ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Clinical Relevance of the cagA,vacA and babA2 Virulence Factors ofHelicobacter pylori in Egyptian Patients with Gastroduodenal Diseases.

Naglaa El-Toukhy[1*], Amal M. Saeed[2] and Nashwa M. Emara[3].

1. Department of Hepatology, Gastroenterology and infectious diseases, Faculty of Medicine, Benha University, Egypt.
2. Department of Microbiology and Immunology, Faculty of Medicine, Benha University, Egypt.
3. Department of Pathology, Faculty of Medicine, Benha University, Egypt.

Manuscript Info

Manuscript History:

Received: 15 February 2016
Final Accepted: 28 March 2016
Published Online: April 2016

Key words:
H.pylori, virulence factors, Gastroduodenal diseases.

Corresponding Author

Naglaa El-Toukhy.

Abstract

Background: Helicobacter pylori (H. pylori) is a gram-negative, micro-aerophilic, curved rod that causes a transmissible bacterial infection of the gastric mucosal surface and affect about one half of the world's population. It induces chronic gastritis in all infected individuals, but only induces clinical diseases in 10-20% of them.

This may be related to differences in genetic susceptibility of the host, environmental factors, and genetic diversity of H. pylori.

Aim: This study was conducted to identify the frequency of the genetic virulence factors (cagA, vacA and babA2) of H. pylori and their possible association with gastroduodenal diseases.

Methods: The study was conducted on 70 adult patients with upper gastrointestinal complaints. All patients were subjected to full history taking, clinical examination, gastroduodenoscopy. Four antral biopsies were taken for genotyping by PCR, histopathological examination and culture.

Results: All the patients (100%) had chronic active H. pylori gastritis by histopathological examination. The most frequent H. pylori genotype was cagA (67.8%) followed by vacA s1a (61%) and vacA m2 (61%), while the least frequent was babA2 (18.6%). CagA was associated with vacA s1a in (83.3%) with statistical significance. Most patients with cagA positive isolates (77.8%) had no heart burn with statistical significance which may support the protective role of cagA against GERD. There was no significant difference between genotypes distribution as regards culture positive and culture negative H. pylori strains. CagA, vacA s1a and vacA m2 had the highest prevalence in patients with PUD, gastritis and duodenitis while babA2 had the least prevalence. Although in patients with PUD and NUD the prevalence of cagA was (65.1%, 75%) and vacA s1 was (62.8%, 56.3%) respectively, the association between these H. pylori genotypes and PUD did not reach a level of statistical significance.

Conclusion: None of H. pylori genetic virulence factors individually can accurately predict clinical outcome and one has to recognize the importance of the bacteria-host interaction in the final outcome.

Introduction:-

Helicobacter pylori (H. pylori) is a transmissible bacterial infection of the gastric mucosal surface. The infection results in progressive mucosal damage with eventual impairment of gastric function **[1].**

H. pyloriis the most successful human pathogen infecting estimated 50% of the global population **[2].** It has been identified as a major cause of peptic ulcer disease and a risk factor for gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma.

Gastric mucus colonization with H. pylori induces chronic gastric inflammation in all infected individuals, but only induces clinical diseases in 10-20% of infected individuals. These include peptic ulcers, acute and atrophic gastritis, intestinal metaplasia, gastric adenocarcinoma and gastric B-cell lymphoma **[3].**

The reasons for this may be related to differences in genetic susceptibility of the host, environmental factors, and genetic diversity of H. pylori**[4].**

Studies show that different genetic virulence attributes of H. pylori are involved in different gastroduodenal disorders **[5]** and the characterization of these markers could aid medical prognosis, which could be extremely important in predicting clinical outcomes and prevention of H. pylori induced gastric injury **[3].**

The aim of this work was to identify the frequency of some genetic virulence factors of H. pyloriand their possible association with gastroduodenal diseases.

Patients and methods:-

PatientsThis study was conducted on 70 adult patients with upper gastrointestinal complaints attending the Department of Hepatology, Gastroenterolgy and Infectious Diseases in Benha University Hospital between January 2013 and May 2013. The committee of ethics of scientific research of Benha Faculty of Medicine approved the study protocol and written consents were obtained from the patients. Patients with dyspeptic complaints as nausea, vomiting, epigastria pain, heart burn, fullness, eructation, etc. aged from 19 to 73 years were selected for this study.

Patients who have received any of the following in the last month prior to endoscopy:

Anti-microbial therapy.
H2 receptor blockers.
Proton pump inhibitors.
Non steroidal anti-inflammatory drugs.
Corticosteroids.were excluded from the study

Methods:

All patients were subjected to

a-Full history taking: stressing on symptoms suggesting upper gastrointestinal disorders e.g. nausea, vomiting, epigastria pain, heart burn, eructation, fullness, hiccough, dyspepsia, early satiety, hematemesis or melena.

b-Clinical examination: Including general and abdominal examination.

Laboratory investigations: Venous blood samples were taken using sterile syringes (each about 5ml) under aseptic conditions. The collected samples were sent immediately to the laboratory of Benha University Hospital for further investigations the following:

c-Gastroduodenoscopy and biopsy:

This was done using disinfected upper gastrointestinal videoscope

Two biopsy specimens were preserved in a container using diluted formaline solution for histopathological examination.

d- Histopathological examination of H. pylori:

Routinely processed, Formalin-fixed, paraffin-embedded gastric antral tissues were used in this study and cut into three to four microns-thick serial sections then mounted on grease-free slides and subjected to:

H&E (Haematoxylin-Eosin) stain:

Examined for the presence of H. pylori (Gram negative spiral to comma-shaped organisms, sometimes cocci), degree of gastritis, presence of atrophy, complete intestinal metaplasia, dysplasia or lymphoid follicles according to Updated Sydney Classification **[6].**

Giemsa Stain: Examined for confirmation of H. pylori [**7].**

Microbiological examination:

Culture for H. pylori on selective media:

e-Identification of H. pylori:

Rapid Urease test: a portion of the grounded materials was inoculated into Christensen's urea agar and incubated microaerophilically for 8 hours at 37˚c. Change of the color from yellow to pink within 8 hours indicates positive urease test.
Bacterial colonies were identified as H. pylori on the basis of:
Growth characteristics: slowly growing organism, requiring excess humidity for better growth.
Colonial morphology: Circular, convex, translucent colonies about 2mm in diameter.
Microscopic examination: Gram negative spiral to comma-shaped organisms.
Oxidase test: This was performed using Oxidase identification sticks. The colony to be examined was touché by the impregnated end of the stick. A positive reaction was shown by the development of a blue purple color within 30 seconds to 3 minutes.
Catalase test: A drop of Hydrogen peroxide solution was placed on a slide and a small amount of bacteriological growth was placed in the solution. The formation of bubbles reflected a positive test.
f-Genotyping: of the following virulence factors of H. pylori in gastric biopsy specimens by Multiplex polymerase chain reaction using sequence specific primer (PCR-SSP):
Cytotoxin associated gene A (cagA)
Blood group antigen binding adhesin A2 (babA2).
Vacuolating toxin (vacA) alleles: s1a, s2, m1, m2.

Statistical Analysis:-
Statistical package (SPSS, version 20.0) was used for data management. Descriptive statistics was presented as mean± standard deviations for continuous variables, number and percentage for categorical variables (frequency distribution). Unpaired Student t-test (two sided) was used to test the significance of difference between the mean value of studied groups and chi-square test was used for comparison of categorical variables. The significance level was set at $p<0.05$.

Results:-

All the patients (100%) presented by different gastroduodenal complaints were positive for H. pylori infection by histopathological examination, but only 59 cases were subjected to genotyping by PCR due to inadequate tissue sampling.
The most frequent genotype was cagA (67.8%) followed by vacA s1a (61%) and vacA m2 (61%), while the least frequent was babA2 (18.6%) in H. pylori infected patients table (1), figure (1,2).

There is no significant difference among various H. pylori genotypes as regards age and gender distribution table(2).
Patients with nausea tend to be cagA positive, vacA m2 positive.Patients with vomiting tend to be vacA m2 positive.
Patients with epigastric pain tend to be cagA positive. However, all are of no statistical significance but patients with cagA positive were most likely to have no heart burn (77.8%) with statistical significance table (3).
Patients with dyspepsia tend to be cagA positive but of no statistical significance.
Patients with early satiety were most likely to be vacA m1 negative and vacA m2 negative with statistical significance.
Patients with epigastric tenderness were most likely to be babA2 negative and vacA s1a positive with statistical significance.
Patients with hematemesis tend to be vacA m2 positive and babA2 negative but of no statistical significance table(4).
There is no significant difference between genotypes distribution as regards culture positive and culture negative H. pylori strains. However, vacA s2 tends to be more common in culture positive strains followed by vacA m2 table (5).
CagA positive strains were more likely to be vacA s1a positive but babA2, vacA s2 and vacA m1 negative with statistical significance table (6).
In PUD patients, cagA positive isolates have the highest frequency (65.1%), followed by vacA s1a (62.8%) and vacA m2 (62.8%) while babA2 positive isolates have the least frequency (18.6%).
In patients with NUD, cagA positive isolates have the highest frequency (75%), followed by vacA s1a (56.3%) and vacA m2 (56.3%) while babA2 and vacA m1 positive isolates have the least frequency (18.8%) table(7).As regards the endoscopic findings
Most of patients with duodenitis (85.7%) tend to be cagA and vacA s1a positive with statistical significance.
Most of patients with gastritis tend to be cagA positive (71.4%) and babA2 negative (82.1%).

Most of patients with pangastritis tend to be cagA positive and vacA s1a positive (61.5%) but vacA s2 negative (69.2%).
Most of patients with antral gastritis tend to be cagA positive (80%) but babA2 negative and vacA m1 negative (86.7%) table(8).As regards histopathological finding All cases had chronic active gastritis figure(3).
Patients with babA2 positive and vacA s2 positive tends to have moderate to severe neutrophil activity. However, there is no statistical significance table (9).
Patients with glandular atrophy tend to be vacA s2 positive in (60%) while all of them were vacA m1 negative(100%)
Ninety seven (97.4%) of patients with cagA positive had no glandular atrophy with statistical significance.
Most of patients with intestinal metaplasia were cagA positive, vacA s1a positive and vacA m2 positive (66.7%) but all of them were babA2 negative and vacA m1 negative (100%).
Most of patients with lymphoid follicles hyperplasia were vacA m2 positive (87.5%).
All patients with parietal cells hyperplasia were cagA positive and vacA s1a positive (100%).
None of the patients had dysplasia table (10),figure(4,5).

Discussion:-

H. pylori is a gram-negative, micro-aerophilic, curved rod that is estimated to infect approximately 50% of the world's population **[8].** Surprisingly, only a fraction of infected individuals develop clinically identifiable symptoms in the course of their infections **[9].**
The well-established H. pylori associated syndromes include peptic ulcer disease (PUD), dyspepsia, non-ulcer dyspepsia (NUD), gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT)-type lymphoma **[10].** There is a possible association with extragastroduodenal diseases like unexplained iron-deficiency anaemia **[11]** and idiopathic thrombocytopenic purpura**[12].**
Specific host polymorphisms, environmental factors as well as pathogen specific virulence factors, appear to dictate the specific development of illness associated with H. pylori infections.
These H. pylori specific virulence factors are paramount for its survival and pathogenicity in the harsh environment of the human stomach [**13].**Themost important are cagA and vacA which present in almost all patients with peptic ulceration **[14]).**
This study was conducted to identify the frequency of the genetic virulence factors (cagA, vacA and babA2) of H. pylori and their possible association with gastroduodenal diseases. The study was conducted on 70 adult patients with upper gastrointestinal complaints as nausea, vomiting, epigastric pain, heart burn, early satiety. They were 35 males and 24 females. Their ages ranged from 19 to 73 years.
All the patients (100%) presented by different gastroduodenal complains were positive for H. pylori infection by histopathological examination. This high frequency was in agreement with (**Perez-Perez, et al. 2004)[15]** who reported that more than 80% of the population were H. pylori positive, even at young ages, in various developing countries and **(Hunt, et al. 2011)[16]** who reported that H. pylori prevalence in adults in Egypt was about 90%**.** Studies in other different areas in Egypt revealed that the frequency of H. pylori infection in adults ranged up to 88.72% **[17], [18]** and **[19].**
Also, the present study was in agreement with the study of **(Podzorski, et al. 2003)[20]** in which all the studied patients were positive for H. pylori and the study of **(Karaman, et al. 2011)[21]** in which all the studied patients were positive for H. pylori.
On the other hand, the frequency of H. pylori infection in the present study was higher than that (49.7%) reported in a study in Kuwait on 362 patients with uninvestigated dyspepsia **[22]**. This could be explained by the lower number of patients in the current study, the difference in socioeconomic status and their higher mean age as the prevalence of H. pylori infection increases with age **[23]**.
In European studies, the prevalenceof infection with H. pylori varied between 7 and 33%**[24], [25] and [26])** and in South American studies, it varied between 48 and 78% **[27]** while in Asian studies it varied between 37.5 and 92% **[28] , [29] ,[30]**and**[31].**
The highfrequency of H. pylori infection in the present study could be explained as most of the patients live in rural areas in and around Benha. Also, the poor socioeconomic status and overcrowded conditions in Egypt which is a developing country, contribute to increase the rate of transmission of infection as indicated by **(Awadallah, et al. 2010)[32]**.
In the present study regarding the frequency of H. pylori genotypes,the most frequent was cagA (67.8%). This percentage of cagA was consistent with an Egyptian study reported by **(Essa, et al. 2008)[33]** in which cagA was (62.2%) in Minofyia compared to (11%) in asymptomatic control.

This result goes in agreement with reports from Europe where cagA positive H. pylori isolates was about 51.8-82% **[34],[35]** and **[36]** and both cagA and vacA s1 were the most predominant H. pylori genotypes **[36]**.

Also, in East and South Asian countries cagA was the most predominant H. pylori genotype but it was presented in higher percentages than the present study **[37]** e.g. 96%in india**[38],** 81.7% in Kingdom Saudi Arabia **[39],** 89.3% in China **[40]** and 90% in South Africa **[41]).**

In the present study, although the frequency of cagA was consistent with percentages fromWest Asian countries e.g. 71.4% **[42],** 72.7% **[43],** 62.2% **[44]** and reports from Americas e.g. 66% **[20],** 67.1% **[45],** 73.2% **[46]**, it was not the most predominant H. pylori genotype in most of them but vacA alleles or babA2.

In general we can conclude that worldwide, the presence of the cagA gene varies from 50% in some Middle Eastern countries to 99% in East Asian countries and results of the present study follow this geographic distribution.

On the contrary, the cagA frequency in the present study was higher than that in other studies of Egypt reported by **Van Doorn, et al. 1999[47]** as it was 35.7%, where most of the isolates were from non-ulcer patients and **Gad and Hassan, 2012[18]** reported a presence of cagA in (46.13%) in Mansoura but the last study was done on asymptomatic apparently healthy adults, so we can conclude that the frequency of cagA in Egypt is related to the clinical presentation of H. pylori infection being lowest in asymptomatic and increasingly prevalent in symptomatic individuals **[33].** It was also higher than that reported in Jordan (26.4%) [**48].**

VacA is the second most extensively studied H. pylori virulence factor **[49].** In the present study, the second most frequent genotype after cagA (67.8%) was vacA s1a (61%) and vacA m2 (61%). It was observed that the frequency of cagA was near to vacA s1a and this may confirm the association between them reported in other studies **[50] and [51].**

The frequency of vacA s1a is consistent with results of other studies e.g. (79.8%) in Iran **[52],** 69%in India **[5],** (79.9%) in Iraq **[43]** and 70.1% in Turkey **[53].**

On the other hand, vacA s1a frequency in the present study was higher than that reported by **(Podzorski, et al. 2003)[20]** in USA (11%) and in China (6%) **[40]** where vacA m1 and vacA s1c were more predominant in these studies respectively.

The least frequent H. pylori genotype in the present study was babA2 (18.6%). This percentage was consistent with a report in Mexico (21.7%) [54]but lower than that reported in South and West Asian countries e.g. (31.4%) in India **[5],** (94.6%) in Iran **[44],** (44.8%)in Iraq **[43]** and **(**40.8%) in Turkey **[55].** It was also lower than babA2 reports from The Far East countries (92%) **[36],** America (57-75%) and Europe (66-73%) **[46].**

All these data confirm the geographic distribution of H. pylori genotypes**[47]** and variable results could be attributed to different samples size, H. pylori genetic polymorphism, evolutionary relationships in different ethnic groups or population migration **[36].**

As regards the association of the distribution of H. pylori genotypes, age and sex in this study, there was no significant statistical association among various H. pylori genotypeand age and sexand this was in agreement with most studies e.g. **[56], [48]** who reported that there was no statistical difference in the prevalence of the cagA gene or vacA alleles observed according to the gender or the age of the patients and in a study by **Mansour, et al. 2010[57],** there was no significant association between cagA gene and sex.

As regards the relationship between H. pylori virulence factors and clinical outcome, patients with nausea tend to be cagA positive, vacA m2 positive, patients with vomiting tend to be vacA m2 positive and patients with epigastric pain tend to be cagA positive. However, all were of no statistical significance but patients with cagA positive strains (77.8%) were most likely to have no heart burn with statistical significance. Heart burn is a typical symptom of GERD **[58]**.

Patients with dyspepsia tend to be cagA positive and patients with hematemesis tend to be vacA m2 positive and babA2 negative but of no statistical significance while patients with early satiety were most likely to be vacA m1 negative and vacA m2 negative and patients with epigastric tenderness were most likely to be babA2 negative and vacA s1a positive with statistical significance.

Unfortunately, all these items of research were not touched by other investigators yet except GERD. Several studies have provided evidence supporting the protecting role of cagA-positive H. pylori strains against GERD **[59,[60],[61], [62]and [63],** but these results were not confirmed by others **[64],[65].** On the contrary,there was a significant increase in cagA-positive H. pylori strains in GERD (70%) **[66].**

These contradictory results could be due to bias, inconsistent tests for the diagnosis of H. pylori infection (culture, histopathology, urease test or serology) and diagnosis of cagA (PCR or serology) or confounding factors.

Although the protective role linked to infection with a cagA- positive H. pylori strain may be explained by the lower gastric acid output due to the more intense gastric lesions induced by these strains, a direct effect of the more virulent strains may not be ruled out if there is a bacterial product that acts, for example, in the prevention of lower esophageal sphincter relaxation **[60].**

There was no significant difference between genotypes distribution as regards culture positive and culture negative H. pylori strains in consistency with other study by **Saxena, et al. 2011[5]**. However, in the present study vacA s2 tends to be more common in culture positive strains followed by vacA m2.

As regards the relationship between different H. pylori virulence factors, cagA positive strains were more likely to be vacA s1a positive but babA2, vacA s2 and vacA m1 negative with statistical significance. This result signifies that both genes (cagA and vacA s1a) work synergistically in causing PUD, gastritis and duodenitis as they had the highest frequency in these patients.

In agreement with the present study, most studiesreported that there was a highly significant association between cagA and vacA s1 presence **[43], [67], [51], [48]and [20].** Additionally, **(Erdoğdu, et al. 2014)[53]** reported that most of cagA positive isolates had vacA s1a whereas only 11.5% strains had vacA s2.

On the contrary, **Paniagua, et al. 2009[54]** reported that no statistically significant association was observed between vacA s1, cagA and babA2 virulence markers and **Abdullah, et al. 2013[43]** reported that babA2 positive strains were significantly more likely to be cagA positive and they did not find a significant association between cagA status and vacA s2, vacA m1, vacA m2 subtypes.

As regards the relationship between H. pylori virulence factors and endoscopic findings, cagA positive isolates had the highest frequency, followed by vacA s1a and vacA m2 while babA2 positive isolates had the least frequency in most patients with PUD, NUD, gastritis and duodenitis.

In the present study the frequencies of vacA s1a and vacA m2 were higher in patients with PUD (62.8%) than NUD (56.3%). However, the association between these H. pylori genotypes and PUD did not reach a level of statistical significance.

This goes in agreement with a study reported by **(Zambon, et al. 2003)[68]** in which cagA and vacA s1 were more frequent in patients with diffuse gastritis, peptic ulcer, or duodenitis.

In agreement with the present study higher frequencies of cagA and vacA s1a in PUD than NUD were reported in other studies as well without confirming the presence of statistical significance e.g. **[69],[70] and [5].**

Additionally, vacA s1 and / or cagA-positive genotypes were significantly associated with PUD in many parts of the world **[47],[59] and [71]).**

Studies from Spain **[72],** Pakistan **[73]** and Iraq **[43]** reported that there was a significant association between cagA positive status and PUD. In Taiwan vacA s1a was also significantly more predominant in PUD patients **[74].**

Also in the present study, the frequency of cagA positive isolates was higher in NUD (75%) than in PUD (65.1%) and this was in agreement with **Sedaghat, et al.2014[44]** who reported that the frequency of cagA was (82.6%) in NUD and (17.4%) in PUD.

In disagreement with the present study, a study of four different countries (Korea, Japan, USA and Colombia) reported that there was no association between cagA or vacA genotype and disease outcome **[75]**. Other studies reported that cagA was higher in PUD than in NUD **[47]** as the cagA gene is a marker for the cag pathogenicity island, which is associated with very severe gastritis, and an increased risk of peptic ulcer disease **[3].**

Also, in disagreement with the present study, **Erdoğdu et al.2014[53]** reported that there was no association between cagA or vacA genotypes and endoscopic findings. In other studies babA2 was significantly associated with PUD **[68],[5] and [43].**

The explanation of these findings in the present study may be due to small sample size, mixed H. pylori infection (presence of more than one genotype in the same patient), the fact that we couldn't confirm the association between genotype and H. pylori virulence genes.

The cause of the variability in these studies is unknown, but may be related to differences in methodology, study populations, bacterial strains **[76]** or may be due to the different state of cagA expression as patients who are cagA positive by PCR may have a relatively low expression level of the virulence gene in situ and this extend the pathogenesis of PUD by demonstrating the importance of cagA expression at the cellular level in addition to traditional isolation **[77].**

All these reports may indicate that cagA cannot be considered as the sole virulence marker for determination of the disease outcome.
It is possible that other some genes of H. pylori and the cag pathogenicity island (PAI) are responsible for pathogenicity and disease outcome **[5].**

As regards the relationship between H. pylori genetic virulence factors and histopathological findings, patients with babA2 positive and vacA s2 positive tends to have moderate to severe neutrophil activity. However, there was no statistical significance.

Patients with glandular atrophy tend to be vacA s2 positive in (60%) while all of them were vacA m1 negative (100%). Ninety seven (97.4%) of patients with cagA positive had no glandular atrophy with statistical significance.

Most of patients with intestinal metaplasia were cagA positive, vacA s1a positive and vacA m2 positive (66.7%) but all of them were babA2 negative and vacA m1 negative (100%). Most of patients with lymphoid follicles were vacA m2 positive (87.5%). None of the patients in the present study had dysplasia.

In agreement of the present study, cagA-positive stains were reported to be involved in the development of intestinal metaplasia **[78]** and precancerous lesions **[79].** Also, **Zambon, et al. 2003[68]** reported that both cagA and vacA s1 positive stains was associated with intestinal metaplasia.

In contrary to the present study, cagA-positive stains were reported to be involved in the development of gastric atrophy **[78].** Also, **Gold, et al. 2001[80]** reported that there was no association between H. pylori strain genotype and histopathologic abnormalities.

Also in contrary to the present study, other studies reported that the presence of cagA had a significant association with increased neutrophil activity but not vacA s1 **[81].** Also, there was a significant association between cagA positivity and neutrophil activity and glandular atrophy, but not with chronic inflammation, and intestinal metaplasia**.** There was no significant relationships observed between vacA s1, vacA s2, vacA m1 and vacA m2 genotypes and histopathological parameters except neutrophil activity which was more severe in the vacA m1 than in the vacA m2 positive strains **[82]**

These variable results may be due to inconsistency in the number, size and site of gastric biopsy specimen as there were many pathologic finding for assessment.

All the patients included in the present study had chronic active gastritis and this observation was in agreement with **[83]** who reported that infection with H. pylori always causes chronic active gastritis.

This discussion signifies that none of the genetic virulence factors individually can accurately predict clinical outcome and that one has to recognize the importance of the bacteria-host interaction in the final outcome **[36].**

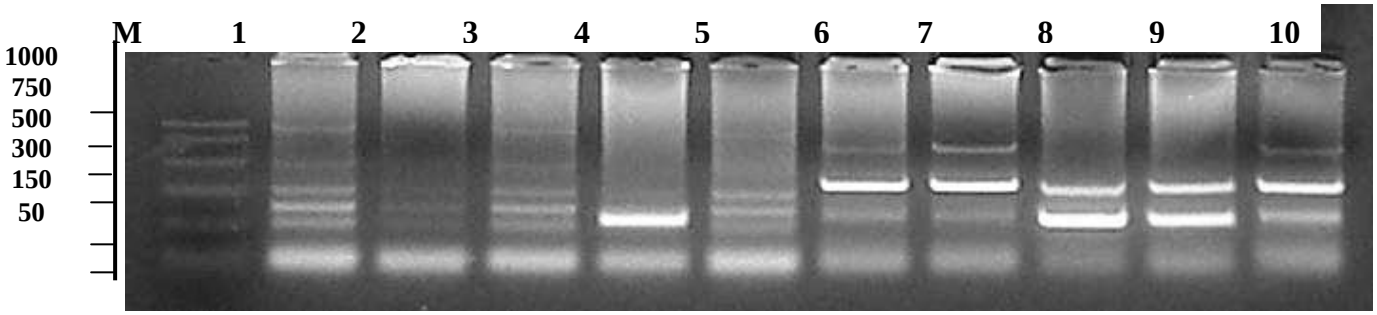

Figure (1): Gel electrophoresis of the amplified products of *H. pylori* virulence genes *(cagA, babA2* and *vacA* alleles: s1a, s2, m1, m2) obtained from biopsies of 10 patients. Lane M (mobility marker) shows DNA ladder 1000bp. Lanes 6,7,8,9,10 show *cagA* positive strains, lanes 4,8,9,10 show *vacA* s1a positive strains, lanes 6,7,10 show *vacA* m2 positive strains and lanes 7,10 show *babA2* positive strains.

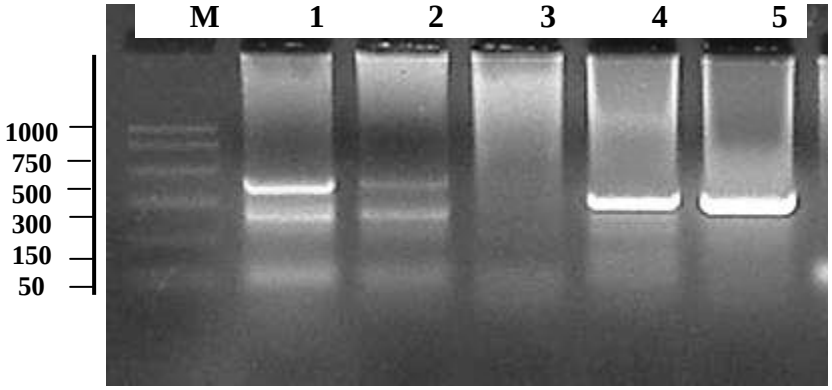

Figure (2): Gel electrophoresis of the amplified products of *H. pylori* virulence genes (cagA, babA2 and vacA alleles: s1a, s2, m1, m2) obtained from biopsies of 5 patients. Lane M (mobility marker) shows DNA ladder 1000bp. Lanes 4,5 show *cagA* positive strains, lanes 1,2 show *vacA* m1 and *vacA* m2 positive strains.

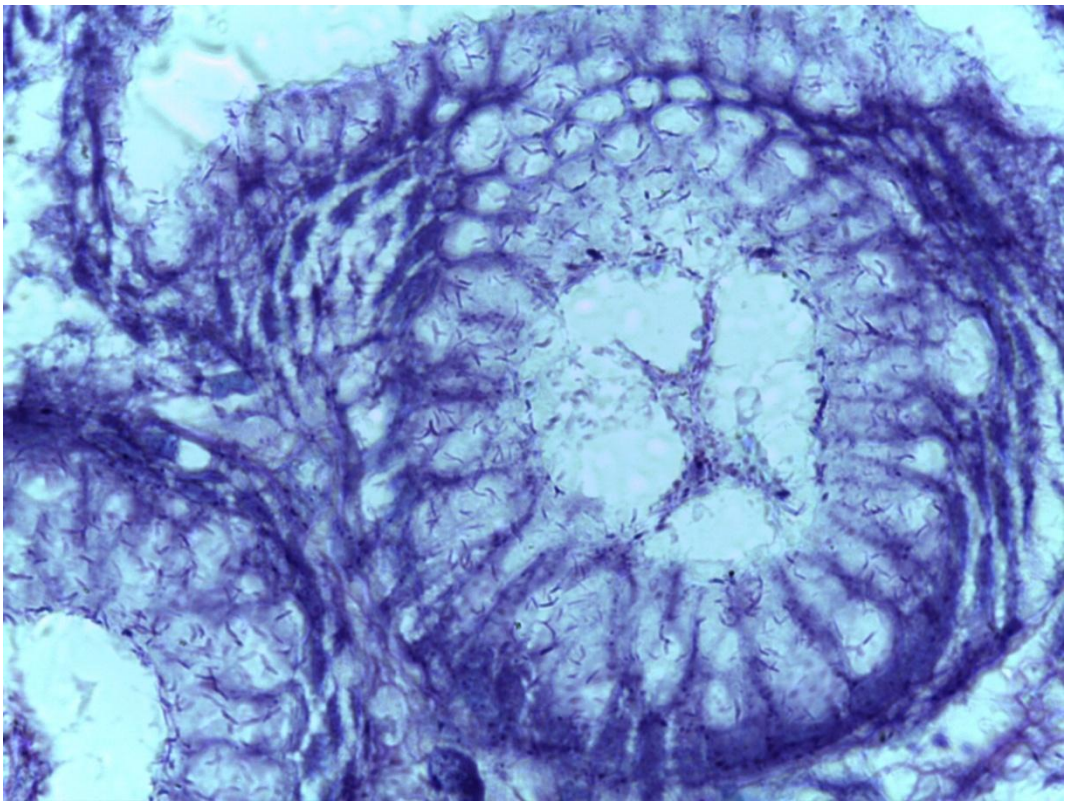

Figure (3): Gastric biopsy specimen showing *H. pylori* organisms adhering to gastric mucosa stained by Giemsa (1000x).

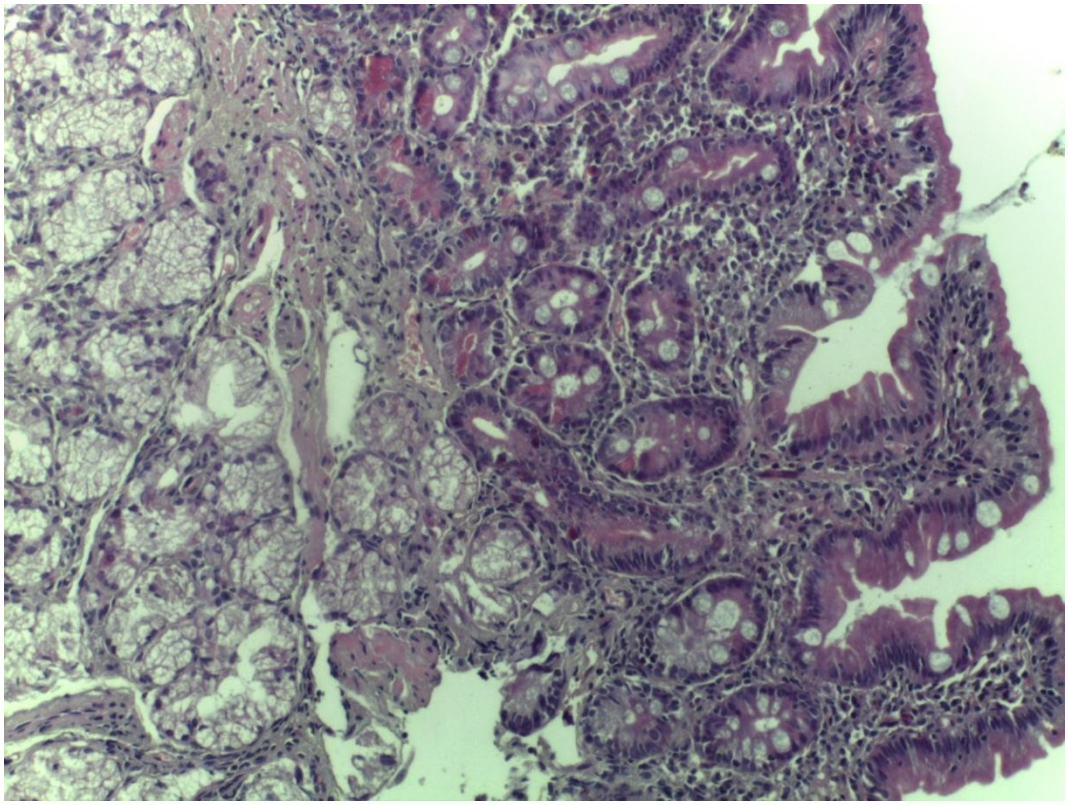

Figure (4): Gastric biopsy specimen showing intestinal metaplasia (Goblet cells) stained by H&E (400x).

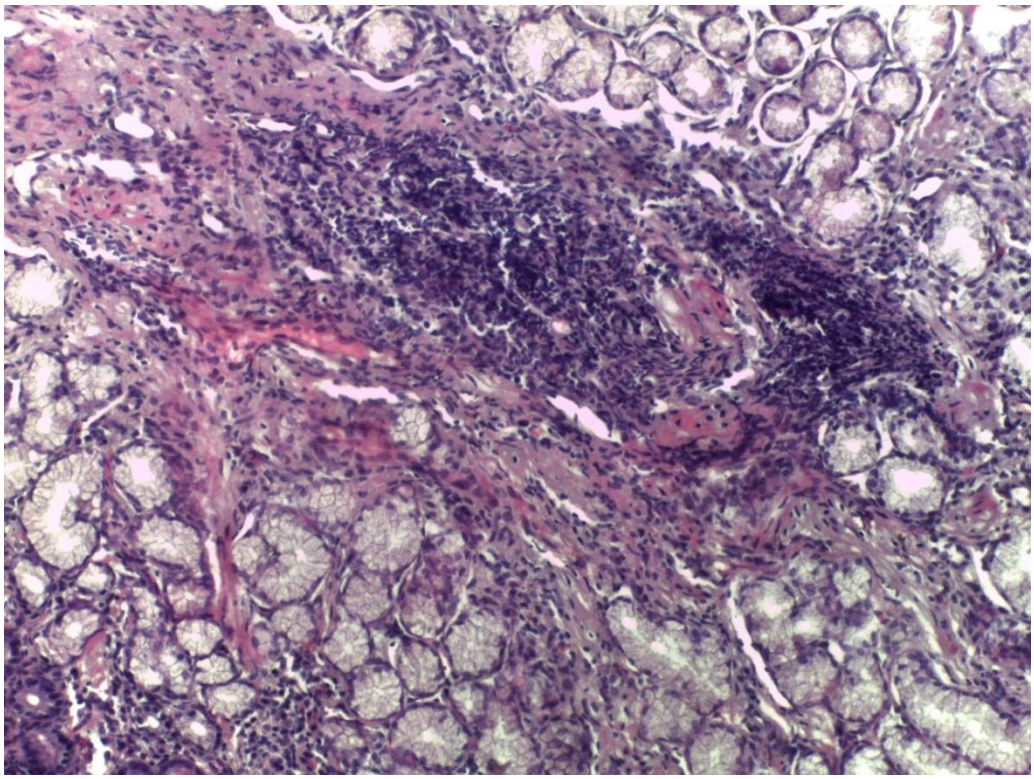

Figure (5): Gastric biopsy specimen showing lymphoid follicular hyperplasia and fusion of muscularis mucosa and muscularis externa suggesting ulceration stained by H&E (400x).

Table (1): Frequency of *H. pylori* genotypes in all cases.

GENOTYPE	TOTAL NUMBER OF CASES =59	
	Positive No. (%)	**Negative No. (%)**
cagA	40(67.8)	19(32.2)
babA2	11(18.6)	48(81.4)
***vacA* s1a**	36 (61)	23 (39)
***vacA* s2**	16(27.1)	43(72.9)
***vacA* m1**	15(25.4)	44(74.6)
***vacA* m2**	36 (61)	23 (39)

Table (2): Age and gender distribution among various *H. pylori* genotypes.

Genotype	CagA		BabA2		VacA s1a		VacA s2		VacA m1		VacA m2	
	+ve No.=40	-ve No.=19	+ve No.=11	-ve No.=48	+ve No.=36	-ve No.=23	+ve No.=16	-ve No.=43	+ve No.=15	-ve No.=44	+ve No.=36	-ve No.=23
Nausea No. (%)	10(52.6)	9(47.4)	3(15.8)	16(48.2)	9(37.4)	10(52.6)	5(26.3)	14(73.7)	4(21.1)	15(78.9)	10(52.6)	9(47.4)
P-value	0.079		0.49		0.116		0.59		0.42		0.265	
Vomiting No. (%)	12(54.5)	10(45.5)	3(13.6)	19(68.4)	13(59.1)	9 (40.9)	5 (22.7)	17(77.3)	4(18.2)	18(81.8)	16 (72.7)	6 (27.3)
P-value	0.08		0.34		0.51		0.394		0.25		0.125	
Epigastric pain No. (%)	36(67.9)	17(32.1)	8(15.1)	45(84.9)	34 (64.2)	19(35.8)	13(24.5)	40(75.5)	13(24.5)	40(75.5)	33(62.3)	20(37.7)
P-value	0.637		0.07		0.153		0.194		0.48		0.434	
Heart burn No. (%)	12(52.2)	11(47.8)	6 (26.1)	17(73.9)	12(52.2)	11(47.8)	9 (39.1)	14(60.9)	5 (21.7)	18(78.3)	14 (60.9)	9 (39)
P-value	0.039*		0.2		0.2		0.08		0.42		0.59	

Table (3): Comparison between different *H. pylori* genotypes regarding gastrointestinal tract symptoms.

Genotypes		CagA		BabA2		VacA s1a		VacA s2		VacA m1		VacA m2	
		+ve No.=40	-ve No.=19	+ve No.=11	-ve No.=48	+ve No.=36	-ve No.=23	+ve No.=16	-ve No.=43	+ve No.=15	-ve No.=44	+ve No.=36	-ve No.=23
Age: Mean ±SD		49.9 ±15	45.3 ±14.9	46.4 ±17.2	48.9 ±14.8	49.2 ±14.1	47.2 ±16.9	48.3 ±14.6	48.5 ±15.5	51 ±15.4	47.5 ±15.1	49.1 ±15.5	49.4 ±14.7
P- value		0.27		0.63		0.62		0.96		0.445		0.68	
Gender No.(%)	**Male**	23(57.5)	12(63.2)	4 (36.4)	31(64.6)	20(55.6)	15(65.2)	9(56.3)	26(60.5)	8 (53.3)	27(61.4)	21 (58.3	14 (60.9)
	Female	17(42.5)	7 (36.8)	7 (63.6)	17(35.4)	16(44.4)	8 (34.8)	7 (43.8)	17 (39.5	7 (46.7)	17 (38.6	15(41.7)	9 (39.1)
P-value		0.45		0.085		0.32		0.49		0.4		0.53	

* significant.

Table (4): Comparison between different *H. pylori* genotypes regarding gastrointestinal tract symptoms (continued).

Genotype	CagA		BabA2		VacA s1a		VacA s2		VacA m1		VacA m2	
	+ve No.=40	-ve No.=19	+ve No.=11	-ve No.=48	+ve No.=36	-ve No.=23	+ve No.=16	-ve No.=43	+ve No.=15	-ve No.=44	+ve No.=36	-ve No.=23
Dyspepsia No. (%)	16(66.7)	8(33.3)	5(20.8)	19(79.2)	14(58.3)	10(41.7)	9(37.5)	15(62.5)	7(29.2)	17(70.8)	14(58.3)	10(41.7)
P-value	0.54		0.48		0.46		0.118		0.4		0.46	
Early satiety No. (%)	16(61.5)	10(38.5)	7(26.9)	19(73.1)	13(50)	1(50)	9(34.6)	17(65.4)	10(38.5)	16(61.5)	11(42.3)	15(57.7)
P-value	0.26		0.133		0.102		0.196		0.04*		0.009*	
Epigastric tenderness No. (%)	37(68.5)	17(31.5)	8(14.8)	46(85.2)	36(66.7)	18(31.3)	13(24.1)	41(75.9)	13(24.1)	41(75.9)	33(61.1)	21(38.9)
P-value	0.52		0.04*		0.007*		0.11		0.376		0.654	
Bleeding No. (%)	6(60)	4(40)	0 (0)	10(100)	7(70)	3(30)	1(10)	9(90)	3(30)	7(70)	8(80)	2(20)
P-value	0.4		0.104		0.39		0.17		0.49		0.16	

* significant.

Table (5): Comparison between different *H. pylori* genotypes regarding the status of culture.

	Culture positive No.=49	Culture negative No.=10	p- value
***cagA*No. (%)**	31(77.5)	9 (22.5)	0.09
***babA2*No. (%)**	9 (81.8)	2 (18.2)	0.6
***vacA* s1aNo. (%)**	29 (80.6)	7 (19.4)	0.39
***vacA* s2No. (%)**	14 (87.5)	2 (12.5)	0.45
***vacA* m1No. (%)**	12 (80)	3 (20)	0.49
***vacA* m2No. (%)**	31(86.1)	5 (13.9)	0.33

Table (6):The relationship between the status of *cagA* and different *H. pylori* genetic virulence factors.

	***Bab*A2**		***VacA* s1a**		***VacA* s2**		***VacA* m1**		***VacA* m2**	
	+ve No.=11	**-ve No.=48**	**+ve No.=36**	**-ve No.=23**	**+ve No.=16**	**- veNo.=43**	**+ve No.=15**	**-ve No.=44**	**+ve No.=36**	**-ve No.=23**
CagA +veNo.%	3 (27.3)	37 (77.1)	30 (83.3)	10(43.5)	4(25)	36(83.7)	7 (46.7)	33 (75)	27 (75)	13 (56.5)
CagA – veNo.%	8 (72.7)	11(22.9)	6 (16.7)	13(56.5)	12 (75)	7 (16.3)	8 (53.3)	11 (25)	9(25)	10 (43.5)
p- value	0.003*		0.002*		0.000*		0.046*		0.116	

* significant.

Table (7):Comparison between PUD and NUD patients regarding the frequency of different *H. pylori* genetic virulence factors.

	CagA		**BabA2**		**VacA s1a**		**VacA s2**		**VacA m1**		**VacA m2**	
	+ve No.=40	**-ve No.=19**	**+ve No.=11**	**-ve No.=48**	**+ve No.=36**	**-ve No.=23**	**+ve No.=16**	**-ve No.=43**	**+ve No.=15**	**-ve No.=44**	**+ve No.=36**	**-ve No.=23**
PUDNo.%	28(65.1)	15(34.9)	8(18.6)	35(81.4)	27(62.8)	16(37.2)	12(27.9)	31(72.1)	12(27.9)	31(72.1)	27(62.8)	16(37.2)
NUDNo.%	12 (75)	4 (25)	3 (18.8)	13(81.2)	9 (56.3)	7 (43.7)	4 (25)	12 (75)	3 (18.8)	13(81.2)	9(56.3)	7(43.7)
p-value	0.347		0.628		0.43		0.55		0.36		0.434	

Table (8):The relationship between different *H. pylori* genotypes and endoscopic findings.

* significant.

Genotype	**CagA**		**BabA2**		**VacA s1a**		**VacA s2**		**VacA m1**		**VacA m2**	
	+ve No.=40	**-ve No.=19**	**+ve No.=11**	**-ve No.=48**	**+ve No.=36**	**-ve No.=23**	**+ve No.=16**	**-ve No.=43**	**+ve No.=15**	**-ve No.=44**	**+ve No.=36**	**-ve No.=23**
Duodenitis No. (%)	12(85.7)	2(14.3)	1 (7.1)	13(92.9)	12(85.7)	2(14.3)	3(21.4)	11(78.6)	3(21.4)	11(78.6)	8(57.1)	6(42.9)
P-value	0.09		0.196		0.028*		0.43		0.496		0.484	
Gastritis No. (%)	20(71.4)	8(28.6)	5(17..9)	23(82.1)	18 (64.3)	10(36.7)	7(25)	21 (75)	7 (25)	21 (75)	17 (60.7)	11(39.3)
P-value	0.387		0.57		0.413		0.479		0.59		0.587	
Pangstritis No. (%)	8 (61.5)	5(38.5)	3 (23.1)	10(76.9)	8 (61.5)	5 (38.5)	4(30.8)	9 (69.2)	5 (38.5)	8 (61.5)	6 (46.2)	7 (53.8)
Antral gastritis No. (%)	12 (80)	3 (20)	2(13.3)	13(86.7)	10(66.7)	5(33.3)	3(20)	12(80)	2(13.3)	13(86.7)	11(73.3)	4 (26.7)
P-value	0.475		0.79		0.85		0.759		0.3		0.336	

Table (9):Comparison between different *H. pylori* genotypes regarding histopathological findings.

Genotype	CagA		BabA2		VacA s1a		VacA s2		VacA m1		VacA m2	
	+ve No.=40	-ve No.=19	+ve No.=11	-ve No.=48	+ve No.=36	-ve No.=23	+ve No.=16	-ve No.=43	+ve No.=15	-ve No.=44	+ve No.=36	-ve No.=23
Mild neutrophil activity	6(15)	0 (0)	0 (0)	6(12.5)	5(13.9)	1(4.3)	0 (0)	6 (72.1)	1 (6.7)	5 (11.4)	4 (11.1)	2 (87)
Moderate & severe neutrophil activity	34(85)	19(100)	11(100)	42(87.5)	31(86.1)	22(95.7)	16(100)	37 (86)	14(93.3)	39(88.6)	32(88.9)	21(91.3)
p- value	0.085		0.27		0.236		0.135		0.36		0.56	
Chronic inflammation	40(100)	19(100)	11(100)	48 (100)	36 (100)	23 (100)	16 (100)	43(100)	15 (100)	44 (100)	36 (100)	23 (100)

Table (10): Comparison between *H. pylori* genotypes regarding histopathological findings (continued).

Genotype	CagA		BabA2		VacA s1a		VacA s2		VacA m1		VacA m2	
	+ve No.=40	-ve No.=19	+ve No.=11	-ve No.=48	+ve No.=36	-ve No.=23	+ve No.=16	-ve N o.=43	+ve No.=15	-ve No.=44	+ve No.=36	-ve No.=23
Glandular atrophy No. (%)	1(20)	4(80)	1(20)	4(80)	1(20)	4(80)	3(60)	2(20)	0 (0)	5(100)	1(20)	4(50)
P-value	0.036*		0.66		0.075		0.123		0.237		0.063	
Intestinal metaplasia No.(%)	2(66.7)	1(33.3)	0(0)	3(100)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	0(0)	3(100)	2(66.7)	1(33.3)
P-value	0.696		0.5		0.665		0.62		0.4		0.665	
Lymphoid follicles hyperplasia No. (%)	4(50)	4(50)	3(37.5)	5(62.5)	4(50)	4(50)	3(37.5)	5(62.9)	2(25)	6(75)	7(87.5)	1(12.5)
P-value	0.222		0.16		0.376		0.372		0.673		0.1	
Parietal cells hyperplasia No.(%)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	0(0)	0(0)	2(100)	1(50)	1 (50)	1 (50)	1 (50)
P-value	0.456		0.659		0.369		0.528		0.447		0.632	

* significant

Refrences:-

1.Nurgalieva, Z. Z.; Goodman, K. J. And Graham, D. Y. (2004): Helicobacter pylori. In Encyclopedia of Gastroenterology. Johnson, L.R. (ed). Academic Press/Elsevier (USA) p272-278.

2.Malfertheiner, P.; Megraud, F.; O'Morain, C.; Atherton, J.; Axon, A.T.; Bazzoli, F.; Gensini, G.F.; Gisbert, J. P. ; Graham, D. Y. ; Rokkas, T; El-Omar, E.M. and Kuipers, E.J. (2012): Management of Helicobacter pylori infection--The Maastricht IV/ Florence Consensus Report. Gut; 61: 646-664.

3.Proença-Modena, J.L.; Acrani, G.O. and Brocchi, M. (2009): Helicobacter pylori: phenotypes, genotypes and virulence genes. Future Microbiol. ; 4(2):223-40.

4.Azuma, T. ; Ito, S. ; Sato, F. ; Yamazaki, Y. ; Miyaji, H. ; Ito, Y.; Suto, H.; Kuriyama, M.; Kato, T.; Kohli, Y. (1998):
The role if the HLA-DQA 1 gene in resistance to atrophic gastritis and gastric adenocarcinoma induced by Helicobacter pylori infection. Cancer, 82: 1013-8.

5.Saxena, A. ; Shukla, S. ; Prasad, K.N. ; Ghoshal, U.C. (2011): Virulence attributes of Helicobacter pylori isolates and their association with gastroduodenal disease. Indian J. Med. Res.; 133:514-520.

6.Dixon, M.F.; Genta, R.M.; Yardley, H. And Correa, P. (1996): Classification and grading of gastritis: the updated Sydney system. Am. J. Surg. Pathol.;20: 1161–1181.

7.Vijaya, D.; Chandrashekar, N.; Nagarantnamma, T. And Shivarudrappa, A.S. (2012):
Simple Stain for Helicobacter Pylori. Journal of Clinical and Diagnostic Research. (Suppl-2);6(4): 664-666.

8.Go, M.F. (2002):
Natural history and epidemiology of Helicobacter pylori infection. Aliment. Pharmacol. Ther. 16 (Suppl. 1), 3-15.

9.Peek, Jr. And Blaser, M.J. (2002):
Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat. Rev. Cancer, 2: 2.8-37.

10.Chey, W.D. and Wong, B.C. (2007):
American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am. J. Gastroenterol. 102:1808-1825.

11.Qu, X.H.; Huang, X.L.; Xiong, P.; Zhu, C.Y.; Huang, Y.L.; Lu, L.G.; Sun, X.; Rong, L.; Zhong, L.; Sun, D.Y.; Lin, H.; Cai, M.C.; Chen, Z.W.; Hu, B.; Wu, L.M.; Jiang, Y.B. and Yan, W.L. (2010):
Does Helicobacter pylori infection play a role in iron deficiency anemia? A meta-analysis. World J Gastroenterol;16:886-96.

12.Pellicano, R.; Franceschi, F.; Saracco, G.; Fagoonee, S.; Roccarina, D. And Gasbarrini, A. (2009):
Helicobacters and extragastric diseases. Helicobacter;14 (Suppl 1):58-68.

13.Couturier, M. R. (2013):
The Evolving Challenges of Helicobacter pylori Disease, Diagnostics,and Treatment, Part I. Clinical Microbiology Newsletter; 35(3**):**19-24.

14.Malfertheiner, P. (2005):
H. Pylori: its diseases and management. In Clinical Gastroenterology and Hepatology. Weinstein, M.W.; Hawkey, C.J.; Bosch, J.(eds), 1st edition. Elsevier, Mosby. Philadelphia, USA. Vol.(1), 34: p193-206.

15.Perez-Perez, G.I.; Rothenbacher, D. And Brenner, H. (2004): Epidemiology of Helicobacter pylori infection. Helicobacter; 9(1):1–6.

16.Hunt, R.H.; Xiao, S.D. ; Megraud, F. ; Leon-Barua, R. ; Bazzoli, F.; van der Merwe, S.; Vaz Coelho, L.G. ; Fock, M.; Fedail, S. ; Cohen, H.; Malfertheiner, P.; Vakil, N.; Hamid, S.; Goh, K.L.; Wong, B.C.Y.; Krabshuis, J. And Le Mair, A. (2011):
Helicobacter Pylori in Developing Countries: World Gastroenterology Organisation Global Guideline. J. Gastrointestin. Liver. Dis.; 20(3): 299-304.

17.Enany, S.M.; Abdalla, S.; and Ali, K. (2005):
The prevalence of Helicobacter pylori and resistance patterns in dyspeptic patients from Ismailia, Egypt. Suez Canal University Medical Journal (SCUMJ); 8(1): 87-92.

18.Gad, Y.Z.; Hassan, A.M. (2012):
caga Helicobacter seropositivity in asymptomatic apparently healthy young adult Egyptian food handlers. Euroasian Journal of hepato-gastroenterology; 2(1):20-23.

19.Sayed, A.S.M.; Abd Al Azeem, M.W.; Noaman, H.A. and Hassan, M.A. (2007): Seroepidemiological study on H. Pylori infection in children and adults in Assiut governorate, Upper Egypt. JASMR, 2:129-133.

20.Podzorski, R. P.; Podzorski, D. S.; Wuerthd, A. And Toliad, V. (2003): Analysis of the vaca, caga, cage, icea, and baba2 genes in Helicobacter pylori from sixty-one pediatric patients from the Midwestern United States.
Diagnostic Microbiology and Infectious Disease 46; 83–88.

21.Karaman, M.; Abacıoğlu, H.; Topalak, O.S. and Simşek, I. (2011): Molecular detection of Helicobacter pylori vaca and caga genes in gastric tissue specimens of patients with peptic ulcer disease and non-ulcer dyspepsia. Mikrobiyol. Bul.;45(1):11-20.

22.Al-Azmi, W.M.; Siddique, I.; Alateeqi, N. And Al-Nakib, B. (2010): Prevalence of Helicobacter pylori infection among new outpatients with dyspepsia in Kuwait.
BMC Gastroenterology, 10(14):1-4.

23.Malaty, H.M. (2010):
Epidemiology of Helicobacter pylori infection. In Advances in Cellular and Molecular Microbiology 17 : Helicobacter in the 21st century. Sutton,P. And Mitchell, H.M.(eds), CAB international, UK. P1-12.

24.Breckan, R.K.; Paulssen, E.J.; Asfeldt, A.M.; Mortensen, L.; Straume, B. And Florholmen, J. (2009):
The impact of body mass index and Helicobacter pylori infection on gastro-oesophageal reflux symptoms: a population-based study in Northern Norway.
Scand. J. Gastroenterol.; 44: 1060-6.

25.Sykora , J.; Siala, K.;Varvarovska,J.; Pazdiora, P.; Pomahacova, R. And Huml, M. (2009):
Epidemiology of Helicobacter pylori infection in asymptomatic children: A prospective population-based study from the Czech Republic. Application of a monoclonal-based antigen-instool enzymeimmunoassay.
Helicobacter; 14: 286-97.

26.Bureš, J.; Kopáčová, M.; Koupil, I.; Seifert, B.; Fendrichová, M.S.; Špirková, J.; Voříšek, V.; Rejchrt, S.; Douda, T.; Král, N. And Tachecí, I. (2012):
Significant decrease in prevalence of Helicobacter pylori in the Czech Republic.
World J. Gastroenterol.;18(32): 4412-4418.

27.Santos, I.S.; Boccio, J.; Davidsson, L.; Hernandez-Triana, M.; Huanca- Sardinas, E.; Janjetic, M.; Moya-Camarena, S.Y.; Paez-Valery, M.C.; Ruiz-Alvarez, V.; Valencia, M.E.; Valle, N.C.; Vargas-Pinto, G.; Solano, L. And Thomas, J. (2009):
Helicobacter pylori is not associated with anaemia in Latin America: results from Argentina, Brazil, Bolivia, Cuba, Mexico and Venezuela.
Public Health Nutr; 12: 1862-70.

28.Jafri, W.; Yakoob, J.; Abid, S.; Siddiqui, S.; Awan, S. And Nizami, S.Q. (2010):
Helicobacter pylori infection in children: population-based age specific prevalence and risk factors in a developing country.
Acta. Paediatr.; 99: 279-82.

29.Javed, M.; Amin, K.; Muhammad, D.; Husain, A. And Mahmood, N. (2010):
Prevalence of H. Pylori.
Professional Med. J.; 17: 431-9.

30.Mishra, S.; Singh, V.; Rao, G.R.; Dixit, V.K.; Gulati, A.K.; and Nath, G. (2008):
Prevalence of Helicobacter pylori in asymptomatic subjects.
A nested PCR based study. Infect. Genet. Evol.; 8: 815-9.

31.Zhang, D.H.; Zhou, L.Y.; Lin, S.R.; Ding, S.G.; Huang, Y.H.; Gu, F.; Zhang, L.; Li, Y.; Cui, R.L.; Meng, L.M.; Yan, X.E. and Zhang J. (2009):
Recent changes in the prevalence of Helicobacter pylori infection among children and adults in high or low-incidence regions of gastric cancer in China.
Chin. Med. J. (Engl); 122: 1759-63.

32.Awadallah, H.I.; Ragab, M.H.; Hanna, L.N. (2010):
Environmental risk factors affecting transmission of Helicobacter pylori in Egypt.
J. Public health; 18: 237-244.

33.Essa , A.S.; Nouh, M. A.; Ghaniam, N.M.; Graham, D.Y. and Sabry, H. S. (2008):
Prevalence of caga in relation to clinical presentation of H. Pylori infection in Egypt. Scandinavian Journal of Infectious Diseases; 40 (9): 730-733.

34.Chiarini, A.; Cala, C.; Bonura, C.; Gullo, A.; Giuliana, G.; Peralta, S.; D'Apra, F. And Giammanco, A. (2009):
Prevalence of virulence associated genotypes of Helicobacter pylori and correlation with severity of gastric pathology in patients from Western Sicili, Italy.
Euro. J. Clin. Microbiol. Infect. Dis.;28(5),437-46.

35.Kolaylı, F.; Karadenizli, A.; Bingöl, R.; Schneider, T. And Kist, M. (2012): Differences of vaca alleles and caga gene positivity of Helicobacter pylori strains isolated from two different countries: Turkey and Germany. Mikrobiyol. Bul.;46(2):332-4.

36.Holton, J. (2013):
Peptic ulcer disease. In Genomic and Personalized Medicine. Ginsberg, G. S. And Willard H.F. (eds), 2nd edition. Academic press, Elsevier, UK, USA, Vol.2 (77): p 914-934.

37.Sahara, S.; Sugimoto, M.; Vilaichone, R.; Varocha Mahachai, V.; Miyajima, H.; Furuta, T. And Yamaoka, Y. (2012):
Role of Helicobacter pylori caga EPIYA motif and vaca genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infectious Diseases, 12:223.
38.Arachchi, H.S.; Kalra.,V.; Lal B.; Bhatia, V. And Baba, C.S. (2007):
Prevalence of duodenal ulcer-promoting gene (dupa) of Helicobacter pylori in patients with duodenal ulcer in North Indian population.Helicobacter; 12(6): 591–597.
39.Kadi, R. H.; Halawani, E. M. And Abdelkader, H. S. (2014):
Prevalence of H. Pylori strains harbouring caga and icea virulence genes in Saudi patients with gastritis and peptic ulcer disease. Microbiol. Discov.;2(2)1-6.
Retrieved from: http://www.hoajonline.com/journals/pdf/2052-6180-2-2.pdf
40.Wei, G.C; Chen, J.; Liu, A.Y.; Zhang, M.; Liu, Z. J.; Liu, D.; Xu, J.; Liu, B. R.; Ling, H.; Wu, H. X. And Du, Y.J. (2012):
Prevalence of Helicobacter pylori vaca, caga and icea genotypes and correlation with clinical outcome. Experimental and therapeutic medicine, 4: 1039-1044.
41.Tanih, N.F.; mcmillan, M.; Naidoo, N.; Ndip, L.M.; Weaver, L.T. and Ndip, R.N. (2010):
Prevalence of Helicobacter pylori vaca, caga, icea genotypes in South African patients with upper gastrointestinal diseases. Acta Tropica; 116: 86-73.
42.Ozbey, G. And Aygun, C. (2012):
Prevalence of genotypes in Helicobacter pylori isolates from patients in eastern Turkey and the association of these genotypes with clinical outcome.
Brazilian Journal of Microbiology,43(4):1332-9.
43.Abdullah, M.S.; Hussein, N.R.; Salih, A. M.; Merza, M.A.; Goreal, A.A.; Odeesh, O.Y.; Majed, H.S.; Assafi, M.A. and Hawrami, K. (2013):
Infection with H. Pylori strains carrying baba2 and caga is associated with an increased risk of peptic ulcer disease development in Iraq. Arab Journal of Gastroenterology.p1-4. Retrieved from: (http://dx.doi.org/10.1016/j.ajg.2012.12.001)
44.Sedaghat, H.; Moniri, R.; Jamali, R.; Arj, A.; Zadeh, M.R.; Moosavi, S.; Rezaei, M. And Pourbabaee, M. (2014):
Prevalence of Helicobacter pylori vaca, caga, cage, icea, baba2, and oipa genotypes in patients with upper gastrointestinal diseases.
Iran. J. Microbiol. Vol. 6, No.1, 14-21.
45.Ribeiro, M.L.; Godoy, A.P.; Benvengo, Y.H.; Mendonca, S. And Pedrazzoli, J. (2003):
Clinical relevance of the caga, vaca and icea genotypes of Helicobacter pylori in Brazilian clinical isolates. FEMS Immunol. Med. Microbiol. 36, 181-185.
46.Torres, L.E.; Melián, K.; Moreno, A.; Alonso, J.; Sabatier, C.A.; Hernández, M.; Bermúdez, L. And Rodríguez , B. L. (2009): Prevalence of vaca, caga and baba2 genes in Cuban Helicobacter pylori isolates. World J. Gastroenterol. ; 15(2): 204–210.
47.Van Doorn, L.; Figueiredo, C.; Mégraud, F.; Pena, S.; Midolo, P.; Queiroz, D.M.; Carneiro, F.; Vanderborght, B.; Pegado, M.D.; Sanna, R.; De Boer, W.; Schneeberger, P.M.; Correa, P.; Ng, E.K.; Atherton, J.; Blaser, M.J. and Quint, W.G. (1999): Geographic distribution of vaca allelic types of Helicobacter pylori. Gastroenterology.;116(4):823-30.
48.Nimri, L.F.; Matalka, I.; Bani Hani, K. And Ibrahim, M. (2006): Helicobacter pylori genotypes identified in gastric biopsy specimens from Jordanian patients.
BMC. Gastroenterol.; 6:27.
49.Suzuki, R.; Shiotaa, S. And Yamaoka, Y. (2012):
Molecular epidemiology, population genetics, and pathogenic role of Helicobacter pylori. Infect. Genet. Evol. ; 12(2): 203–213.
50.Cover, T.L. (1996):
The vacuolating cytotoxin of Helicobacter pylori. Mol. Microbiol.; 20:241-246.
51.Jafari, F.; Shokrzadeh, L.; Dabiri, H.; Baghaei, K.; Yamaoka, Y.; Zojaji, H.; Haghazali, M.; Molaei, M. And Zali, M.R. (2008):
vaca genotypes of Helicobacter pylori in relation to caga status and clinical outcomes in Iranian populations. Jpn. J. Infect. Dis.;61(4):290–3.
52.Siavoshi, F.; Asgharzadeh, A.; Ghadiri, H.; Massarrat, S.; Latifi-Navid, S. And Zamani, M. (2011):
Helicobacter pylori genotypes and types of gastritis in first degree relatives of gastric cancer patients. International Journal of Medical Microbiology; 301:506-512.

53.Erdoğdu, C.; Saribaş, Z. And Yilmaz, Y.A. (2014):
Detection of caga and vaca genotypes of Helicobacter pylori isolates from a university hospital in Ankara region, Turkey. Turk. J. Med. Sci.; 44: 126-132.
54.Paniagua, G.L. ; Monroy, E. ; Rodríguez, R. ; Arroniz, S. ; Rodríguez, C.; Cortés, J.L.; Camacho, A.; Negrete, E. And Vaca, S. (2009): Frequency of vaca, caga and baba2 virulence markers in H. Pylori strains isolated from Mexican patients with chronic gastritis. Ann. Clin. Microbiol. Antimicrob. 30;8:14.
55.Ozbey, G.; Dogan, Y. And Demiroren, K. (2013):
Prevalence of Helicobacter pylori virulence genotypes among children in Eastern Turkey. World J. Gastroenterol.;19(39): 6585-6589.
56.Ahmad, T.; Sohail, K.; Rizwan, M.; Mukhtar, M.; Bilal, R.; Khanum, A. (2009): Prevalence of Helicobacter pylori pathogenicity-associated caga and vaca Genotypes among Pakistani dyspeptic patients. FEMS Immunol. Med. Microbiol.;55 : 34–38.
57.Mansour, K.B; Fendri, C.; Zribi, M.; Masmoudi, A.; Labbene, M.; Fillali, A.; Mami, N.B.; Najjar, T.; Meherzi, A.; Sfar, T. And Burucoa, C. (2010):
Prevalence of Helicobacter pylori vaca, caga, icea and oipa genotypes in Tunisian patients. Ann. Clin. Microbiol. Antimicrob., 9:10.
58.Katz, P.O.; Gerson, L.B. and Vela, M.F. (2013):
Guidelines for the Diagnosis and Management of Gastroesophageal Reflux Disease. Am. J. Gastroenterol.; 108:308 – 328.
59.Arents, N.L.; van Zwet, A.A.; Thijs, J.C.; Kooistra-Smid, A.M.; van Slochteren, K.R.; Degener, J.E.; Kleibeuker, J.H. and van Doorn, L.J. (2001):
The importance of vaca, caga, and icea genotypes of Helicobacter pylori infection in peptic ulcer disease and gastroesophageal reflux disease.
Am. J. Gastroenterol. ;96(9):2603-8.
60.Queiroz, D.M.; Rocha, G.A.; Oliveira, C.A.; Rocha, A.M.; Santos, A.; Cabral, M.M.; Nogueira, A.M. (2002):
Role of corpus gastritis and caga-positive Helicobacter pylori infection in reflux esophagitis. J. Clin. Microbiol.;40(8):2849-53.
61.Loffeld, R.J.; Werdmuller, B.F.; Kuster, J.G.; Pérez-Pérez, G.I.; Blaser, M.J. and Kuipers, E.J. (2000):
Colonization with caga-positive Helicobacter pylori strains inversely associated with reflux esophagitis and Barrett's esophagus. Digestion.; 62(2-3):95-9.
62.Rasmi, Y.; Sadreddini, M.; Shahsavari, Z. And Raeisi, S.(2009):
Prevalence of Helicobacter pylori and cytotoxin-associated gene A in Iranian patients with non-erosive and erosive reflux disease. Indian J Med Sci.;63(9):402-7
63.Somi, M.H.; Fattahi, E.; Fouladi, R.F.; Karimi, M.; Bonyadi, R. And Baballou, Z. (2008):
An inverse relation between caga+ strains of Helicobacter pylori infection and risk of erosive GERD. Saudi. Med. J.;29(3):393-6.
64.Kiltz, U.; Pfaffenbach, B.; Schmidt, W.E. and Adamek, R.J. (2002):
The lack of influence of caga positive Helicobacter pylori strains on gastro-oesophageal reflux disease. Eur. J. Gastroenterol. Hepatol. ;14(9):979-84.
65.Rubenstein, J.H. ; Inadomi, J.M. ; Scheiman, J. ; Schoenfeld, P. ; Appelman, H.; Zhang, M.; Metko, V. And Kao, J.Y. (2014):
Association between Helicobacter pylori and Barrett's esophagus, erosive esophagitis and gastroesophageal reflux symptoms. Clin. Gastroenterol. Hepatol. ;12(2):239-45.
66.Mahdi, B.M. (2011):
The relationship between Helicobacter pylori infection and gastro-esophageal reflux disease. N. Am. J. Med. Sci.; 3(3): 142–145.
67.Atherton, J.C.; Cao, P.; Peek, R. M. Jr.; Tummuru, M.K.; Blaser, M. J. And Cover, T. L. (1995):
Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vaca types with cytotoxin production and peptic ulceration.
J. Biol. Chem.; 270:17771–17777.
68.Zambon, C.F.; Navaglia, F.; Basso, D.; Rugge, M. And Plebani, M. (2003): Helicobacter pylori baba2, caga, and s1 vaca genes work synergistically in causing intestinal metaplasia.J. Clin. Pathol.; 56(4): 287–291.
69.Chomvarin, C.; Namwat, W.; Chaicumpar, K.; Mairiang, P.; Sangchan, A.; Sripa, B.; Tor-Udom, S. And Valaichone, R. (2008):
Prevalence of Helicobacter pylori vaca, caga, cage, icea, baba2 genotypes in Thai dyspeptic patients. International Journal of Infectious Diseases; 12:30-36.
70.Hussein, N.R. and Talebkhan, Y. M.; Talebkhan, Y.; Doraghi, M.; Letley, D.P.; Muhammad, M.K.; Argent, R.H. and Atherton, J.C. (2008):

Differences in virulence markers between H. Pylori strains from Iraq and those from Iran: potential importance of regional differences in H. Pylori associated disease. J. Clin. Microbiol. ; 46(5): 1774-9.

71.Saribasak, H.; Salih, B.A.; Yamaoka, Y. And Sander E. (2004):
Analysis of Helicobacter pylori genotypes and correlation with clinical outcome in Turkey.J. Clin. Microbiol. ; 42(4): 1648-51.

72.Martin, J.M.; Hergueta D.P, Esteban, C. J.; Romero C. R.; Pellicer, F.J.; Herrerías, J.M. (2000):
Clinical relevance of Helicobacter pylori caga-positive strains: gastroduodenal peptic lesions marker.Rev. Esp. Enferm. Dig.; 92(3):160-73.

73.Yakoob, J.; Abid, S.; Abbas, Z.; Jafri, W.; Ahmad, Z.; Ahmed, R. And Islam, M. (2009):
Distribution of Helicobacter pylori virulence markers in patients with gastroduodenal diseases in Pakistan.BMC Gastroenterology, 9:87.

74.Chao, C.; Pai, Y.; Chi, T.; Zuo, C.; Liu,2 Chih, Y.; Yi, T. And Nien, T. (2005):
Clinical Relevance of the vaca, icea, caga, and flaa Genes of Helicobacter pylori Strains Isolated in Eastern Taiwan. J Clin Microbiol. Jun; 43(6): 2913–2915.

75.Yamaoka, Y.; Kodama, T.; Gutierrez, O.; Kim, J.G.; Kashima, K.; Graham, D.Y. (1999) :
Relationship between Helicobacter pylori icea, caga, and vaca status and clinical outcome: studies in four different countries. J. Clin. Microbiol. ;37(7):2274-9.

76.Hatakeyama, M. And Higashi, H. (2005):
Helicobacter pylori caga: a new paradigm for bacterial carcinogenesis. Cancer Sci.;96:835–84.

77.Rick, J. R.; Goldman, M.; Semino-Mora, C.; Liu, H.; Olsen, C.; Rueda-Pedraza, E. , Sullivan, C. And Dubois, A. (2010):
In situ expression of caga and risk of gastroduodenal disease in Helicobacter pylori infected children.J. Pediatr. Gastroenterol. Nutr.; 50(2): 167–172.

78.Scholte, G.H.; van Doorn, L.J.; Cats, A.; Bloemena, E.; Lindeman, J.; Quint, W.G.; Meuwissen, S.G. and Kuipers, E.J. (2002):
Genotyping of Helicobacter pylori in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. Am. J. Gastroenterol.;97(7):1687-95.

79.Atherton, J.C. (2006):
The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Annu. Rev. Pathol.;1:63–96.

80.Gold, B.D.; van Doorn, L.J.; Guarner, J.; Owens, M.; Pierce-Smith, D.; Song, Q.; Hutwagner, L.; Sherman, P.M.; de Mola, O.L. and Czinn, S.J. (2001):
Genotypic, clinical, and demographic characteristics of children infected with Helicobacter pylori. J. Clin. Microbiol.;39(4):1348-52.

81.Warburton, V. J.; Everett, S.; Mapstone, N. P.; Axon, A. T. ; Hawkey, P. And Dixon, M.F. (1998):
Clinical and histological associations of caga and vaca genotypes in Helicobacter pylori gastritis. J. Clin. Pathol.;51:55–61.

82.Umit, H.; Tezel, A.; Bukavaz, S.; Unsal, G.; Otkun, M.; Soylu, A.R.; Tucer, D.; Otkun, M. And Bilgi, S. (2009):
The relationship between virulence factors of Helicobacter pylori and severity of gastritis in infected patients. Dig Dis Sci.;54(1):103-10.

83.Meining, A.; Riedl, B. And Stolte, M. (2002):
Features of gastritis predisposing to gastric adenoma and early gastric cancer.
J. Clin. Pathol.; 55: 770-773.