

16Srna		Groups		Total
		Controls	Patients	
Positive	N%	3(3.3%)	106(96.4%)	109(54.5%)
Negative	N%	87(96.7%)	4(3.6%)	91(45.5%)

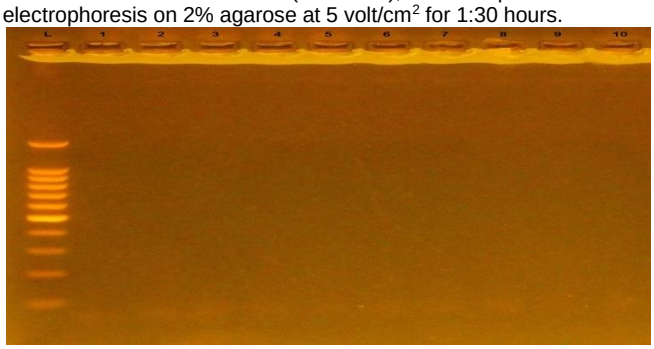
Pvalue=0.001*** RR=0.03
OR=0.001 Sn=3%
Sp= 36%

Figure (1): Agarose gel electrophoresis of DNA from biopsy tissue directly showing PCR products for 515bp of 16SrRNA when compared to the molecular ladder(2000-100), The product was electrophoresis on 2% agarose at 5 volt/cm^2 for 1:30 hours.



The virulence genes of bacteria *H. pylori* were detected in all samples of patients who had positive results in diagnostic detection using 16sRNA gene (109 patients from 200 studies group), according to that the virulence gene were identified for *H. pylori* in biopsies contain this bacteria. While CagA gene ,which the size (1320bp). This gene was identified in(20)patients that it is infected with *H. pylori*, and percentage (18.20%), the statistical analysis is high significant (p 0.001).

Figure (2): Agarose gel electrophoresis of DNA from biopsy tissue directly showing PCR products for 411bp of CagA when compared to the molecular ladder(2000-100), The product was electrophoresis on 2% agarose at 5 volt/cm^2 for 1:30 hours.



In the current study, was high percentage for diagnosis infection with *H. pylori* among patients ,was by detecting the *H. pylori* in biopsy through PCR which percentage (96.36%) and by rapid diagnostic test which percentage (99.09%), and rapid urease test which percentage (78.18%) and histology was(9.0%), while culture test which is low percentage (3.60%), table(6).

Table 6: Comparative positivity of tests specific for *H. pylori* of patients

Positivity of tests NT of patients		Patients
Rapid Ureases Test	NP%	86 [b](78.18%)
Rapid diagnostic test	NP%	109 [a] (99.09%)
16sRNA	NP%	106 [a](96.36%)
CagA	NP%	20 [c](18.18%)
Histology test	NP%	10(9.0%)
Culture test	NP%	3(3.60%)

In the current study, two isolates from three isolate appears resistance for all antibiotic in this study, while one isolate appear resistance for clarithromycin and amoxicillin and sensitive for tetracycline and metronidazole, show the table(7).

Table 7 : The resistance of *H. pylori* isolate for antibiotic.

Antibiotics	Samples		
	1	2	3
Clarithromycin	Resistance	Resistance	Resistance
Amoxicillin	Resistance	Resistance	Resistance
Tetracycline	Resistance	Sensitive	Resistance
Metronidazole	Resistance	Sensitive	Resistance

DISCUSSION

In the present study, we used five method for diagnosis *H. pylori* , these method are pcr include (16srRNA, UreA, CagA) ,rapid urease test, rapid diagnostic test, histology test and culture test. Also used ELISA test for detect vitamin b12 deficiency and associated with *H. pylori* . The highest detection rate of *H. pylori* infection recorded in the age group(45-60). The higher infection were among males than females, the increase rate of infection in males is due to the daily effort of males compared to female, and other factors such as smoking and alcohol ,the low infection in females compared to males may be due to antibiotics , taken by females over the course of life[13]. The high infection in patients with diabetes was percentage (54.10%) , this indicate that *H. pylori* infection associated with

diabetes patients. There is evidence that *H. pylori* infection may contribute to the development of diabetes through the effect of hormones regulating the intestinal insulin . As a result ,*H. pylori* infection are common in patients with diabetes[14]. The incidence *H. pylori* in patients suffering from pressure and diabetes was percentage (15.60%). Also in patients suffering from pressure, asthma and sensitivity , the incidence *H. pylori* was rate (1.80%), in people not suffering from chronic diseases and those infected with *H. pylori* (26.60%), the incidence *H. pylori* infection in the patients with cancer was (15.5%) but in patients without cancer (84.5%). The results of RDT showed that**,** out of the 200 studied patients,109 (54.5 %) were RDT positive and 91 (45.5 %) were RDT negative. The present study agree with[14]. The detection of *H. pylori* infection by RDT from different countries revealed variation in the positivity rate that range between(61% - 87%)[15]. The reason due to the diversity of the immune response from one person to another, and that the period of exposure to infection may affect the result[16], this may effect on the sensitivity and specificity of this test. In the present study, *H. pylori* has been isolated and diagnosed from gastric biopsy by culture method using skirrows medium. The specificity of this test is influenced by the skill of the person working in this field and its ability to determine the form of bacteria or associated changes[17,18].

CONCLUSION

H. pylori infection is strongly related with chronic gastritis of the stomach, which causes impairment in gastric acid and secretion of pepsin, and linked to malabsorption. Isolate *H. pylori* from patients suffering from gastric ulcer in high rate, The presence of this gene may be due to its primary role in carrying *H. pylori* of acid environment located within the stomach cavity , where this gene are important genes of this bacteria, which is responsible for the production of secondary unit involved in the synthesis of urease enzyme. In the current study the percentage with CagA is very low because the patients infected with gastric cancer is low.

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Data Availability: Data obtainment for patients with different age groups who attended Baquba Teaching Hospital in Diyala Province.

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