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Evolution of virulence factors and defection factor H.pylori among gastrointestinal disease patients in Waist province

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Abstract

Background:

Helicobacter Pylori (H. pylori) infection is the most important factor affecting clinical outcome in patients with gastric mucosal lesions The aim of this study is to employ conventional PCR to determine the presence of Helicobacter pylori through molecular analysis of the 16S rRNA gene. Additionally, the existence of virulence genes, cagA, vacA, IceA, will be investigated.

Mateials and methods

Total sample was 40 positive h. Pylori infection in different stage range from mild until sever or chronic among gastrointestinal disease patient from waist province, the age of participant range from 12 to 62 years attended to gastrointestinal section in AL-karma testing hospital during 2022/2023. saliva spacemen was collected to Primers of Polymerase Chain Reaction (PCR) for detection of 16sRNA, CagA,and VagA s1/s2 as a detection and virulence factor of h.pylori infection.

Results

The study found 16sRNA positive 40(100%), and CagA most of samples positive 23(57.5%), and the negative 17(42.5%), and the distribution of gastroenteritis patients according to VagA s1/s2 most of samples negative 32(80%) compare to positive 8(20%). there is no significant relationship between CagA (p=0.288) and VagA s1/s2 (p=0.825) tests when considering different age groups. the PCR product for 16sRNA showing difference in nine cases from all positive patients at line 446 bp, that equal to (22.5%) have deferent strain of H. Pylori and the PCR product for CagA showing difference in seven cases from all positive patients at line 599 bp that means 30.4% suffer from sever H. Pylori conclusion : One of fourth of H. Pylori infection have a chance to sever or chronic disease through presence of, CagA, and VagA s1/s2 gene in strain and female's patients has more risk to suffer from sever H. Pylori infection through change in CagA gene.

Keyword: H.

pylori, gastric disease, Molecular detection, virulence factor ,detection factor

Helicobacter pylori (H. pylori) infection, a bacterium with widespread distribution, has been identified as the primary determinant of clinical outcomes in individuals with stomach mucosal lesions. A meta-analysis conducted in 2018 revealed a global prevalence rate of 44.3% [1,2]. Gastrointestinal disorders, ranging from gastritis to stomach cancer, frequently exhibit this infection [3]. Helicobacter pylori possesses a repertoire of elements that contribute to its ability to remain within the gastric environment, guarantee its survival, and initiate interactions with its cells in the host. The presence of virulence factors is correlated with a heightened susceptibility to developing peptic ulcer, gastric adenocarcinoma, or MALT-type lymphoma. Furthermore, the development of gastric disease is also linked to other factors, including the host inflammatory response, host genetic diversity, and environmental factors [4,5,6]. CagA, which is encoded by the cagA gene, serves as both a constituent and an indicator of the cagA Pathogenicity Island (PAI). Helicobacter pylori strains that are positive for the CagA protein have been found to be correlated with pronounced inflammation and an elevated susceptibility to the development of ulcers and cancer in the human population. The co-occurrence of cagA is typically observed alongside other virulence factors, such as vacA [13].

Materials and methods

Total sample was 40 positive h. Pylori infection in different stage range from mild until sever or chronic among gastrointestinal disease patient from waist province, the age of participant range from 12 to 62 years attended to gastrointestinal section in alkarma testing hospital during 2022/2023. saliva spacemen was collected to Primers of Polymerase Chain Reaction (pcr) for detection factor of 16sRNA, and CagA,and VagA s1/s2 as a virulence factor of h.pylori infection.

Primers of Polymerase Chain Reaction

The PCR primers used for diagnosis H.pylori and detection of virulence genes were designed according to references and NCBI- Genbank. These primers were synthesized by (Scientific Researcher Co. Ltd, Iraq) as following table.

Table (2-3) Primers used in PCR to amplify 16SrRNA and virulence factors genes

Target gene	Primer pair (5'-3')	Product Size(bp)
16SrRNA	F: CTGGAGAGACTAAGCCCTCC R: AGGATCAAGGTTTAAGGATT	446
CagA	F: CAAGCCCTAGCCGAACTCAA R: GCTTCTGACACTGCCTGACT	599
vacAs1/S2	F: ATGGAAATACAACAAACACAC R: CTGCTTGAATGCGCCAAAC	259/286

Conditions of PCR Thermocycler

PCR thermocycler conditions protocol for each gene was calculated by using Optimase Protocol Writer™ online application and done by using conventional PCR thermocycler as clarified in the following tables

Table (2-6) Touchdown protocol of 16SrRNA gene

Touch Down PCR step	Temp.	Time	Repeat
Initial Denaturation	95ºC	2min.	1
Denaturation	95 ºC	30sec.	14 cycle
Annealing	57.1 ºC decrease 0.5 per cycle	30sec	
Extension	72 ºC	50sec	
Denaturation	95 ºC	30sec.	19 cycle
Annealing	50.1 ºC	30sec	
Extension	72 ºC	50sec	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	-

Table (2-7) PCR Thermocycler conditions of cagA gene

Target gene	Cycling Conditions						
	Denaturation		Annealing	Extension	Final Extension	Hold	No. Of cycles
cagA	95C° 3 min	95C° 30 S	58 C° 30S	72C° 1 min	72C° 5 min	4 °C	35

Table (2-8) Touchdown protocol of VACA S1/S2 gene

Touch Down PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	2min.	1
Denaturation	95 °C	30sec.	14 cycle
Annealing	58.3 °C decrease 0.5 per cycle	30sec	
Extension	72 °C	30sec	
Denaturation	95 °C	30sec.	19 cycle
Annealing	51.3 °C	30sec	
Extension	72 °C	30sec	
Final extension	72 °C	5min.	1
Hold	4 °C	Forever	-

Electrophoresis

2.2.5.4.5.1 Preparation of gel

N	Step
1	1.5% agarose bowder was dissolive in (1X) TBE and melted in microwave for 3min and then left to cool at 50C .
2	5µl of safeview dye was add to the agarose gel solution
3	The agarose gel solution was poured into a tray and the comb was then fixed in the appropriate position and left to solidify at room temperature for 15 minutes. The comb was gently removed from the tray
4	The gel tray was fixed in the electrophoresis chamber, after which it was filled with 1X TBE buffer solution".
5	A volume of 5µl (DNA tag ladder) was added into the first well, and then 10µl of PCR product was loaded into each well in an agarose gel and electrophoresed at 100V for 40 min.
6	After the time was up, the PCR products were photographed using gel documentation.

2.3 Statistical analysis

The data were subjected to analysis using "version 26 of the Statistical Package for the Social Sciences (SPSS). The data were analyzed by calculating basic statistical measures such as percentages, means, standard deviations, and employing the chi-square test Statistical significance was determined by considering p-values that were less than 0.05."

Results

Total of participants was 40 positive patients, the result finding that the distribution of gastroenteritis patients according to 16sRNA positive 40(100%), and CagA most of samples positive 23(57.5%), and the negative 17(42.5%), and the distribution of gastroenteritis patients according to VagA s1/s2 most of samples negative 32(80%) compare to positive 8(20%).

Table 1.1 distribution of gastrointeritis patients according to 16sRNA, CagA,and VagA s1/s2

N	Test		Frequency	Percent
1	16sRNA	positive	40	100%
2	CagA	positive	23	57.5%
		negative	17	42.5%
3	VagA s1/s2	positive	8	20%
		negative	32	80%

The results suggest that there is no significant relationship between CagA (p=0.288) and VagA s1/s2 (p=0.825) tests when considering different age groups. As show in Table 1.2

Table 1.2 compares between 16sRNA, CagA, and VagA s1/s2 gene according to age group

N	Test			Age group			X2	Sig
				15-30	31-45	46--60		
1	16sRNA	positive	Count	16	19	5	N/s	N /s
			%	40.0%	47.5%	12.5%		
2	CagA	positive	Count	8	11	4	1.405[a]	0.288
			%	34.8%	47.8%	17.4%		
		negative	Count	8	8	1		
			%	47.1%	47.1%	5.9%		
3	VagA s1/s2	positive	Count	4	3	1	0.461	0.825
			%	50.0%	37.5%	12.5%		
		negative	Count	12	16	4		
			%	37.5%	50.0%	12.5%		

The findings of table 1.3 indicate a noteworthy relationship between CagA (p=0.007) and the absence of a substantial relationship with VagA s1/s2 (p=0.350) tests across differs gender

Table 1.3 compares between 16sRNA, CagA,and VagA s1/s2 Test according to sex

N	Test			sex		x2	odds ratio	sig
				female	Male			
1	16sRNA	positive	Count	25	15	n/s	n/s	n/s
			%	62.5%	37.5%			
2	CagA	positive	Count	15	8	0.171[a]	1.313	0.007
			%	65.2%	34.8%			
		negative	Count	10	7			
			%	58.8%	41.2%			
3	VagA s1/s2	positive	Count	6	2	0.667[a]	2.053	0.350
			%	75.0%	25.0%			
		negative	Count	19	13			
			%	59.4%	40.6%			

DNA amplified of detection factor 16sRNA gene

to indicted if there are any indel (insertion, deletion) nucleotide or any changes in 16sRNA gene, the peripheral genomic DNA amplified with specific primers targeting 16sRNA gene., the PCR product showing difference in nine cases from all positive patients at line 446 bp, compared to leader line range from 100 to 2000 bp. This result indicates that 16sRNA can affect the progression and the severity the disease by change the expression level that means that (22.5%) have sever H. Pylori As it showing in figure (1.1)

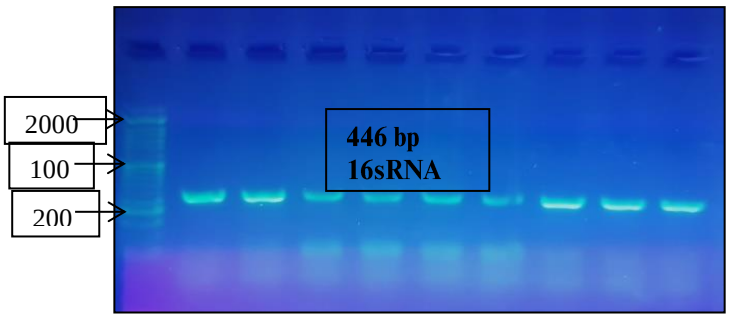

Figure (1.1) 1.5% Agarose gel for NOS3 PCR amplification /16sRNA of H. Pylori test

DNA amplified of virulence factor (CagA, VagA s1/s2)

Also for, indicted if there are any indel (insertion, deletion) nucleotide or any changes in CagA gene, the peripheral genomic DNA amplified with specific primers targeting CagA gene. As it showing in figure (1.2), the PCR product showing difference in seven cases from all positive patients at line 599 bp, compared to leader line range from 100 to 1000 bp. This result indicates that CagA can affect the progression and the severity the disease by change the expression level that means 30.4% suffer from sever H. Pylori infection

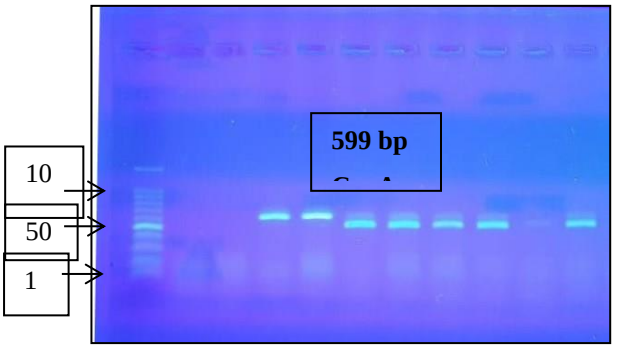

Figure (1.2) 1.5% Agarose gel for NOS3 PCR amplification /CagA of H.pylori test

Again for, indicted if there are any indel (insertion, deletion) nucleotide or any changes in VagA s1/s2 gene, the peripheral genomic DNA amplified with specific primers targeting VagA s1/s2 gene. As it showing in figure (1.2), the PCR product showing difference in sex cases at line 259 bp, compared to leader line range from 100 to 1000 bp. This result indicates that VagA s1/s2 can affect the progression and the severity the disease by change the expression level that means 30.4% suffer from sever H. Pylori infection that means 75% suffer from sever H. Pylori infection

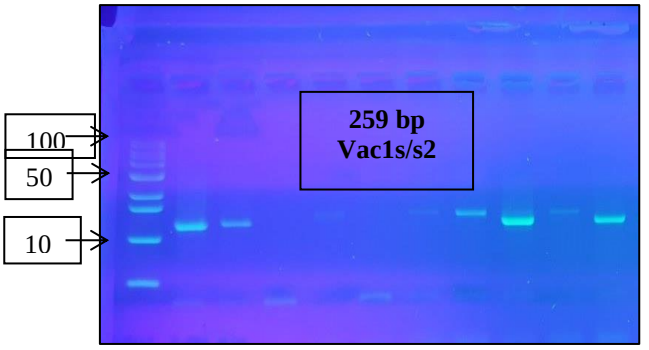

Figure (1.3) 1.5% Agarose gel for NOS3 PCR amplification / VagA s1/s2 of H. Pylori test

Discussion

Helicobacter pylori is considered to be one of the most genetically diverse bacterial species, exhibiting significant genetic variations across different geographic regions[20] Numerous studies conducted in various countries have revealed distinct distributions of vacA alleles and the presence of the cagA gene within H. pylori genotypes strains. These findings have been closely linked to the occurrence of gastro duodenal diseases. Similar to other studies, there is compelling evidence suggesting that the detection of multiple strains of H. pylori is prevalent in clinical samples. Several studies have reported a potential association between multiple genotypes and duodenal ulcers [21] It is possible to speculate that the presence of multiple strains may contribute to an increased likelihood of pathogen infection. The colonization of various receptors expressed on gastric epithelial cells by m1 and m2 strains is likely to result in the induction of pathological alterations. The presence of multiple strains during eradication treatment poses a significant burden on patients and significantly increases the risk

of malignant tumors in the digestive tract among adult patients. However, based on our data analysis, there is no significant evidence to suggest that the occurrence of multiple strain infections increases the risk of developing diseases ($p>0.05$). Moaddeb et al. utilized polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to perform genotyping of the Helicobacter pylori cagA gene obtained from paraffin-embedded sections of gastric cancer. The study populations consisted of patients from Brazil and Japan. The results of the PCR-RFLP analysis conducted on the cagA gene indicated a notable disparity in the prevalence of the east Asia subtype between the Japanese subjects and the Brazilians [22] Based on these findings, the authors reached the conclusion that this particular genotype plays a significant role in the advancement of gastritis within the region. Helicobacter pylori possesses various virulence factors, among which cagA and vacA have been extensively investigated. Multiple studies have documented the variability in the H. pylori cagA/vacA genotype [23,24]. The predominant genotype observed in both the antrum and corpus was vacA s1m1/cagA+ in this study. Additional studies conducted in the southern region of Mexico have documented prevalence rates of 71.1% and 69.7% [25,26]. Moreover, when examining gastric pathology, the prevalence of this particular genotype was found to be 70.7% in cases of chronic gastritis, 57.9% in gastric ulcers, and 81.3% in gastric cancer [27]. Based on the findings, it can be inferred that the vacA s1m1/cagA+ genotype is the prevailing genotype in the Mexican population. It is important to acknowledge that the distribution of vacA/cagA genotype combinations may differ across different geographical regions. As an illustration, the prevalence of the genotype vacA s1m1/cagA+ in Wenzhou, China was reported to be 90.9% [28]. In southern Vietnam, this genotype was found in 51.5% of cases [29]. In northern Spain, it was detected in 20.6% and 54.5% of cases associated with mild and severe disease, respectively [30]. In a similar vein, the prevalence of the combination of the s and m alleles of vacA also exhibits variations when analyzed. The vacA m1 allele is frequently observed in North Asian countries such as Japan and South Korea, while the m2 allele is more prevalent in Southeast Asian regions including Taiwan, China, and Vietnam [31]. In this study, it is noteworthy that one patient exhibited the vacA s1m1/s2m2/cagA+ genotype in both the antrum and corpus. Additionally, the vacA s1m1/s1m2/cagA- genotype was detected in the corpus of two patients. Previous studies have documented the existence of mixed genotypes [32,33], although such occurrences are sporadic. However, it should be noted that in certain cases, the presence of the s or m allele was not detected, which is consistent with findings observed in other populations [34].

Conclusion and recommendation

One of fourth of H. Pylori infection have a chance to sever or chronic disease through presence of 16sRNA, and virulence factor CagA, and VagA s1/s2 gene in strain and female's patients has more risk to suffer from sever H. Pylori infection through change in CagA gene. The study recommended that early detection of 16sRNA, CagA, and VagA s1/s2 to decrease complication and stomach cancer

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