IL-8 heterozygous polymorphism in children with H. pylori

By

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Abstract:

Background & Aim: Helicobacter pylori infection is a common problem in pediatric practice. Although the organism is thought to be responsible for many diseases, only a handful of them have a direct causal relationship. This work aimed to investigate the association between interleukin-8 (IL-8) -251 T/A and +781 C/T polymorphism and Helicobacter pylori (H. pylori) infection in children. **Methods:** This cross section study was conducted on 60 children with gastritis caused by H. pylori-infection and 60 gastritis children patients without H. pylori infection as a control group. All included patients were subjected to full history taking, complete clinical examination, abdominal ultrasound, laboratory assessment and upper GIT endoscopy, histopathology of gastric and duodenal biopsies and assessment of IL-8 -251 A/T and +781 C/T polymorphism**. Results:** IL-8 (-251 and +781) genotype polymorphism, were significantly associated with H.pylori infection. Patients with IL-8 (-251) A/T mutant type and patients with IL-8 (+781) C/T mutant type had statistically higher grades of chronic inflammation in stomach and higher frequencies of positive polymorph nuclear cell activity. Multivariate regression model showed that positive H. pylori stool antigen, mutant IL-8-251A/T and mutant IL-8+781C/T, were significant predictors of gastritis in the study participants. **Conclusion:** Heterozygous gene polymorphisms (-251 A/T and +781 C/T) was associated with the risk of developing gastritis in children with H. pylori infection. IL-8-251A/T and +781 C/T were significantly associated with severe gastritis in comparison with patient without H.pylori & gastritis.

Keywords: Interleukin-8; polymorphism; Helicobacter Pylori; children

Introduction

Helicobacter pylori infection affects more than half of the world population and it occurs generally in childhood. It is associated with gastroduodenal ulcer, gastric atrophy, intestinal metaplasia, gastric adenocarcinoma and lymphoid tissue-associated lymphoma. It is difficult to eradicate this bacterium due to its high antimicrobial resistance. In children, the infection is asymptomatic in the majority of cases and complications are less common (1).

Helicobacter pylori infection is a common problem in pediatric practice, and its acquisition is related with poor socioeconomic conditions. Although the organism is thought to be responsible for many diseases, only a handful of them have a direct causal relationship. At present, only a small number of children with well-defined clinical syndromes are benefited from testing and treatment. The treatment should include at least two antibiotics with a proton pump inhibitor (2).

Initiation of H. pylori causes an inflammatory reaction in the mucosa of the stomach; that is defined by the increase of production of cytokines, such as interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha (TNF-), and is mediated by neutrophils and mononuclear cells. These cytokines produce reactive oxygen species (ROS) in the gastric mucosa. Gastric mucosal injury results from ROS damaging vital biomolecules like DNA, lipids, and proteins during the inflammatory response. On the other hand, ROS also cause the production of the cytotoxic molecule malondialdehyde (MDA). Nitric oxide (NO) and prostaglandin E2 are upregulated in conjunction with this inflammatory response and are brought on by the increased expression of NO synthase and cyclooxygenase-2 (COX-2). Chronic H. pylori infection alters gastric acid secretion, which may have an impact on both children and adults' gastric microbiota (3).

Interleukin-8 (IL-8), which is a member of C-X-C chemokine subfamily, is an important activator and chemoattractant for neutrophils and has been implicated in a variety of inflammatory diseases. Numerous reports show that various cells express IL-8 mRNA and produce IL-8 protein

rapidly, including monocytes, T lymphocytes, neutrophils, fibroblasts, endothelial cells and epithelial cells (4).

IL-8 gene is located on chromosome 4q13-21 and consists of four exons, three introns, and a proximal promoter region. It's gene coding exhibits several functional polymorphisms, fifteen of them have been characterized. *IL-8* polymorphisms of -251T/A and +781C/T are located on the promoter and intron 1 regions, respectively (5). Several SNPs have been reported in the IL-8 gene and some of them, such as −251 T/A (rs4073) and +781 C/T (rs2227306), can regulate the IL-8 production (6).

This work aimed to investigate the association between interleukin-8 (IL-8) -251 T/A and +781 C/T polymorphism and Helicobacter pylori infection in children.

Ethical consideration

- The study protocol was approved by the Ethics Committee of the Benha Faculty of Medicine **(No: Ms.30-10-2022).**
- Informed consents were obtained from all participants' parents before their enrollment.
- The patient has the right to refuse the study.
- No conflict of interest regarding the study or publication .
- No fund regarding the study or publication.
- Confidentially of the results & data of the patient.

Sample size equation:

The sample size for this study was adapted from the methodology of a previous study by Supriatmo et al., which investigated the association between interleukin-8 (-251 T/A and +781 C/T) polymorphisms and the risk of Helicobacter pylori-induced gastritis in children in a case-control design. Their study used a similar sample size of 60 cases (H. pylori-positive) and 60 controls (H. pylori-negative), calculated based on the expected prevalence of IL-8 polymorphisms in each group, aiming to detect a moderate effect size with 80% statistical power and a significance level of 0.05.

Inclusion criteria

Children age between 1 and 18 years.

Children with dyspeptic symptoms that motivate upper GIT endoscopy as

Abdominal and/or epigastric pain.

Nausea.

Vomiting.

Heartburn.

Exclusion criteria

Any patient with history of non steroidal anti inflammatory drugs intake .

Any cause of gastritis other than H. pylori.

Clinical or laboratory signs suggestive of any infectious diseases that leads to (fever, leukocytosis, positive acute phase reactants).

Patients and methods

This is a prospective cross section study that was conducted on 120 children. They were recruited from the attendance of Pediatrics Gastroenterology and Hepatology Unit in Benha University Hospitals, during the period from November 2022 to August 2024.

This study included 2 group:

Group 1: H. pylori +ve group; included 60 children with gastritis caused by H. pylori-infection (31 males and 29 females), their mean age was 9.17 ± 3.4 years

Group 2: H. pylori -ve group; included 60 children with gastritis without H. pylori infection (22 males and 38 females), their mean age was 10.05 ± 1.74 years.

All included patients were subjected to the following:

- Full history taking, with stress on personal H., GIT symptoms, family history of H.P. infection.
- Complete clinical examination, including general examination as; vital signs and anthropometric measurement, systemic examination; abdominal, chest, Ht.& neurological. Ex.
- Laboratory assessment as complete blood count, ESR, stool analysis and culture, urine analysis and culture, H. pylori stool Ag test.
- Abdominal ultrasound.

- Upper GIT endoscopy and histopathology of gastric and doudenal biopsies.
- Assessment of IL-8 -251 A/T and +781 C/T polymorphism was done for all patients according to the manufacturer's instructions.

Sample collection:

A venous blood sample (2 mls) were withdrawn from each subject and were collected into sterile ethylene diaminetetra acetate "EDTA" (vacutainer) tube and were used for DNA extraction. DNA was extracted from fresh samples and stored at -20 °C till time of assay for determination of IL-8 -251 A/T and +781 C/T polymorphism by using real time Polymerase chain reaction (rt PCR) technique.

DNA Extraction:

The blood samples were used for DNA extraction using the Gene JET Whole Blood Genomic DNA purification Mini Kit (Thermo Fisher Scientific, Germany) according to the manufacturer's protocol**.**

Determination of DNA concentration:

Using Nanodrop One spectrophotometer (thermoscientific, USA). A concentration of (1µg) from each sample was used for genotyping by rtPCR assay.

Genotyping by q PCR:

Genotyping of IL-8 -251 A/T and +781 C/T polymorphism was performed using TaqMan Predesigned SNP Genotyping Assay (Applied Biosystems, USA) and TaqMan Universal PCR Master Mix, No AmpErase UNG, using the following primers:

IL8 -251T/A forward primer 5'-ATTGGCTGGCTTATCTTCA-3'; reverse primer 5'-CAAATACGGAGTATGACGAAAG-3'

IL8 +781C/T forward primer 5'-GTGGTATCACAGAGGATTATGC-3'; reverse primer 5'-CAGTCATAACTGACAACATTGATC-3'.

IL-8 -251 A/T polymorphism; Step One plus allelic discrimination software was used for calculation of normalized dye fluorescence (ΔRn) for Allele T (wild-type) or Allele A (mutant). The software makes an automatic call of either allele T (homozygous T/T), Allele A (homozygous A/A) or heterozygous (A/T).

IL-8 +781 C/T polymorphism; Step One plus allelic discrimination software was used for calculation of normalized dye fluorescence (ΔRn) for Allele C (wild-type) or Allele T (mutant). The software makes an automatic call of either allele C (homozygous C/C), Allele T (homozygous T/T) or heterozygous (C/T).

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 soft ware (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages, Chi square (χ^2) and Fisher's exact tests were used to analyze them, odds ratio (OR) and the corresponding 95%CI were calculated when appropriate. Quantitative data were tested for normality using Shapiro-Wilks test assuming normality at P>0.05. Normally distributed variables were expressed as mean ±standard deviation and analyzed by Student "t"for 2 independent groups, while non parametric data were presented as median and range, and analyzed by Kruskal Wallis (KW) test for 3 independent groyups. Binary logistic regression analysis was used to detect the significant predictors of H .pylori. P ≤0.05 was considered significant. Genotype distributions in the studied groups were in Hardy-Weinberg equilibrium for gene polymorphisms (data not shown).Hardy-Weinberg equilibrium was calculated using Simple Hardy-Weinberg Calculator-Court Lab (Washington State University College of Veterinary Medicine, Pullman, WA, USA). Hardy-Weinberg equilibrium was calculated according to OEGE - Online Encyclopedia for Genetic Epidemiology studies**.**

Results

Our results will be demonstrated in the following tables.

Table 1: Demographic data of the studied groups.

		H. pylori +ve group (n = 60)	H. pylori-ve group (n=60)	Statistical test	P value
Age (year)	**Mean ± SD**	9.17 ± 3.4	10.05±1.74	U= 1620	0.341
	Range	5 – 14	6-12		
Sex	**Male**	31 (52%)	22 (37%)	X^2 = 2.74	0.098
	Female	29 (48%)	38 (63%)		
Residence	**Urban**	23 (38%)	36 (60%)	X^2 =5.64	**0.018***
	Rural	37(62%)	24(40%)		
Consanguinity	**Yes**	25 (41%)	17 (28.3%)	X^2 = 1.79	0.180
	No	35 (58.3%)	43 (71.6%)		

This table show that there was no statistically significant difference in age, sex, and consanguinity between the studied groups. The number of patients living in rural area in H. pylori +ve group was statistically significantly higher compared to H. pylori -ve group (p=0.018).

Table 2: Clinical presentation in the studied groups

	H. pylori +ve group (n =60)	**H. pylori -ve group (n =60)**	**Statistical test**	**P value**
Nausea	46 (76.7%)	22 (36.7%)	χ^2 =19.5	**<0.001***
Vomiting	37 (61.7%)	15 (25.0%)	χ^2 =16.4	**<0.001***
Abdominal distention	19 (31.7%)	17 (28.3%)	χ^2 =0.16	0.690
Anorexia and weight loss	21 (35.0%)	11 (18.3%)	χ^2 =4.26	**0.039***
Heart burn	43 (71.7%)	38 (63.3%)	χ^2 =0.94	0.329
GIT bleeding	9 (15.0%)	14 (23.3%)	χ^2 =1.34	0.246
Diarrhea	7 (11.7%)	11 (18.3%)	χ^2 =1.05	0.306
Constipation	8 (13.3%)	13 (21.7%)	χ^2 =1.4	0.229
Anemia	38 (63.3%)	27 (45.0%)	χ^2 =4.06	**0.044***
Faltering growth	28 (46%)	16 (26.7%)	χ^2 =4.34	**0.037***

This table shows that there is significant difference between the two studied groups regarding nausea, vomiting, anorexia, weight loss & anemia.

Table 3: Laboratory and radiological investigations of the studied groups

		H. pylori +ve group (n =60)	H. pylori -ve group (n =60)	Statistical test	P value
Hb (g/dL)	**Mean ± SD**	11.6 ± 1.3	12.6 ± 2.5	U =1318	**0.011***
	Range	8.1 – 13	7.6 – 15.8		
MCV (fl)	**Mean ± SD**	75.4 ± 3.8	79.5 ± 7	U =1124	**<0.001***
	Range	58 - 80	69 – 83		
MCH (pg/cell)	**Mean ± SD**	26.5 ± 2.9	26.1 ± 3.5	U =1577	0.238
	Range	17 – 30	18 - 33		
WBCs $\times 10^3$ (cells/µL)	**Mean ± SD**	6.7 ± 2.1	6.4 ± 1.8	U =2142	0.534
	Range	4 – 9.9	3.9 – 10.6		
PLT $\times 10^6$ (cells/µL)	**Mean ± SD**	275 ± 69.5	289 ± 56.5	U =1454	0.073
	Range	152 - 491	148 - 387		
Stool analysis and culture	**Normal**	51 (85%)	55 (91.7%)	χ^2 =1.75	0.417
	E. histolytica	7 (11.7%)	3 (5%)		
	Meat fibers and vegetables, follicular starch granules from plants	2 (3.3%)	2 (3.3%)		
Abdominal U/S	**Normal**	60 (100%)	60 (100%)	-	-
	Abnormal	0 (0.0%)	0 (0.0%)		

SD: Standard deviation, U: Mann-Whitney test, χ^2: Chi-square test, *Statistically significant as p value <0.05.

This table shows significant difference between both groups regarding Hb% & M.C.V, while no statistical difference regarding the other lab. Findings.

Table 4: Upper GIT endoscopy and histopathological findings in the studied groups

Upper GIT endoscopy		H. pylori +ve group (n =60)	H. pylori -ve group (n =60)	Statistical test	P value
Esophagus	**Normal**	42 (70%)	49 (81.7%)	χ^2 =1.64	0.201
	Esophagitis	18 (30%)	11 (18.3%)		
Stomach	**Normal**	0 (0.0%)	17 (28.3%)	χ^2 =19.8	**<0.001***
	Mild hyperemia	36 (60%)	32 (53.3%)	χ^2 =0.54	0.461
	Moderate hyperemia	21 (35%)	11 (18.3%)	χ^2 =4.26	**0.039***
	Ulcer	3 (5%)	0 (0%)	χ^2 =3.08	0.079
Duodenum	**Normal**	32 (53.3%)	53 (88.3%)	χ^2 =16.1	**<0.001***
	Duodenitis	28 (46.7%)	7 (11.7%)		
Histopathological findings					
Chronic inflammation grade	**Normal**	0 (0.0%)	15 (25.0%)	χ^2 =38.4	**<0.001***
	Mild chronic active gastritis	19 (31.7%)	27 (45.0%)		
	Moderate chronic active gastritis	19 (31.7%)	18 (30.0%)		
	Severe chronic active gastritis	22 (36.7%)	0 (0.0%)		
Polymorph nuclear cell activity	**Present**	52 (86.7%)	10 (16.7%)	χ^2 =56.1	**<0.001***
	Absent	8 (13.3%)	50 (83.3%)		
Atrophy	**Present**	0 (0%)	0 (0%)	-	-
	Absent	60 (100%)	60 (100%)		
Metaplasia	**Present**	0 (0%)	0 (0%)	-	-
	Absent	60 (100%)	60 (100%)		

χ^2: Chi-square test, *Statistically significant as p value <0.05.

This table shows that there is significant difference regarding endoscopic finding in stomach, duodenum & chronic inflammatory changes with polymorph nuclears cell activity, between both studied groups.

Table 5: Interleukin-8 polymorphism (+781) and (-251) genotypes in the studied groups

		H. pylori +ve group (n =60)	H. pylori -ve group (n =60)	Statistical test	P value
IL-8 (+781) genotype	**CC (Wild)**	13 (21.7%)	44 (73.3%)	χ^2 =32.1	**<0.001***
	TT (Homozygous)	4 (6.7%)	4 (6.7%)	-	1.000
	CT (Heterozygous)	43 (71.7%)	12 (20.0%)	χ^2 =32.3	**<0.001***
Allele	**C**	69 (57.5%)	100 (83.3%)	χ^2 =18	**<0.001***
	T	51 (42.5%)	20 (16.7%)		
IL-8 (-251) genotype	**TT (Wild)**	9 (15%)	33 (55.0%)	χ^2 =21.1	**<0.001***
	AA (Homozygous)	10 (16.7%)	7 (11.7%)	χ^2 =0.62	0.432
	AT (Heterozygous)	41 (68.3%)	20 (33.3%)	χ^2 =14.7	**<0.001***
Allele	**A**	61 (50.8%)	34 (28.3%)	χ^2 =11.8	**<0.001***
	T	59 (49.2%)	86 (71.7%)		

χ^2: Chi-square test, *Statistically significant as p value <0.05.

this table shows that there is significant difference between both studied groups regardingIL-8(+781)genotype C.C ,C.T ,allele C.T.also genotype IL.8 -281, TT(wild) ,AT(heterozygous)&allele A-Twas significantly different between both groups.

Table 6: Upper GIT endoscopy and histopathological findings in correlation to the presence of mutations in IL-8 (+781).

Upper GIT endoscopy		IL-8 (+781)CC wild type (n =57)	IL-8 (+781)CT mutant type (n =63)	Statistical test	P value
Esophagus	**Normal**	44 (77.2%)	47 (74.6%)	$\chi^2 = 0.11$	0.741
	Esophagitis	13 (22.8%)	16 (25.4%)		
Stomach	**Normal**	13 (22.8%)	4 (6.3%)	$\chi^2 = 5.38$	**0.02***
	Mild hyperemia	31 (54.4%)	37 (58.7%)	$\chi^2 = 0.08$	0.768
	Moderate hyperemia	13 (22.8%)	19 (30.2%)	$\chi^2 = 0.49$	0.482
	Ulcer	0 (0.0%)	3 (4.8%)	$\chi^2 = 1.17$	0.278
Duodenum	**Normal**	45 (78.9%)	40 (63.5%)	$\chi^2 = 3.46$	0.063
	Duodenitis	12 (21.1%)	23 (36.5%)		
Histopathological findings					
Chronic inflammation grade	**Normal**	9 (15.8%)	6 (9.5%)	$\chi^2 = 16.09$	**0.001***
	Mild chronic active gastritis	26 (45.6%)	20 (31.7%)		
	Moderate chronic active gastritis	20 (35.1%)	17 (27.0%)		
	Severe chronic active gastritis	2 (3.5%)	20 (31.7%)		
Polymorph nuclear cell activity	**Present**	18 (31.6%)	44 (69.8%)	$\chi^2 = 17.5$	**<0.001***
	Absent	39 (68.4%)	19 (30.2%)		
Atrophy	**Present**	0 (0%)	0 (0%)	-	-
	Absent	57 (100%)	63 (100%)		
Metaplasia	**Present**	0 (0%)	0 (0%)	-	-
	Absent	57 (100%)	63 (100%)		

χ^2: Chi-square test, *Statistically significant as p value <0.05.

This table show that patients with IL-8 (+781) CT mutant type had statistically higher frequencies of positive H. pylori stool antigen, , higher number of patients with severe gastritis and patients with PMNC activity compared to patients with IL-8 (+781) CC wild. While there

no statistical significance difference between patients with IL-8 (+781) CC wild type and IL-8 (+781) CT mutant type regarding esophagus and duodenum endoscopy findings.

Table 7: Upper GIT endoscopy and histopathological findings in correlation to the presence of mutations in IL-8 (-251)

Upper GIT endoscopy		**IL-8 (-251)TT wild type (n =42)**	**IL-8 (-251)AT mutant type (n =78)**	**Statistical test**	**P value**
Esophagus	**Normal**	33 (78.6%)	58 (74.4%)	$\chi^2 = 0.26$	0.607
	Esophagitis	9 (21.4%)	20 (25.6%)		
Stomach	**Normal**	11 (26.2%)	6 (7.7%)	$\chi^2 = 6.24$	**0.013***
	Mild hyperemia	21 (50.0%)	47 (60.3%)	$\chi^2 = 0.79$	0.374
	Moderate hyperemia	10 (23.8%)	22 (28.2%)	$\chi^2 = 0.09$	0.762
	Ulcer	0 (0.0%)	3 (3.8%)	$\chi^2 = 0.45$	0.5
Duodenum	**Normal**	32 (76.2%)	53 (67.9%)	$\chi^2 = 0.89$	0.343
	Duodenitis	10 (23.8%)	25 (32.1%)		
Rapid urease test	**Positive**	9 (21.4%)	51 (65.4%)	$\chi^2 = 21.09$	**<0.001***
	Negative	33 (78.6%)	27 (34.6%)		
Histopathological findings					
Chronic inflammation grade	**Normal**	8 (19.0%)	7 (9.0%)	$\chi^2 = 8.4$	0.045*
	Mild chronic active gastritis	18 (42.9%)	28 (35.9%)		
	Moderate chronic active gastritis	12 (28.6%)	25 (32.1%)		
	Severe chronic active gastritis	4 (9.5%)	18 (23.1%)		
Polymorph nuclear cell activity	**Present**	13 (31.0%)	49 (62.8%)	$\chi^2 = 11.1$	**<0.001***
	Absent	29 (69.0%)	29 (37.2%)		

Atrophy	**Present**	0 (0%)	0 (0%)	-	-
	Absent	42 (100%)	78 (100%)		
Metaplasia	**Present**	0 (0%)	0 (0%)	-	-
	Absent	42 (100%)	78 (100%)		

χ^2: Chi-square test, *Statistically significant as p value <0.05.

This table show that patients with IL-8 (-251)AT mutant type had statistically higher frequencies of H. pylori stool antigen , positive rapid urease test, had statistically higher grades of chronic inflammation in stomach and higher frequencies of positive polymorph nuclear cell activity compared to patients with IL-8 (-251) TT wild type. While there no statistical significance difference between patients with IL-8 (-251)TT wild type and IL-8 (-251)AT mutant type regarding esophagus and duodenum endoscopy findings

Table 8: Univariate and multivariate regression analysis of the factors that can predict the occurrence of gastritis in the study participants

	Univariate analysis		**Multivariate analysis**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Abdominal pain relieved by antacid	3.22 (1.11 – 10.73)	**0.039***	1.87 (0.88 – 10.6)	0.09
Positive H. pylori stool antigen	6.59 (2.15 – 24.8)	**0.002***	3.96 (1.15 – 16.3)	**0.037***
Mutant IL-8+781 C/T	4.36 (1.4 – 16.3)	**0.015***	2.9 (0.52 –7.89)	**0.042***
Mutant IL-8-251A/T	4.26 (1.5 – 13.3)	**0.008***	3.19 (1 – 11.1)	**0.027***

*Statistically significant as p value <0.05.

This table show that in univariate regression analysis, all patients assessed factors were analyzed and only abdominal pain relieved by antacid, Positive H. pylori stool antigen, mutant IL-8+781C/T, and mutant IL-8-251A/T were significant predictors of gastritis in the study participants. All significant predictors in univariate analysis were analyzed in multivariate regression model and only positive H. pylori stool antigen and mutant IL-8+781C/T, and mutant IL-8-251A/T were significant predictors of gastritis in the study participants.

Discussion

Interleukin 8 (IL-8) plays an important role in the pathogenesis of *H pylori* infection. IL-8, a major host mediator inducing neutrophil chemotaxis and neutrophil activation, is produced by gastric epithelial cells as an early response to *H pylori* infection. IL-8 is also considered to attract and activate phagocytes and cause mucosal damage by releasing reactive oxygen radicals (8).

In the present study, regarding IL-8 (+781) genotype polymorphism, the number of patients who were CT (Heterozygous) genotype was statistically significantly higher in H. pylori +ve group compared to H. pylori -ve group, but the number of patients who were CC (Wild) genotype was statistically significantly higher in H. pylori -ve group compared to H. pylori +ve group. the prevalence of T allele was significantly higher in H. pylori +ve group compared to H. pylori -ve group. Regarding IL-8 (-251) genotype polymorphism, the number of patients who were AT genotype was statistically significantly higher in H. pylori +ve group compared to H. pylori -ve group, but the number of patients who were TT genotype was statistically significantly higher in H. pylori -ve group compared to H. pylori +ve group. There was no statistically significant difference in AA genotype between both studied groups. The prevalence of T allele was significantly higher in H. pylori -ve group compared to H. pylori +ve group, and the prevalence of A allele was significantly higher in H. pylori +ve group compared to H. pylori -ve group.

Our results were also agreed with Ramis et al., (11), who studied polymorphisms of the IL-6, IL-8 and IL-10 genes and the risk of gastric pathology in patients infected with Helicobacter pylori, observed that the presence of the A allele at position −251 of the IL-8 gene was significantly associated with H. pylori infection. Also Chang et al. (12), showed that having IL-8 -251 AT polymorphism increased risk in developing severe gastritis.

In contrast to Naito et al., (13), who studied associations of plasma IL-8 levels with Helicobacter pylori seropositivity, gastric atrophy, and IL-8 T-251A genotypes, showed that the IL-8 levels in A allele carriers of the IL-8 T-251A polymorphism were slightly higher than in subjects with the T/T genotype but the difference was not statistically significant (mean: 17•5 and

17•0 pg/ml, respectively), p>0.05. And Taguchi et al. (14), did not find any association between IL-8 -251 AT polymorphism with atrophic gastritis and also gastric cancer. Also, Xue et al., (15) showed in a meta-analysis that the IL-8 -251AA genotype is not associated with the H. pylori infection status. However, Xue found that this genotype is associated with the overall risk of developing gastric cancer.

Song et al., (9)**,** who studied association of interleukin-8 with cachexia from patients with low-third gastric cancer, showed that the serum level of IL-8 and its +781 C/T polymorphism have a significant association with the presence of cachexia in low-third gastric cancer patients (*P* = .009), and a significantly increased frequency of +781 T allele was noted in patients with cachexia (OR = 2.247, 95% CI: 1.351–3.737, *P* = .002). The +781 TT genotypes were observed to be associated with a significantly increased risk of cachexia (OR = 3.167, 95% CI: 1.265–7.929, *P* = .011), and with odds ratio of 3.033 (95% CI: 1.065–8.639, *P* = .038) for cachexia after adjusting for patients' actual weight and carcinoma stage.

In the current study, In univariate regression analysis, all patients assessed factors were analyzed and only abdominal pain relieved by antacid, Positive H. pylori stool antigen, mutant IL-8+781C/T, and mutant IL-8-251A/T were significant predictors of gastritis in the study participants. All significant predictors in univariate analysis were analyzed in multivariate regression model and only positive H. pylori stool antigen and mutant IL-8+781C/T, and mutant IL-8-251A/T were significant predictors of gastritis in the study participants.

H. pylori-induced gastric mucosal inflammation is mediated by an array of pro- and anti-inflammatory cytokines. In general, studies show that this inflammation is exacerbated in patients with a high production of alleles of proinflammatory cytokines and a low production of alleles of anti-inflammatory cytokines, which result in a higher risk of peptic ulcer or gastric carcinoma. Ulcer occurs because of a disequilibrium between defensive mucosa-protective factors and aggressive injurious factors; carcinogenesis occurs because of the accumulation of genetic alterations and the dysfunction of cellular mechanisms that normally maintain human genome integrity (10)

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Conclusion

Our results showed that heterozygous gene polymorphisms (-251 AT and +781 CT) was associated with the risk of developing gastritis in children with H. pylori infection. IL-8-251A/T and +781 CT were significantly associated with severe gastritis.

Limitation

Some limitations of this study should be noted. This was a single center study with a relative small sample size, which didn't allow for a better analysis, also this study did not perform any follow-up of esophagogastroduodenoscopy (EGD) after the establishment of the diagnosis and eradication therapeutic interventions.

Recommendations

- Larger metacentric studies are warranted to approve these results
- Future studies should assess the role of these mutations in respond to treatment and in complications

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