AIM OF THE WORK

The aim of the work is to correlate the virulence factors, (cagA, vacA and babA2) with:

1- The clinical presentation.

2- The endoscopic picture.

3- The histopathological parameters of *H. pylori* related gastritis.

SUBJECTS

Over a period of 12months, between June 2011 and June 2012 ,this study was conducted on:

Ninety five (95) children who were referred to our Gastroenterology department of Alexandria University Children's Hospital for upper GI endoscopy. The symptoms reported by these patients were hematemsis abdominal pain and/or vomiting *H. pylori* have been looked for during endoscopy. 50 symptomatic *H. pylori* positive children, their age range 1-13 and mean age 3.09±1.85, were detected. The second group are 45 symptomatic *H. pylori* negative children, their age range 0.01-12 and mean age 3.04+3.37. Hematemsis patients had upper endoscopy after exclusion of bleeding disorder and systemic causes of hematemsis). Cases of abdominal pain were selected according to the definition of Apley[161], (the presence of at least three discrete episodes of pain, debilitating enough to interfere with routine activity and occurring 3 months or more during the year preceding clinical examination).

Exclusion criteria:

Children with history of anti-secretory, antimicrobial, or anti-inflammatory medication, within 3 months preceding the endoscopy were excluded.

Children with malabsorption were also excluded.

These patients were compared to 25 asymptomatic *H. pylori* positive children. They are both age and sex matched and apparently healthy children attending the outpatient clinic of surgery of Alexandria University Children's Hospital (free from any gastrointestinal symptoms). Stool samples were taken for detection of *H. pylori* genes (cagA, vacA and babA2).

An informed consent was obtained from the subject's parents and ethical approval was obtained from the Ethics Committee of Alexandria, Faculty of Medicine.

METHODS

Symptomatic children were subjected to the following:

1. Detailed history taking: with emphasis on:

a. Onset, duration, amount, and the colour of hematemsis
b. Abdominal pain and its site, time of onset (night time or postprandial), duration, aggravation, relieving factors and associated symptoms.
c. Family history especially of a similar condition in siblings, or history of peptic disease in adults as evidenced by:
   - A diagnosed case of peptic ulcer or hyperacidity
   - Or: history of chronic or recurrent epigastric pain and heart burn

Exclusion of patients who received proton pump inhibitor or antibiotic for the last three months.

2. Through physical examination:

This included vital signs, height, weight and systemic examination to rule out cases of secondary hematemsis.

3. Investigations:

a. Routine investigations:

- Complete blood count
- Bleeding time and activity
- Aspartate transaminase (AST). Alanine transaminase (ALT)

b. Specific investigations:

- **Isolation of DNA from feces:**

It is the isolation of high quality DNA from fecal samples. Bacterial, protest, as well as host DNA can be isolated from <150mg sample of mammalian feces by using ISOLATE Fecal DNA Kit.

Procedure:

Fecal samples are added directly to a Bashing Beads Lysis Tube and rapidly lysed by bead beating in a vortex, without the use of organic denaturants or proteinases. The DNA is then bound, isolated and purified using spin columns. The eluted DNA, free of contaminants and enzyme inhibitors, is used for downstream molecular biology applications including PCR, arrays, genotyping, etc.

- **Stool antigen test**

It is an in vitro chromatographic immunoassay diagnostic test for qualitative detection of *H. pylori* antigens in human feces specimens by One Step *H. pylori* Antigen Test Device (ABON).

Principle:

In this test the membrane is pre-coated with anti *H. pylori* antibodies on the test line region of the test. During testing, the specimen react with the particle coated with the anti-*H. pylori* antibodies. The mixture migrates upward on the membrane by capillary action to react with anti-*H. pylori* antibodies on the membrane and generate a colored line in the test region

Interpretation of the result

The presence of this colored line indicates a positive result, while its absence indicates a negative result .To serve as a procedural control, A colored line will always appear in the control line region. If the control line does not appear, the test result is not valid.

- **Upper gastrointestinal endoscopy (after written consent):**

By using Olympus esophagogastroduodenoscope type GIF-XQ20 under sedation after preparation.

Biopsies were taken systemically from duodenum, gastric antrum, body and esophagus.3 antral biopsies were taken, One for rapid urease test the second was processed, paraffin blocked, sectioned into 5 micron thick section and submitted for histopathological assessment according to modified Sydney classification for gastritis and the third was stored in saline container and frozen for PCR.

Dietary precautions for sedation and endoscopy

Children up to 36 months: no milk or solid for 6 hours prior to scheduled procedure.

Children older than 36 months: no milk or solids for 8 hours prior to scheduled procedure.

Sedation:

A mixture of pethidine 1-2 mg/kg intramuscular, midazolam 0.1 mg/kg intramuscular and in some cases oral choral hydrate 75mg/kg as premedication, and diazepam 0.3 mg/kg was administrated intravenously immediately prior to the procedure.

Technique:

The endoscope was checked for proper functioning and then the first 20_30 cm of the tip and shaft were lubricated with Jelly

A bite guard was inserted between the teeth. The patient was turned on his left side, the tip of the endoscope was lubricated and introduced into the mouth, the endoscope was

steadily advanced along the length of esophagus, and the location of gastroesophageal junction was identified by recognizing the origin of the gastric folds, the Z line and diaphragmatic pinch. The endoscope was then advanced to examine the pylorus and duodenum, the antrum, stomach and esophagus.

The biopsies were subjected to histopathological examination under light microscopy to identify the underlining pathology.

One of the biopsies specimens was subjected to rapid urease test for detection of helicobacter pylori immediately after it was taken.

- **Rapid urease test:**

It is in vitro diagnostic test for detection of the urease enzyme of helicobacter pylori for the presumptive evidence of *H. pylori* in gastric biopsy by using Hp kit.

Principle:

A gastric mucosal endoscopic biopsy is placed in an agar gel urea, two pH dye indicators,

Bromthymol blue and methyl red and aparaben preservative

$(NH_2)\ CO$ (urea) + $H_2O \rightarrow NH_3$ (ammonia) + HCO_3

The urease in *H. pylori* converts the urea onto ammonia which raises the pH and changes the agar colour indicating a positive test.

Interpretation of the result:

Suggestive reading time are 15, 30, 60 minute and 24 hours after biopsy insertion.

Once a positive reaction has been obtained, no further readings are required.

The sensitivity and specificity of rapid urease test is 75-98% and 95-100%, respectively.

- **Pathological methods:**

Each paraffin block was cut at 5 micron thick sections and stained with the following:

1- H&E stain for histopathological examination.
2- Masson trichome stain for better evaluation of the degree of fibrosis the diagnosis of gastritis was based on the histopathological findings. Each of these findings was scored following the updated Sydney system [116] and using visual analogue scales.

1. Severity score:
   1: Mild
   2: Severe

2. Depth: depth of involvement of the gastric mucosa (above or below the gastric pits)
   1: Superficial
   2: Deep

3. Activity: (i.e. presence of neutrophils)
   1: No activity
   2: Active

4. Lymphoid follicles
   1: Yes
   2: No

5. Eosinophils
   1: Yes
   2: No

- **PCR assay**

DNA extraction:

DNA was extracted from frozen biopsies by using QIAmp **DNA Mini Kit** (QIAGEN, USA) according to the manufacturer's instructions. The sample was put in 180 µl of buffer ATL with 20 µl of proteinase K and then incubated at 56°C for with occasional vortexing until the pellet was completely lysed, which usually took 30 minutes. For the biopsy samples incubation was extended for overnight incubation. After lysis of the sample, 200 µl of buffer AL was added to the sample and the mixture was incubated for 10 min at 70°C. The mixture was then combined with 200 µl of absolute ethanol and mixed by pulse-vortexing for 15 s. The mixture was applied to a spin column, which holds a silica gel membrane, and spun for 1 min at 6,000 × *g*. The spin column was washed with 500 µl of buffer AW1 and then AW2 by centrifugation at 20,000 × *g* for 1 and 3 minutes respectively. The DNA bound on a membrane was eluted by centrifugation with 50 µl of buffer AE after 5-min incubation at room temperature. The resulting DNA extracts were stored at -20°C until PCR assessment.

DNA amplification and gel electrophoresis:

1) Initially, the DNA extracts were amplified by *Helicobacter pylori glmM (UreC)* [162] primers as previously described:

glmM	GlmM1-R	GCTTACTTTCTAACACTAACGCGC	
	GlmM2-F	GGATAAGCTTTTAGGGGTGTTAGGGG	296

The forward and reverse primers amplified a product of approximately 296 bp. (figure 1). Amplification was performed in a final volume of 50 µl of PCR mixture containing: 0.8 µM of each primer, 10 mM of each deoxynucleotide triphosphate (dATP, dGTP, dTTP, and dCTP). Ten mM tris HCl, 50 mM Kcl, 0.1% triton X-100, 1.5 ml MgCl2, 1 unit of DNA polymerase (Promega) and 10 µl of template DNA. Thirty-five cycles of amplification were performed, each consisting of an initial denaturation at 94ºC for 4 min, followed by denaturation at 94 ºC for 1 min, primer annealing at 55 ºC for 1.5 min, extension at 72 ºC

for 2 min, and a final extension step at 72 ºC for 10 min. in a Techne Progene thermal cycler.

2) The samples that generated a positive result in *H. pylori* were subjected to 3 separate PCR reactions (for *vacA s1/2, vacA m1/2, cagA* genes) performed with different sets of primers (table1)[163,164] and amplified under the following conditions: 3 minutes at 94° C for initial denaturation followed by 35 cycles of 1 minute at 94° C, 1 minute at 55° C, and 1 minute at 72° C, in a Techne Progene thermal cycler.

- The PCR products were resolved by 2% agarose gel electrophoresis and were visualized after ethium bromide (0.5 µg/ml) staining, using a U/V transilluminator and photographed by a polaroid camera. (Fig. 2)

Table (1): Primers used for the amplification of vacA alleles and cagA

DNA region(s) amplified	Primer name	Primer sequence	Amplicon Size(s) (bp)
vacA s1/*vacA* s2	VA1-F VA1-R	5'-ATGGAAATACAACAAACACAC-3' 5'-CTGCTTGAATGCGCCAAAC-3'	259/286
vacA m1/*vacA* m2	VAG-F VAG-R	5'-CAATCTGTCCAATCAAGCGAG-3' 5'-GCGTCAAAATAATTCCAAGG-3'	567/642
cagA	cag5c-F cag3c-R	5'-GTTGATAACGCTGTCGCTTC-3' 5'-GGGTTGTATGATATTTTCCATAA-3'	350

3) Lastly, the samples were subjected to a PCR reaction for babA2 gene, using the following t sets of primers [165]

bab7-F	CCAAACGAAACAAAAAGCGT	60	271
bab7-R	GCTTGTGTAAAAGCCGTCGT		

and amplified under the following conditions: 3 minutes at 94° C for initial denaturation followed by 30 cycles of 1 minute at 94° C, 1 minute at 45° C, and 1 minute at 72° C, in a Techne Progene thermal cycler.

- The PCR products (271 bp) were resolved by 2% agarose gel electrophoresis and were visualized after ethium bromide (0.5 µg/ml) staining, using a U/V transilluminator and photographed by a polaroid camera. (Figure. 3)

Statistical analysis [166]

Data were fed to the computer and analyzed using IBM ***SPSS software package version 20.0.***[167] Qualitative data were described using number and percent. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For abnormally distributed data Kruskal Wallis test was used to compare between different groups. Agreement of the different predictives with the outcome was used and was expressed in sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Significance of the obtained results was judged at the 5% level.

RESULTS

Over a period of 12months, between June 2011 and June 2012, 95 pediatric patients were referred to our pediatric gastroenterology clinic for upper gastrointestinal endoscopy because of hematemsis, abdominal pain and /or vomiting. *H. pylori* have been looked for during endoscopy. 50 symptomatic *H. pylori* positive children and 45 symptomatic *H. pylori* negative children were detected via rapid urease test, histopathology and PCR). Among the studied lab parameters, only Hb was affected in the *H. pylori* positive children (mean ± SD =10.81 ±1.14). These patients compared to 25 asymptomatic *H. pylori* positive children diagnosed by stool antigen. The three groups were age and sex matched as shown in table (2).

Hematemsis is the most common clinical manifestation in patient who referred to our pediatric gastroenterology clinic for upper gastrointestinal endoscopy (54.7%, 52 out of 95 children) as shown in table (3). In symptomatic *H. pylori* positive children with hematemsis, abdominal pain was an associated symptom in 48.7% (19 out of 39) and vomiting was an associated symptom in 69.2% (27 out of 39).

The amount of vomited blood was either moderate (34%) or sever (32%), while mild hematemsis was reported in (34%).

Hematemsis cases (52) were classified as: 39 (75%) had *H. pylori* gastritis, 6 (11.5%) had GERD, 5(9.6%) had varices, a case of vasculitis and a case of esophageal polyp.

As shown in table (3), of the total 50 symptomatic *H. pylori* positive patients, 31cases (62%) presented with recurrent abdominal pain. Vomiting was an associated symptom in 38.7 % (12 out of 31) patients and hematemsis in 61.3% (19 out of 31) patients. Recurrent abdominal pain was the only complaint in 8 patients (25.8%). While in symptomatic *H. pylori* negative cases, 13.3% (6 out of 45 cases) presented with RAP. Family history of abdominal pain in other sibling was found in 27% and history suggestive of peptic disease in adults of the same family was obtained in 57% of patients in symptomatic *H. pylori* positive cases.

Table (4) shows the characteristics of abdominal pain in the symptomatic children. Night- time pain with nocturnal awakening and fasting pain relived by food are significantly associated with *H. pylori* infection ($p<0.05$). The abdominal pain associated with meals is less common in children with *H. pylori* than in children without the infection ($p<0.05$).

Hematemsis is significantly higher in *H. pylori* positive cases compared to *H. pylori* negative cases ($p<0.05$). There is no difference between H. Pylori- positive and –negative groups with regard to abdominal pain and vomiting as shown in table (3).

Table (2): Comparison between the studied groups according to demographic data

	H pylori positive Symptomatic (n = 50)		Non helicobacter (n = 45)		H pylori positive non symptomatic (n = 25)		Test of sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	21	42.0	27	60.0	14	56.0	χ^2= 3.310	0.191
Female	29	58.0	18	40.0	11	44.0		
Age								
Min. – Max.	1.0 – 13.0		0.01 – 12.0		1.0 – 10.0		$^{KW}\chi^2$= 4.959	0.084
Mean ± SD.	3.09 ± 1.85		3.04 ± 3.37		3.42 ± 2.25			
Median	3.0		2.0		3.0			

χ^2: Chi square test
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
*: Statistically significant at $p \leq 0.05$
Z: Z for Mann Whitney test

Table (3): Comparison between symptomatic *H. pylori* positive and symptomatic *H. pylori* negative according to clinical presentation;

	Symptomatic *H. pylori* positive (n = 50)		Symptomatic *H. pylori* negative (n = 45)		Total (n = 95)		χ^2	P
	No.	%	No.	%	No.	%		
Vomiting$^{\$}$								
Negative	30	75.0	19	70.4	**49**	**73.1**	0.176	0.675
Positive	10	25.0	8	29.6	**18**	**26.9**		
Abdominal pain$^{\#}$								
Negative	19	70.4	39	86.7	**58**	**80.6**	2.861	0.091
Positive	8	29.6	6	13.3	**14**	**19.4**		
Hematemsis ♦								
Negative	11	22.0	32	71.1	**43**	**45.3**	23.057*	<0.001*
Positive	39	78.0	13	28.9	**52**	**54.7**		

#: Cases of Vomiting and Hematemsis were excluded from abdominal pain
$: Cases of Abdominal pain and Hematemsis were excluded from Vomiting
♦: Cases of Hematemsis ± cases of vomiting and abdominal pain
χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (4): Characteristics of abdominal pain in Symptomatic *H. pylori* positive and Symptomatic *H. pylori* negative children:

Abdominal pain	Symptomatic *H. pylori* positive (n = 31)		Symptomatic *H. pylori* negative (n = 6)		χ^2	P
	No.	%	No.	%		
Abdominal pain associated with meals	9	29.0	5	83.3	6.302*	0.021*
Postprandial pain	15	48.4	3	44.2	0.005	1.000
Nigh time pain associated with nocturnal awakening	16	51.6	0	0.0	5.456*	0.027*
Fasting pain relived by food	17	54.8	0	0.0	6.087*	0.022*

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

I. Gross endoscopic results

Table (5), shows comparison of the result of endoscopic examination, between symptomatic *H. pylori* positive and *H. pylori* negative children.

There is a significant negative association between *H. pylori* infection and esophageal erythema. While, we found a significant association between endoscopic nodular antrum and symptomatic *H. pylori* infection ($p<0.05$).

Normal antrum is seen in only 7 cases of symptomatic *H. pylori* positive cases (14%), while it seen in 66.7% (30 of 45) of symptomatic *H. pylori* negative cases. It is a statistically significant difference ($p<0.05$).

The sensitivity, the specificity, the PPV, the NPV and the accuracy of antral nodularity for *H. pylori* infection are 82, 100, 100, 83.33, 90.53% respectively.

Significant relation between *H. pylori* and duodenal erythema is also present.

Table (5): Comparison between symptomatic *H. pylori* positive and symptomatic *H. pylori* negative children according to endoscopic findings

	Symptomatic *H. pylori* positive (n = 50)		**Symptomatic *H. pylori* negative (n = 45)**		**χ^2**	**P**
	No.	**%**	**No.**	**%**		
Esophagus						
Normal	24	48.0	4	8.9	17.429*	<0.001*
Erythema	26	52.0	41	91.1		
Fundus						
Normal	26	52.0	21	46.7	0.270	0.604
Erythema	24	48.0	24	53.3		
Antrum/Pylorus						
Normal	7	14.0	30	66.7	27.628*	<0.001*
Erythema	22	44.0	15	33.3	1.133	0.287
Nodularity	41	82.0	0	0.0	64.917*	<0.001*
Duodenum						
Normal	16	32.0	29	64.4	10.00*	0.002*
Erythema	34	68.0	16	35.6		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

II. Histopathological findings:

The histopathological analysis was performed in all of the 50 symptomatic h pylori positive cases and the 45 symptomatic h pylori negative children.

Esophagus:

Histologically, 78% (39 out of 50) of the symptomatic h pylori positive cases had picture suggestive of reflux esophagitis in comparison to 68.9% (31 out of 45) of the symptomatic h pylori negative cases with the same picture ($p>0.05$).

Antrum

Antral predominant gastritis is the main pattern of gastritis in our samples histologically 92% (46 out of 50). While, gastritis in *H. pylori* negative cases was eosinophilic (15 cases), chemical (10 cases), hypersecretory (5 cases), PHG (5 cases), **Meneterier's (one case), Combined (Eos/chemical) (one case).**

Duodenum

Histologically, 78% (39 out of 50) symptomatic h pylori positive cases had duodenitis in comparison to 66.7% (30 out of 45) symptomatic h pylori negative cases had duodenitis.

Gastric biopsy specimens were observed from the antrum and 5 histopathological parameters were applied on the samples according to modified Sydney classification which are:
(1) Severity of inflammation
(2) Depth of inflammation
(3) Activity of inflammation
(4) Presence of lymphoid follicles
(5) Presence of eosinophils.

The comparison of histopathological parameters between symptomatic H. Pylori-positive and negative groups are summarized in table (6)

50% of the symptomatic *H. pylori* positive specimens are associated with severe inflammation compared to 15% of the *H. pylori* negative specimens. This is statistically significant difference.

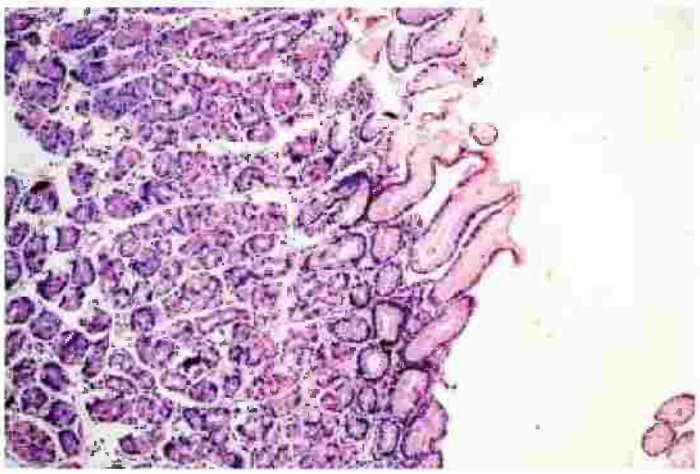
The activity of inflammation is significantly higher in symptomatic *H. pylori* positive group than the symptomatic *H. pylori* negative other.

Lymphoid follicles are significantly more prevalent in biopsies from H. Pylori-infected children than the non infected children.

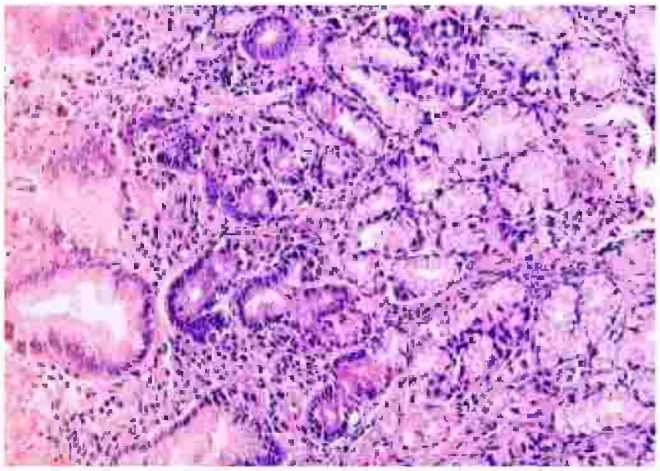
Biopsies from *H. pylori* infected subjects revealed a great number of cases with deep inflammation (46%) in relation to non infected children (32.5%), although not statistically significant.

No statistically significant difference between the two groups as regard the eosinophils.

Cases of h pylori realted gastritis showed disorganization of the surface epithelium with a chronic nonspecific infiltrate of the lamina propria by a lymphohistiocytic infiltrate which was superficial or deep (reaching beyond the level of the gastric pits).



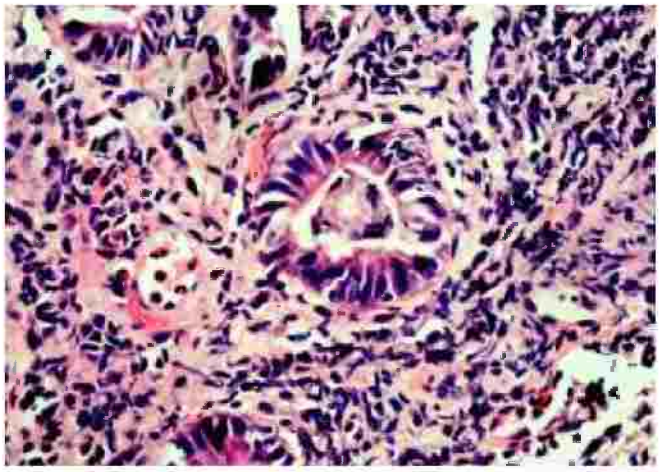
A



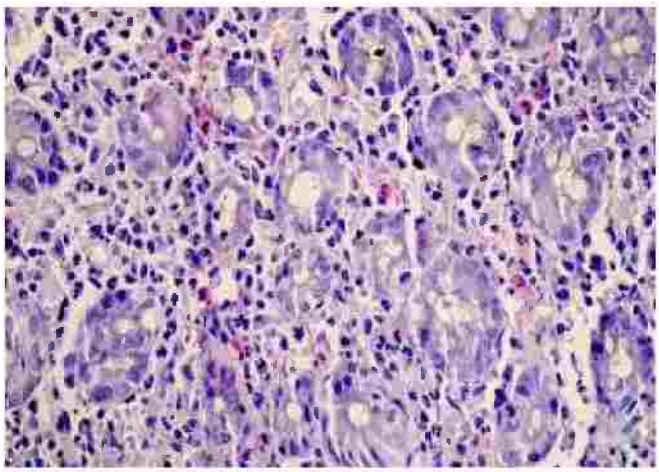
B

Figure (2): The inflammatory infiltrate was seen limited to the upper gastric mucosa in superficial cases (a) and extending beyond the gastric pits (b) in the deep cases. (H&E, x100).

A minor eosinophilic element was noted.



A



B

Figure (3): Eosinophils are seen (a) marginating in the blood vessels (x400) and (b) in the lamina propria (x200). (H&E).

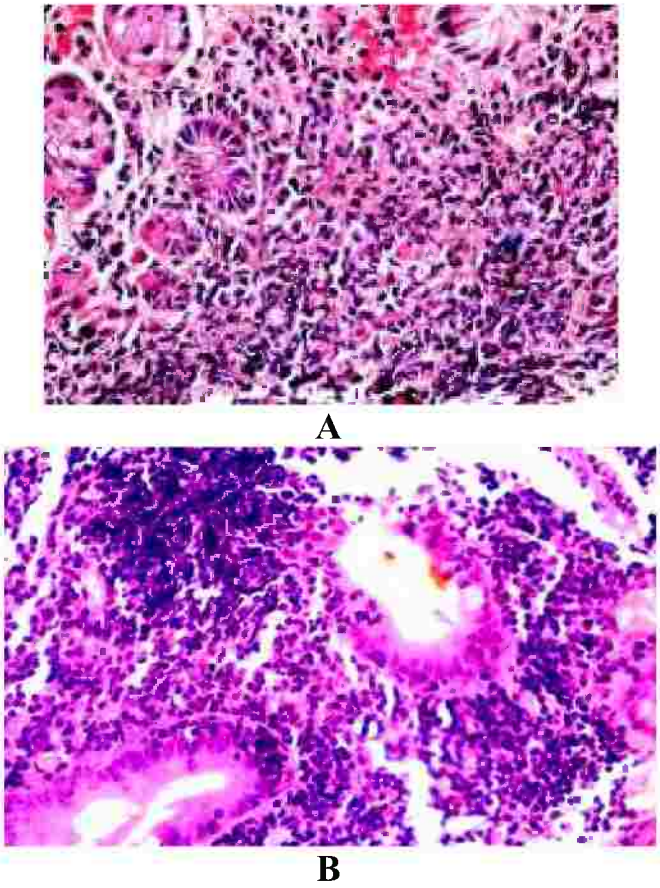


Figure (4): Signs of activity were seen in the form of a neutrophilic infiltrate (a) in the lamina propria and (b) infiltrating the glandular epithelium. (H & E x200)

Lymphoid follicles with reactive germinal centers were observed also.

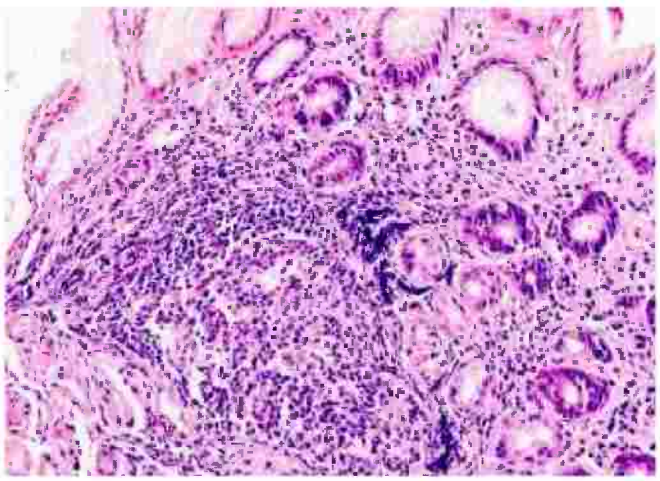


Figure (5): Lymphoid follicles with reactive germinal centers in the mucosa.(H&E, x100).

Table (6) details the frequency of these histopathological findings in the symptomatic H pylori infected and non infected groups.

Table (6): Comparison between symptomatic *H. pylori* positive and symptomatic *H. pylori* negative according to histopathological findings in the antrum

	Symptomatic *H. pylori* positive (n = 50)		Symptomatic *H. pylori* negative (n = 45)		χ^2	P
	No.	%	No.	%		
Severity of Infl						
Mild	25	50.0	34	85.0	12.056*	0.001*
Severe	25	50.0	6	15.0		
Depth of infl						
Superficial	27	54.0	27	67.5	1.688	0.194
Deep	23	46.0	13	32.5		
Activity						
No Activity	28	56.0	36	90.0	12.505*	<0.001*
Active	22	44.0	4	10.0		
Lymphoid follicles						
Yes	33	66.0	4	10.0	28.785*	<0.001*
No	17	34.0	36	90.0		
Eosinophils						
Yes	2	100.0	22	55.0	1.575	FEp=0.498
No	0	0.0	18	45.0		

χ^2: Chi square test
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

III. Relation between the clinical presentations and the pathology among symptomatic *H. pylori* positive children.

III. A- Relation between the clinical presentations and the endoscopic picture among symptomatic *H. pylori* positive children:

H. pylori positive patients with and without vomiting and abdominal pain cannot be differentiated according to endoscopic picture as shown in tables (7,8).

As regard hematemsis, we found a negative association between hematemsis in *H. pylori* positive cases and erythema of the esophagus as shown in table (9).

Table (7): Relation between vomiting and the endoscopic picture among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	P
	Without vomiting (n=30)		With vomiting (n=20)			
	No.	%	No.	%		
Esophagus						
Normal	12	40.0	12	60.0	1.923	0.166
Erythema	18	60.0	8	40.0		
Fundus						
Normal	15	50.0	11	55.0	0.120	0.729
Erythema	15	50.0	9	45.0		
Antrum/Pylorus						
Normal	2	6.7	5	25.0	3.350	0.100
Erythema	14	46.7	8	40.0	0.216	0.642
Nodularity	25	83.3	14	70.0	3.252	0.130
Duodenum						
Normal	12	40.0	4	20.0	2.206	0.137
Erythema	18	60.0	16	80.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (8): Relation between abdominal pain and endoscopic picture among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	P
	With abdominal pain (n=19)		Without abdominal pain (n=31)			
	No.	%	No.	%		
Esophagus						
Normal	7	36.8	17	54.8	1.529	0.216
Erythema	12	63.2	14	45.2		
Fundus						
Normal	8	42.1	18	58.1	1.202	0.273
Erythema	11	57.9	13	41.9		
Antrum/Pylorus						
Normal	3	15.8	4	12.9	0.014	1.000
Erythema	9	47.4	13	41.9	0.030	0.863
Nodularity	13	68.4	26	83.9	1.057	0.412
Duodenum						
Normal	5	26.3	11	35.5	0.455	0.500
Erythema	14	73.7	20	64.5		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (9): Relation between hematemsis and the endoscopic picture among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	P
	Without hematemsis (n=11)		With hematemsis (n=39)			
	No.	%	No.	%		
Esophagus						
Normal	0	0.0	24	61.5	13.018*	<0.001*
Erythema	11	100.0	15	38.5		
Fundus						
Normal	3	27.3	23	59.0	3.455	0.063
Erythema	8	72.7	16	41.0		
Antrum/Pylorus						
Normal	1	9.1	6	15.4	0.282	FEp=1.000
Erythema	7	63.6	15	38.5	2.207	FEp=0.178
Nodularity	10	90.9	31	79.5	0.758	FEp=0.662
Duodenum						
Normal	3	27.3	13	33.3	0.145	FEp= 1.000
Erythema	8	72.7	26	66.7		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

III. B-Relation between the clinical presentations and the histopathological findings among symptomatic *H. pylori* positive children:

There was no significant association between vomiting and the any of the histopathological parameters as shown in table (10). The histolopathogical picture of *H. pylori* infected children with RAP characterized by more severe inflammation compared with those not complaining of RAP (p<0, 05) as shown in table (11).

The histopathological picture of symptomatic h pylori positive cases reveals a significant relation between hematemsis and the severity of inflammation in comparison to those not complaining of hematemsis (p<0.05) as shown in tables (12).

Table (10): Relation between vomiting and the histopathology among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	**P**
	Without vomiting (n=30)		**With vomiting (n=20)**			
	No.	**%**	**No.**	**%**		
Severity of infl						
Mild	14	46.7	11	55.0	0.333	0.564
Severe	16	53.3	9	45.0		
Depth of infl						
Superficial	17	56.7	10	50.0	0.215	0.643
Deep	13	43.3	10	50.0		
Activity						
No Activity	17	56.7	11	55.0	0.014	0.907
Active	13	43.3	9	45.0		
Lymphoid follicles						
Yes	19	63.3	14	70.0	0.238	0.626
No	11	36.7	6	30.0		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (11): Relation between abdominal pain and the histopathology among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	P
	Without abdominal pain (n=19)		With abdominal pain (n=31)			
	No.	%	No.	%		
Severity of infl						
Mild	13	68.4	12	38.7	4.160*	0.041*
Severe	6	31.6	19	61.3		
Depth of infl						
Superficial	12	63.2	15	48.4	1.035	0.309
Deep	7	36.8	16	51.6		
Activity						
No Activity	11	57.9	17	54.8	0.045	0.833
Active	8	42.1	14	45.2		
Lymphoid follicles						
Yes	11	57.9	22	71.0	0.897	0.344
No	8	42.1	9	29.0		
Eosinophils						
Yes	2	100.0	2	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (12): Relation between hematemsis and the histopathology among symptomatic *H. pylori* positive children (n=50):

	Symptomatic *H. pylori* positive				χ^2	P
	Without hematemsis (n=11)		With hematemsis (n=39)			
	No.	%	No.	%		
Severity of infl						
Mild	9	81.8	16	41.0	5.711*	0.017*
Severe	2	18.2	23	59.0		
Depth of infl						
Superficial	8	72.7	19	48.7	1.991	0.158
Deep	3	27.3	20	51.3		
Activity						
No Activity	7	63.6	21	53.8	0.344	^{FE}p = 0.734
Active	4	36.4	18	46.2		
Lymphoid follicles						
Yes	5	45.5	28	71.8	2.653	^{FE}p=0.151
No	6	54.5	11	28.2		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

IV. Virulence factors of H. Pylori:

IV. A- Prevalence of cagA, vacA, babA2 in *H. pylori* positive children:

By using multiplex PCR, we examined the prevalence of cagA, vacA and babA2 genes to define the different genotypes of H. Pylori.

As shown in table (13), the most prevalent virulent gene is vacA (72%) then cagA (52%) then babA2 (44%).

Also, the most common s allele is s2 and the most common m allele is m2.

As regard vacA s+m combined genotypes, s2/m2 is the most common, then s1/m1 then s1/m2 and s2/m1 with equal prevalence.

Table (13): Distribution of the *H. pylori* positive cases according to the studied genes (n = 75)

	No.	%
babA2		
Negative	42	56.0
Positive	33	44.0
CagA		
Negative	36	48.0
Positive	39	52.0
VacA		
Negative	21	28.0
Positive	54	72.0
VacA s		
Negative	30	40.0
Positive	45	60.0
S1		
Negative	59	78.7
Positive	16	21.3
S2		
Negative	46	61.3
Positive	29	38.7
VacA m		
Negative	25	33.3
Positive	50	66.7
M1		
Negative	52	69.3
Positive	23	30.7
M2		
Negative	48	64.0
Positive	27	36.0
VacA alleles		
s1/m1	12	32.4
s1/m2	3	8.1
s2/m1	3	8.1
s2/m2	19	51.4

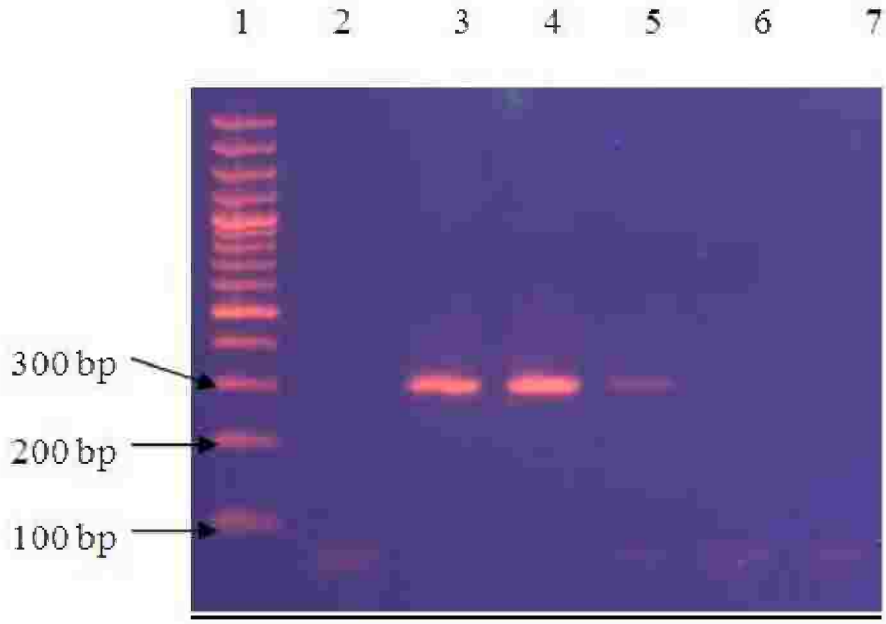


Figure (6): 2% agarose gel electrophoresis showing 296 bp PCR products of the glmM (UreC) gene (lanes 3, 4 and 5). Lane 1 shows 100 bp molecular weight marker (Fermentas).

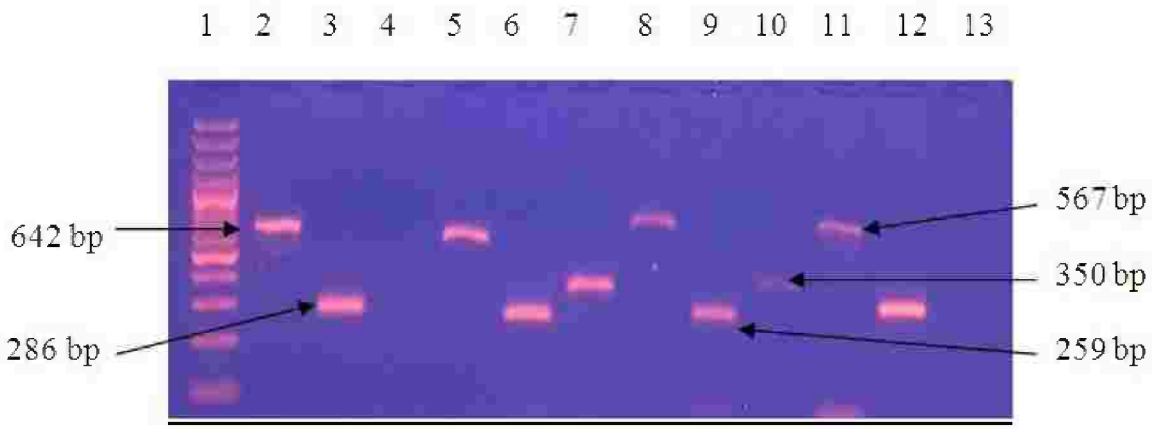


Figure (7): 2% agarose gel electrophoresis showing PCR products of the cagA gene (lanes7 and 10), vacA s1 (lanes 6, 9 and 12) ,vacA s2 (lane 3) ,vacA m1 (lane 5 and 11) ,vacA m2 alleles (lane 2 and 8) respectively. Lane 1 shows 100 bp molecular weight marker (Fermentas).

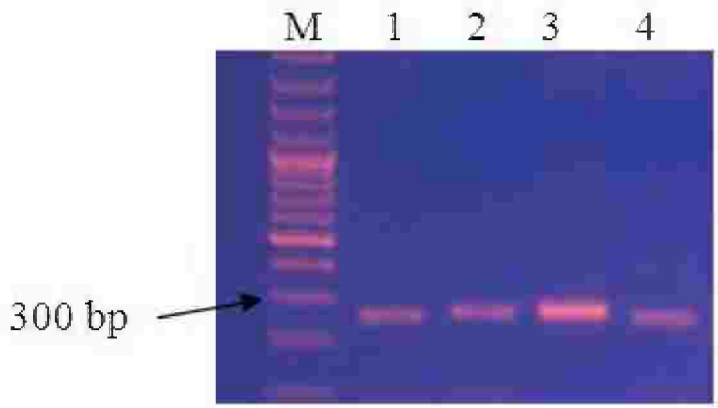


Figure (8): 2% agarose gel electrophoresis showing 271 bp PCR products of the babA2 gene (lanes 1,2,3 and 4). Lane M shows 100 bp molecular weight marker (Fermentas).

IV. B-Virulence factors of *H. pylori* and it's relation to clinical presentations among *H. pylori* positive children:

By using multiplex PCR, we examined the prevalence of cagA, vacA and babA2 genes in symptomatic *H. pylori* positive cases and compare the results to the asymptomatic group, to define the different genotypes of *H. pylori* and to relate them with the presence of symptoms in *H. pylori* positive patients, as shown in table (14).

CagA positive strains are present in 70% of the symptomatic cases while it is present in only 16% of the asymptomatic group, the difference is statistically significant.

There is a statistical significant relation between babA2 positivity and the presence of clinical symptoms. BabA2 is present in 60% (30 out of 50) cases in symptomatic *H. pylori* positive cases while it is present in only 12% (3 out of 45) cases in asymptomatic children.

Most of the symptomatic cases are vacA *positive* (80%, of the symptomatic group and 56% of the non symptomatic group with a statistically significant difference ($p<0.05$).

As shown in table (14), the most common s allele in the symptomatic group is s2 46% (23 out of 50 cases), and the most common m allele is m2 46 % (23 out of 50).

There is a significant relationship between presence of clinical symptoms and presence of s1 positive strains. S1genotype is detected in 30% of the symptomatic group in relation to 4% of the asymptomatic *H. pylori* positive patients.

Table (14) also shows vacA s/m combined genotypes in relation to the presence or absence of the symptoms in the two *H. pylori* positive groups. There is a higher prevalence of vacA s2/m2 positive strains over other vacA alleles (38%), this is followed by s1/m1then s2/m1 +s1/m2 (equal prevalence of 4%).There is a statistically significant relation between presence of symptoms and both s2/m2 positivity and s1/m1 positivity.

Hematemsis:

While estimating the relationship between potentially virulent *H. pylori* strains and clinical presentations, significant relations are found between babA2, cagA, vacA s, vacA m, s1/m1 and s2/m2 positivity and hematemsis, as shown in table (15).

Vomiting:

As shown in table (16), There is a high percentage of babA2 among vomiting cases (65%) compared to children not complaining of vomiting (36.4%), it is a significant relationship ($p<0,05$). Neither of the vacA s- and m- alleles are associated with vomiting; in addition, cagA status does not appear to be correlated with vomiting. A lack of correlation with vomiting is also found with all of the vacA s and m combinations.

Abdominal pain:

Presence of babA2, cagA, vacA s1 or s1/m1 positive strains is significantly associated with abdominal pain, as a complaint in symptomatic *H. pylori* positive children as shown in table (17).

Table (14): Comparison between symptomatic *H. pylori* positive cases and asymptomatic *H. pylori* positive children according to genes (n=75):

	Symptomatic *H. pylori* positive (n = 50)		Asymptomatic *H. pylori* positive (n = 25)		χ^2	P
	No.	%	No.	%		
babA2						
Negative	20	40.0	22	88.0	15.584*	<0.001*
Positive	30	60.0	3	12.0		
CagA						
Negative	15	30.0	21	84.0	19.471*	<0.001*
Positive	35	70.0	4	16.0		
VacA						
Negative	10	20.0	11	44.0	4.762*	0.029*
Positive	40	80.0	14	56.0		
VacA s						
Negative	12	24.0	18	72.0	16.000*	<0.001*
Positive	38	76.0	7	28.0		
s1						
Negative	35	70.0	24	96.0	6.713*	0.010*
Positive	15	30.0	1	4.0		
s2						
Negative	27	54.0	19	76.0	3.401	0.065
Positive	23	46.0	6	24.0		
VacA m						
Negative	13	26.0	16	64.0	10.148*	0.001*
Positive	37	74.0	9	36.0		
m1						
Negative	33	66.0	19	76.0	0.784	0.376
Positive	17	34.0	6	24.0		
m2						
Negative	27	54.0	21	84.0	6.510*	0.011*
Positive	23	46.0	4	16.0		
VacA alleles						
s1/m1	12	24.0	0	0.0	7.143*	^{FE}p = 0.006*
s1/m2	2	4.0	1	4.0	0.0	^{FE}p = 1.000
s2/m1	2	4.0	1	4.0	0.0	^{FE}p = 1.000
s2/m2	19	38.0	0	0.0	12.723*	<0.001*

χ^2: Chi square test
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (15): Relation between hematemsis and different genotypes among *H. pylori* positive children (n=75):

	H. pylori positive(n=75)				χ^2	P
	Without hematemsis (n=36)		With hematemsis (n=39)			
	No.	%	No.	%		
babA2						
Negative	28	77.8	14	35.9	12.326*	<0.001*
Positive	8	22.2	25	64.1		
CagA						
Negative	23	63.9	13	33.3	7.002*	0.008*
Positive	13	36.1	26	66.7		
VacA s1						
Negative	32	88.9	27	69.2	4.311*	0.038*
Positive	4	11.1	12	30.8		
VacA s2						
Negative	28	77.8	18	46.2	7.984*	0.005*
Positive	8	22.2	21	53.8		
VacA m1						
Negative	29	80.6	23	59.0	4.101*	0.043*
Positive	7	19.4	16	41.0		
VacA m2						
Negative	29	80.6	19	48.7	8.236*	0.004*
Positive	7	19.4	20	51.3		
VacA alleles						
s1/m1	1	16.7	11	35.5	9.006*	0.003*
s1/m2	2	33.3	1	3.2	0.404	^{FE}p = 0.610
s2/m1	1	16.7	2	6.5	0.269	^{FE}p = 1.000
s2/m2	2	33.3	17	54.8	14.316*	<0.001*

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (16): Relation between vomiting with different genotypes among *H. pylori* positive children (n=75):

	H. pylori positive				χ^2	P
	Without vomiting (n=55)		With vomiting (n=20)			
	No.	%	No.	%		
babA2						
Negative	35	63.6	7	35.0	4.881*	0.027*
Positive	20	36.4	13	65.0		
CagA						
Negative	28	50.9	8	40.0	0.699	0.403
Positive	27	49.1	12	60.0		
VacA s1						
Negative	45	81.8	14	70.0	1.221	$^{FE}p = 0.341$
Positive	10	18.2	6	30.0		
VacA s2						
Negative	35	63.6	11	55.0	0.461	0.497
Positive	20	36.4	9	45.0		
VacA m1						
Negative	39	70.9	13	65.0	0.241	0.624
Positive	16	29.1	7	35.0		
VacA m2						
Negative	37	67.3	11	55.0	0.959	0.327
Positive	18	32.7	9	45.0		
VacA alleles						
s1/m1	6	26.1	6	42.9	3.977	$^{FE}p=0.072$
s1/m2	3	13.0	0	0.0	1.136	$^{FE}p=0.560$
s2/m1	3	13.0	0	0.0	1.136	$^{FE}p=0.560$
s2/m2	11	47.8	8	57.1	3.102	0.078

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (17): Relation between abdominal pains with different genotypes among *H. pylori* positive children (n=75):

	H. pylori positive				χ^2	P
	Without abdominal pain(n=44)		With abdominal pain(n=31)			
	No.	%	No.	%		
babA2						
Negative	30	68.2	12	38.7	6.411*	0.011*
Positive	14	31.8	19	61.3		
CagA						
Negative	26	59.1	10	32.3	5.246*	0.022*
Positive	18	40.9	21	67.7		
VacA s1						
Negative	39	88.6	20	64.5	6.305*	0.012*
Positive	5	11.4	11	35.5		
VacA s2						
Negative	30	68.2	16	51.6	2.105	0.147
Positive	14	31.8	15	48.4		
VacA m1						
Negative	33	75.0	19	61.3	1.608	0.205
Positive	11	25.0	12	38.7		
VacA m2						
Negative	31	70.5	17	54.8	1.925	0.165
Positive	13	29.5	14	45.2		
VacA alleles						
s1/m1	3	21.4	9	39.1	6.667*	0.022*
s1/m2	2	14.3	1	4.3	0.082	^{FE}p=1.000
s2/m1	1	7.1	2	8.7	0.827	^{FE}p=0.566
s2/m2	8	57.1	11	47.8	8.929*	0.003*

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

IV. C-Endoscopic picture and different genotypes of *H. pylori* among symptomatic *H. pylori* positive children:

As shown in tables (18-25):

Esophagus:

There is a strong association between the presence of cagA and the esophageal erythema (p=0.019).The same relation is present between the vacA s allele and esophageal erythema endoscopically. However the frequency of esophageal erythema is lower in children with babA2 positive *H. pylori* infection (13 out of 30).

Fundus:

Significant relation is present between s2 and erythema of the fundus.

Antrum:

There is a negative relationship between normal antrum and the cagA positivity (p=0.020). Also, none of our vacA s1 cases are associated with normal antrum.

Antral erythema is strongly associated with cagA positivity (p=0.004). This relation cannot be found between vacA or babA2 and the antral erythema (p>0.05).

Endoscopically, antral nodularity is more common in cagA positive patients (p<0.05). Also, significant relation is found between antral nodularity and vacA s1. No relationships are found between vacAs2, vacA m or babA2 and nodularity.

Duodenum

No statistically significant relationships are found between any of the discussed genes and duodenal erythema (p>0.05).

Table (18): Relation between CagA status and endoscopic findings among symptomatic *H. pylori* positive children(n=50)

	CagA				χ^2	P
	-ve (n=15)		+ve (n=35)			
	No.	%	No.	%		
Esophagus						
Normal	11	73.3	13	37.1	5.510*	0.019*
Erythema	4	26.7	22	62.9		
Fundus						
Normal	9	60.0	17	48.6	0.549	0.459
Erythema	6	40.0	18	51.4		
Antrum/Pylorus						
Normal	5	33.3	2	5.7	6.652*	FEp=0.020*
Erythema	2	13.3	20	57.1	8.179*	0.004*
Nodularity	8	53.3	33	94.3	11.931*	FEp=0.002*
Duodenum						
Normal	7	46.7	9	25.7	2.118	FEp=0.191
Erythema	8	53.3	26	74.3		

χ^2: value for Chi square
FE: Fisher Exact test

Table (19): Relation between VacA s1 status and endoscopic findings among symptomatic *H. pylori* positive children(n=50)

	VacA s1				χ^2	P
	-ve (n=35)		+ve (n=15)			
	No.	%	No.	%		
Esophagus						
Normal	20	57.1	4	26.7	3.907*	0.048*
Erythema	15	42.9	11	73.3		
Fundus						
Normal	19	54.3	7	46.7	0.244	0.621
Erythema	16	45.7	8	53.3		
Antrum/Pylorus						
Normal	7	20.0	0	0.0	3.488	FEp=0.087
Erythema	14	40.0	8	53.3	0.758	0.384
Nodularity	26	74.3	15	100.0	4.704*	FEp=0.030*
Duodenum						
Normal	12	34.3	4	26.7	0.280	0.746
Erythema	23	65.7	11	73.3		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (20): Relation between vacA s2 status and endoscopic findings among symptomatic *H. pylori* positive children (n=50)

	VacA s2				χ^2	P
	-ve (n=27)		+ve (n=23)			
	No.	%	No.	%		
Esophagus						
Normal	8	29.6	16	69.6	7.936*	0.005*
Erythema	19	70.4	7	30.4		
Fundus						
Normal	10	37.0	16	69.6	5.265*	0.022*
Erythema	17	63.0	7	30.4		
Antrum/Pylorus						
Normal	4	14.8	3	13.0	0.032	FEp=1.000
Erythema	13	48.1	9	39.1	0.410	0.522
Nodularity	22	81.5	19	82.6	0.011	FEp=1.000
Duodenum						
Normal	9	33.3	7	30.4	0.048	0.827
Erythema	18	66.7	16	69.6		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (21): Relation between vacA m1 status and endoscopic findings among symptomatic *H. pylori* positive children(n=50)

	VacA m1				χ^2	P
	-ve (n=33)		+ve (n=17)			
	No.	%	No.	%		
Esophagus						
Normal	17	51.5	7	41.2	0.480	0.488
Erythema	16	48.5	10	58.8		
Fundus						
Normal	19	57.6	7	41.2	1.209	0.272
Erythema	14	42.4	10	58.8		
Antrum/Pylorus						
Normal	6	18.2	1	5.9	1.410	^{FE}p= 0.398
Erythema	14	42.4	8	47.1	0.098	0.754
Nodularity	27	81.8	14	82.4	0.002	^{FE}p=1.000
Duodenum						
Normal	11	33.3	5	29.4	0.079	0.778
Erythema	22	66.7	12	70.6		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (22): Relation between vacA m2 status and endoscopic findings among symptomatic *H. pylori* positive children (n=50)

	VacA m2				χ^2	P
	-ve (n=27)		+ve (n=23)			
	No.	%	No.	%		
Esophagus						
Normal	11	40.7	13	56.5	1.239	0.266
Erythema	16	59.3	10	43.5		
Fundus						
Normal	11	40.7	15	65.2	2.981	0.084
Erythema	16	59.3	8	34.8		
Antrum/Pylorus						
Normal	4	14.8	3	13.0	0.032	^{FE}p=1.000
Erythema	13	48.1	9	39.1	0.410	0.522
Nodularity	21	77.8	20	87.0	0.709	^{FE}p= 0.479
Duodenum						
Normal	9	33.3	7	30.4	0.048	0.827
Erythema	18	66.7	16	69.6		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (23): Relation between s1/m1 status and endoscopic findings among symptomatic *H. pylori* positive children (n=50)

	s1m1				χ^2	P
	-ve (n = 38)		+ve (n = 12)			
	No.	%	No.	%		
Oesphagus						
Normal	20	52.6	4	33.3	1.361	0.243
Erythema	18	47.4	8	66.7		
Fundus						
Normal	21	55.3	5	41.7	0.675	^{FE}p= 0.411
Erythema	17	44.7	7	58.3		
Antrum/Pylorus						
Normal	7	18.4	0	0.0	2.570	^{FE}p=0.174
Erythema	17	44.7	5	41.7	0.035	^{FE}p=1.000
Nodularity	27	71.1	12	100.0	4.453*	^{FE}p=0.046*
Duodenum						
Normal	13	34.2	3	25.0	0.356	^{FE}p=0.728
Erythema	25	65.8	9	75.0		

χ^2: value for Chi square
FE: Fisher Exact test

Table (24): Relation between VacA alleles and endoscopic findings among symptomatic *H. pylori* positive children (n=50)

	VacA alleles							
	s1/m1 (n=12)		s1/m2 (n=2)		s2/m1 (n=2)		s2/m2 (n=19)	
	No.	%	No.	%	No.	%	No.	%
Esophagus								
Normal	4	33.3	0	0.0	2	100.0	12	63.2
Erythema	8	66.7	2	100.0	0	0.0	7	36.8
Fundus								
Normal	5	41.7	1	50.0	1	50.0	14	73.7
Erythema	7	58.3	1	50.0	1	50.0	5	26.3
Antrum/Pylorus								
Normal	0	0.0	0	0.0	0	0.0	3	15.8
Erythema	5	41.7	2	100.0	2	100.0	5	26.3
Nodularity	12	100.0	2	100.0	1	50.0	16	84.2
Duodenum								
Normal	3	25.0	1	50.0	2	100.0	5	26.3
Erythema	9	75.0	1	50.0	0	0.0	14	73.7

Table (25): Relation between babA2 status and endoscopic findings among symptomatic *H. pylori* positive children (n=50)

	babA2				χ^2	P
	-ve (n = 20)		+ve (n = 30)			
	No.	%	No.	%		
Esophagus						
Normal	7	35.0	17	56.7	2.257	0.133
Erythema	13	65.0	13	43.3		
Fundus						
Normal	10	50.0	16	53.3	0.053	0.817
Erythema	10	50.0	14	46.7		
Antrum/Pylorus						
Normal	3	15.0	4	13.3	0.028	$^{FE}p = 1.000$
Erythema	9	45.0	13	43.3	0.014	$^{FE}p = 1.000$
Nodularity	17	85.0	22	73.3	0.952	$^{FE}p = 0.489$
Duodenum						
Normal	5	25.0	11	36.7	0.751	0.386
Erythema	15	75.0	19	63.3		

χ^2: Chi square test
FE: Fisher Exact test

IV. D- *H. pylori* genotypes and histopathological findings

Relation between histological parameters determined in gastric biopsy specimens and *H. pylori* genetic status are shown in tables (26- 33).

1- Severity of inflammation:

The severity of inflammation is assessed in gastric samples. 60% of the cagA positive strains are associated with severe inflammation compared to 26.7% of the cagA negative strains. This is statistically significant difference.

There are significant relationships between vacA s1 positivity and vacA m1 positivity and severity of inflammation. No relationships are found between vacAs2 or babA2 and severity of inflammation.

The presence of s1/m1 positive strains are associated with severe inflammation. VacA m2 positive strains are not associated with any of the histopathological parameters.

2- Depth: depth of involvement of the gastric mucosa (above or below the gastric pits)

VacA s1 and vacA m1 positive genotypes are associated with the depth of inflammation ($p<0.05$). No relationships are found between cagA, vacA m2 or babA2 and the depth of inflammation.

There is a significance difference between m1 positive and m1 negative samples as regard the depth of inflammation. Also a significant relation is found between the depth of inflammation and s2 positivity.

There is a significant difference between s1/m1 positive samples and s1/m1 negative as regard the depth of inflammation.

3- Activity: (i.e. presence of neutrophils)

CagA –positive genotypes are strongly associated with higher activity in the antrum ($p<0.05$).

Also there is a significant relationship between vacA s1 positivity and the activity. While, a lack of correlation with the activity is found with vacA s2, vacA m and babA2 in the studied samples.

4- Lymphoid follicles:

There is a strong association between presence of lymphoid follicles in the antrum and individual vacA s1 positive genotypes, compared to the negative genotypes ($p=0.0011$). The same relationship could not be observed between cagA, vacA s2, vacA m or babA2 and the presence of lymphoid follicles.

5- Eosinophils;

There is no relationship between any of the studied genes and the presence of eosinophils ($p>0.05$).

Table (26): Relation between CagA status and histopathological findings among symptomatic *H. pylori* positive children (n=50)

	CagA				χ^2	P
	-ve (n=15)		+ve (n=35)			
	No.	%	No.	%		
Severity of Infl						
Mild	11	73.3	14	40.0	4.667*	0.031*
Severe	4	26.7	21	60.0		
Depth of infl						
Superficial	10	66.7	17	48.6	1.384	0.239
Deep	5	33.3	18	51.4		
Activity						
No Activity	13	86.7	15	42.9	8.179*	0.004*
Active	2	13.3	20	57.1		
Lymphoid follicles						
Yes	10	66.7	23	65.7	0.004	0.948
No	5	45.5	12	34.3		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (27): Relation between vacA s1 status and histopathological findings among symptomatic *H. pylori* positive children (n=50)

	VacA s1				χ^2	P
	-ve (n=35)		+ve (n=15)			
	No.	%	No.	%		
Severity of Infl						
Mild	21	60.0	4	26.7	4.667*	0.031*
Severe	14	40.0	11	73.3		
Depth of infl						
Superficial	23	65.7	4	26.7	6.445*	0.011*
Deep	12	34.3	11	73.3		
Activity						
No Activity	23	65.7	5	33.3	4.468*	0.035*
Active	12	34.3	10	66.7		
Lymphoid follicles						
Yes	20	57.1	13	86.7	4.079*	0.043*
No	15	42.9	2	13.3		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (28): Relation between vacA s2 and histopathological findings among symptomatic *H. pylori* positive children (n=50)

	VacA s2				χ^2	P
	-ve (n=27)		+ve (n=23)			
	No.	%	No.	%		
Severity of Infl						
Mild	14	51.9	11	47.8	0.081	0.777
Severe	13	48.1	12	52.2		
Depth of infl						
Superficial	11	40.7	16	69.6	4.154*	0.042*
Deep	16	59.3	7	30.4		
Activity						
No Activity	13	48.1	15	65.2	1.469	0.226
Active	14	51.9	8	34.8		
Lymphoid follicles						
Yes	19	70.4	14	60.9	0.500	0.480
No	8	29.6	9	39.1		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (29): Relation between VacA m1 and pathological findings among symptomatic *H. pylori* positive children (n=50)

	VacA m1				χ^2	P
	-ve (n=33)		+ve (n=17)			
	No.	%	No.	%		
Severity of Infl						
Mild	21	63.6	4	23.5	7.219*	0.007*
Severe	12	36.4	13	76.5		
Depth of infl						
Superficial	23	69.7	4	23.5	9.628*	0.002*
Deep	10	30.3	13	76.5		
Activity						
No Activity	20	60.6	8	47.1	0.836	0.361
Active	13	39.4	9	52.9		
Lymphoid follicles						
Yes	20	60.6	13	76.5	1.258	0.262
No	13	39.4	4	23.5		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test
*: Statistically significant at $p \leq 0.05$

Table (30): Relation between VacA m2 status and pathological findings among symptomatic *H. pylori* positive children (n=50)

	VacA m2				χ^2	P
	-ve (n=27)		+ve (n=23)			
	No.	%	No.	%		
Severity of Infl						
Mild	15	55.6	10	43.5	0.725	0.395
Severe	12	44.4	13	56.5		
Depth of infl						
Superficial	14	51.9	13	56.5	0.109	0.741
Deep	13	48.1	10	43.5		
Activity						
No Activity	14	51.9	14	60.9	0.410	0.522
Active	13	48.1	9	39.1		
Lymphoid follicles						
Yes	18	66.7	15	65.2	0.012	0.914
No	9	33.3	8	34.8		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test

Table (31): Relation between s1/m1 status and pathological findings among symptomatic *H. pylori* positive children (n=50)

	s1m1				χ^2	p
	-ve (n = 38)		+ve (n = 12)			
	No.	%	No.	%		
Severity of Infl						
Mild	23	60.5	2	16.7	7.018*	0.008*
Severe	15	39.5	10	83.3		
Depth of infl						
Superficial	25	65.8	2	16.7	8.860*	0.003*
Deep	13	34.2	10	83.3		
Activity						
No Activity	24	63.2	4	33.3	3.292	0.070
Active	14	36.8	8	66.7		
Lymphoid follicles						
Yes	22	57.9	11	91.7	4.635*	^{FE}p= 0.039*
No	16	42.1	1	8.3		
Eosinophils						
Yes	1	100.0	1	100.0	-	-
No	0	0.0	0	0.0		

χ^2: value for Chi square
FE: Fisher Exact test
*: Statistically significant at $p \leq 0.05$

Table (32): Relation between VacA alleles and histpathological finding among symptomatic *H. pylori* positive children

	VacA alleles							
	s1/m1 (n=12)		s1/m2 (n=2)		s2/m1 (n=2)		s2/m2 (n=19)	
	No.	%	No.	%	No.	%	No.	%
Severity of Infl								
Mild	2	16.7	1	50.0	1	50.0	8	42.1
Severe	10	83.3	1	50.0	1	50.0	11	57.9
Depth of infl								
Superficial	2	16.7	1	50.0	2	100.0	12	63.2
Deep	10	83.3	1	50.0	0	0.0	7	36.8
Activity								
No Activity	4	33.3	0	0.0	2	100.0	12	63.2
Active	8	66.7	2	100.0	0	0.0	7	36.8
Lymphoid follicles								
Yes	11	91.7	2	100.0	1	50.0	12	63.2
No	1	8.3	0	0.0	1	50.0	7	36.8
Eosinophils								
Yes	1	100.0	0	0.0	0	0.0	1	100.0
No	0	0.0	0	0.0	0	0.0	0	0.0

Table (33): Relation between babA2 status and histopathological findings among symptomatic *H. pylori* positive children (n=50)

	BabA2				χ^2	P
	-ve (n = 20)		+ve (n = 30)			
	No.	%	No.	%		
Severity of Infl						
Mild	10	50.0	15	50.0	**0.0**	1.000
Severe	10	50.0	15	50.0		
Depth of infl						
Superficial	10	50.0	17	56.7	**0.215**	0.643
Deep	10	50.0	13	43.3		
Activity						
No Activity	9	45.0	19	63.3	**1.637**	0.201
Active	11	55.0	11	36.7		
Lymphoid follicles						
Yes	13	65.0	20	66.7	**0.015**	0.903
No	7	35.0	10	33.3		
Eosinophils						
Yes	2	100.0	0	0.0	-	-
No	0	0.0	0	0.0		

χ^2: Chi square test

Clinical presentations in symptomatic *H. pylori* positive children and triple positive combination:

Table 34 shows that, the triple positive combination (simultaneous presence of babA2+cagA+S1) is present in 5 of the symptomatic cases but not present in the Asymptomatic *H. pylori* positive children.

Table (34): Comparison between the symptomatic *H. pylori* positive cases and the asymptomatic children according to triple positive gene combination

	symptomatic *H. pylori* positive (n = 50)		Asymptomatic *H. pylori* positive (n = 25)		Total (n = 75)		χ^2	p
	No.	%	No.	%	No.	%		
babA2 + cagA + S1 positive	5	10.0	0	0.0	**5**	**6.7**	2.679	$^{FE}p=0.162$

FE: Fisher Exact test

*: Statistically significant at $p \leq 0.05$