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The Interleukin-8 -251 A Allele is Associated with Increased Risk of Different Gastroduodenal Diseases in *H. pylori* Infected Bangladeshi Patients

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Abstract Persistent *Helicobacter pylori* infection leads to chronic inflammation of the gastric mucosa which is mediated by various inflammatory cytokines. Host susceptibility along with bacterial virulence factors are also regarded as contributing factor of developing severe *H. pylori* induced gastroduodenal diseases like peptic ulcer diseases and gastric adenocarcinoma. Polymorphisms in genes that code cytokines influence cytokine secretion levels. Of the inflammatory cytokines, Interleukin-8 (IL-8) plays an important role in gastric mucosal inflammation induced by *H. pylori* infection. The IL8 gene has been described as having a polymorphism of an A/T base pair in the promoting region (−251) which is associated with an increased synthesis of interleukin by gastric epithelial cells. So the study was conducted to investigate the association of IL-8–251 A/T polymorphism with gastroduodenal diseases in *H. pylori* infected Bangladeshi patients. **Methods:** Endoscopic gastroduodenal biopsy sample of 113 dyspeptic patients were used (54 was infected with *H. pylori* and 59 was not infected with *H. pylori*). *H. pylori* infection was detected by Rapid urease test, PCR of *ureC* gene and histology. Gastroduodenal disease was diagnosed by histopathological examination. Il-8 gene polymorphism (at -251 position) was detected by Polymerase Chain Reaction restriction fragment length polymorphism. **Result:** A significant association was found between host IL-8 genotypes (T/T, T/A, A/A and A carrier) and the presence of *H. pylori* infection (p =0.001). The IL-8 A allele carriers infected with *H. pylori* had an increased risk of developing gastritis (p =0.003) and peptic ulcer diseases (p= .002). We did not find a correlation between IL-8 gene polymorphism and a higher risk of gastric carcinoma and or precancerous lesions. **Conclusion:** The *H. pylori* infected patients carrying A allele may increase risk of developing gastritis and peptic ulcer disease in Bangladeshi patients.

Keywords: *Helicobacter pylori infection, gastroduodenal disease, Interleukin-8, Genetic polymorphism*

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1. Introduction

The spiral-shaped Gram negative bacteria *Helicobacter pylori* colonize specially in the human gastric mucosa and may causes a variety of clinical outcomes ranging from asymptomatic carriage to gastritis, peptic ulcers, and cancer [1]. However, less than 20% of infected patients present with clinical symptoms suggesting that, the variation in the clinical outcome of *H. pylori* induced pathology is multifactorial, involving a complex interplay between the host immune responses, pathogen virulence factors, and niche characteristics [2]. The key pathophysiological event in *H. pylori* infection of gastric mucosa is the induction of a gastric mucosal inflammatory response. Since *H. pylori* is a noninvasive bacterium, the direct interaction of the bacterium with the epithelium or soluble products from the bacterium may initiate the inflammatory process by inducing the production of pro-inflammatory cytokines [e.g. interleukin (IL)-1β, IL-2, IL-6, IL-8 and tumor necrosis factor (TNF)-α) ([3,4]) those are released from neighboring neutrophils and mononuclear cells that are activated by *H. pylori*.

Of the many cytokines that can be induced by a bacterial infection; chemokines contribute to the pathologic changes seen in inflammation following the contact of bacteria with the epithelium [5]. Interleukin- 8, a member of the CXC chemokine family is a potent chemoattractant for neutrophils and lymphocytes, induces cell proliferation, migration and angiogenesis ([6,7]). IL-8 is also secreted by gastric epithelial cells; which has been suggested to be an important factor in the immunopathogenesis of peptic ulcer disease and involved in gastric carcinogenesis [8]. So this cytokine plays an important role in the initiation, modulation and maintenance of the gastrointestinal inflammatory responses.

Genetic polymorphisms related to some inflammatory cytotoxins have been studied and associated with an increase in the synthesis of these interleukins. The IL-8

gene has been described as having a polymorphism of an A/T base pair in the promoting region (−251) which is associated with an increase in the synthesis of this interleukin by gastric epithelial cells ([9,10]).

Several studies conducted in some coutries have found association of IL-8–251 A/T polymorphism with *H. pylori* induced gastric carcinogenesis and peptic ulcer disease ([11,14]) whereas some countries did not find any significant relationship between IL-8–251 A/T polymorphism and risk of gastric cancer ([15,16]). These conflicting results suggest that IL-8–251 A/T polymorphism may be differently associated with gastric carcinogenesis depending on the ethnicity [17].

So, it is important to know the pattern of IL-8 gene polymorphism in Bangladeshi population for identifying the *H. pylori* infected patients who tend to develop severe gastroduodenal diseases. Early identification of persons having IL-8 gene polymorphism and proper treatment can reduce the severity of diseases. So the study was designed to see the association of host interleukin-8 gene polymorphism with gastroduodenal disease severity in *H. pylori* infection.

2. Methods

2.1. Patients and Gastric Biopsy Samples:

The present study included 113 Bangladeshi patients (72 men and 41 women with a mean age of 39.4± 12.8 years) who underwent upper gastrointestinal endoscopic examination at outpatient department of Department of Gastroenterology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka, and Dhaka Medical college Hospital, Dhaka. Out of 113 patients 65 patients were endoscopically reported as gastritis followed by 18 duodenal ulcer, 17 gastric ulcer and 13 gastric carcinoma. Patient aged from 18 to onward presenting with symptoms of **dyspepsia** more than 1 month were included in the study. Patients who received *H. pylori* eradication treatment in the previous 2 months [18], Elderly individuals who had age more than 65 years, had severe medical or surgical illnesses or had used proton pump inhibitors, nonsteroidal anti-inflammatory drugs, colloidal bismuth compounds, or antibiotics within 4 weeks of enrollment were excluded from the study [17].

The study populations were categorized into 2 groups based on the case definition used in this study: *H. pylori* positive patients (n = 54) & *H. pylori* negative patients (n = 59). Patients were considered as *H. pylori* positive when positive results were obtained in at least two of the three tested methods (Rapid urease test, histology for *H. pylori* and PCR for *ureC* gene) and considered as negative when the results of all diagnostic tests were negative. Gastroduodenal diseases was diagnosed by endoscopic and histopathological examinations and established in accordance with the Sydney System Classification.

Three pieces of gastric tissue were taken from nonlesional mucosa of the lesser curvature side of the antrum and midbody from each patient for *H. pylori* detection. Additional biopsies were taken from margins of malignant looking ulcers or proliferative growths for histopathological examination to confirm the diagnosis. Two specimen, each from the antrum and body were fixed in 10% buffered formalin and send to the Pathology Department of BSMMU and DMC hospital for histopathological examination. One specimen each from the body and antrum were examined for the presence of *H. pylori* by rapid urease test and one specimen from the antrum and body were preserved in 1.5ml microcentrifuge tube containing 1 ml phosphate buffer solution for detection of *H. pylori ureC* gene and host IL-8 gene polymorphism by PCR and PCR-RFLP. All biopsy samples were stored at -20°C until DNA extraction from the samples were performed.

2.2. DNA Extraction and PCR for ureC Gene

DNA from gastric tissues was extracted by using the QlAamp (QIAGEN®) DNA Mini Kit according to the manufecturer's instructions as mentioned by Akada et al. [19] , Salih et al. [20]. For confirming the presence of *H. pylori* DNA in tissue, the *ureC* gene was identified by PCR using the following primers [21]

Forward primer:

5′ - AAGCTTTTAGGGGTGTTAGGGGTTT -3′

Reverse primer:

5′- AAGCTTACTTTCTAACACTAACGC -3′.

The DNA were denatured at 94°C for 5 minutes, followed by 35 cycles at 93°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute with a final extension at 72°C for 10 minutes.The PCR product was analyzed in 2% agarose gel with ethidiurn bromide which was prepared in 1xTAE (Tris Acetic acid EDTA- Ethylene diamine tetra acetic acid) buffer by electrophoresis for 30 minutes to detect specific band of 294 bp.

2.3. PCR-RFLP for IL-8 Gene Polymorphism Analysis

To analyze the polymorphism of the IL-8 gene at position -251, the primers used were

Forward primer: 5′ -TTCTAACACCTGCCACTCTAG-3′

Reverse primer: 5′-CTGAAGCTCCACAATTTGGTG-3′.

The PCR was performed as previously reported in another study [9]. The DNA were denatured at 94°C for 4 minutes, followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds with a final extension at 72°C for 7 minutes.

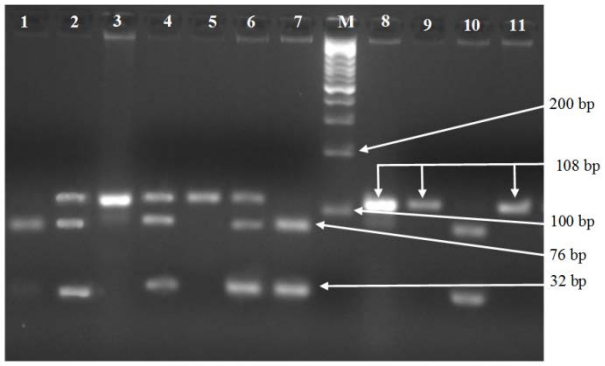

Figure 1. PCR- RFLP analysis of IL-8 (-251) polymorphism by *MfeI* enzyme after gel electrophoresis.(M = ladder marker). Lane 2, 4, 6 indicate **A/T genotype** (3 bands of 108 bp, 76 bp and 32 bp); lane 7 & 10 indicate **A/A genotype** (2 bands of 76 bp & 32 bp) & lane 5, 9 & 11 indicate **T/T genotype (**single band of 108 bp). PCR product of lane 3 & 8 were not digsested by restriction enzyme and show a single band of 108 bp

The PCR products were digested with the restriction enzyme *MfeI*, and then visualized by electrophoresis on 5% agarose gel stained with ethidium bromide. The genotypes were coded as follows: T/T, a single band consisting of 108 bp; T/A, three bands consisting of 108 bp, 76 bp, and 32 bp; and A/A, two bands consisting of 76 bp and 32 bp, as shown in Figure 1.

This work was ethically approved by Institutional Review Board, BSMMU; process number BSMMU/2015/10008. Written, informed consent was obtained from all patients.

2.4. Statistical Analysis:

The sample size was calculated by using the following formula $n = \frac{Z^2 pq}{e^2}$. A 95% confidence interval was used. The seroprevalence of *H. pylori* infection in Bangladesh was 92% and acceptable error was 5%.

The statistical analysis which was based on the creation and categorization of variables was performed by using SPSS version 20.0 (Statistical Program for the Social Sciences Inc., Chicago, USA). The Chi-square test or Fisher's exact test were used to compare between proportions. Values of $p < 0.05$ were considered statistically significant. The effect of IL-8–251 genotypes on the risk of each gastroduodenal disease was expressed as the odds ratio (OR) with a 95% confidence interval (CI) using unconditional logistic regression analysis.

3. Results

Among 113 patients enrolled in this study the female with male female ratio was 2:1. Among the gastritis patients male and female distribution was almost equal (Figure 2). All the study population was in the ages ranging from 18-65 years. The highest rate of *H. pylori* infection was found in the age group from 51 to 60years (27.8%), while the lowest percentage of *H. pylori* infection (5.6%) was in the age group of <20 years (Table 1).

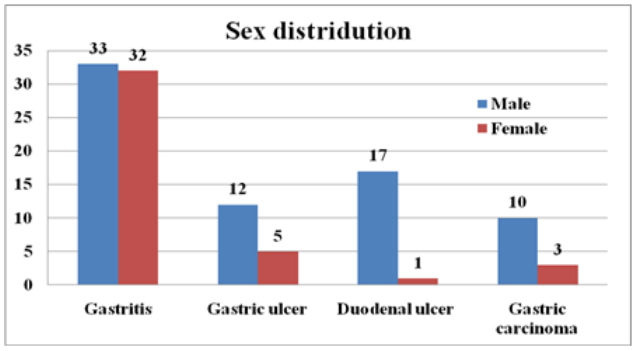

Figure 2. Sex distribution of the study group (*n* = 113) and their endoscopic findings

Table 1. Rate of *H. pylori* infection in different age groups of study population (n=113)

Age Group (Years)	*H. pylori* positive cases n (%)	*H. pylori* negative cases n (%)
< 20	3 (5.6)	2 (3.4)
21 – 30	12 (22.2)	8 (13.6)
31 -40	7 (13)	15 (25.4)
41 – 50	6 (11.1)	12 (20.3)
51 – 60	15 (27.8)	11 (18.6)
>60	11 (20.4)	11 (18.6)
Total (n=113)	**54 (100)**	**59 (100)**

Among the 113 patients, Majority (*n*=61) was histopathologically diagnosed as gastritis followed by duodenal ulcer (*n*=17), gastric carcinoma (*n*=14), chronic gastric ulcer (*n*=12), intestinal metaplasia (n=8) and gastric dysplasia (*n*=1).

For evaluation of results gastroduodenal diseases are categorized into three groups on the basis of histopathological findings: Gastritis (N=61), Peptic ulcer diseases (N=29) (chronic gastric ulcer and duodenal ulcer) and Gastric carcinoma & or pre-cancerous lesions (N=23) (Intestinal metaplasia, Gastric dysplasia and Gastric adenocarcinoma).

Table 2. The frequency of host IL-8 gene polymorphisms at -251 position in *H. pylori* positive cases and *H. pylori* negative cases (*n* =113)

Genotypes IL-8 (-251)	Total cases n(%)	*H. pylori* positive cases n(%)	*H. pylori* negative cases n(%)	Odds Ratio (95% CI)	*P* value[b]
T/T	46(40.7)	9 (16.7)	37 (62.7)	1.0	Ref
T/A	48(42.5)	30 (55.7)	18 (30.5)	6.8 (2.6-7.4)	<.001*
A/A	19(16.8)	15 (27.8)	4 (6.8)	15.4(4.1-57.8)	<.001*
A carrier	67 (59.3)	45 (83.3)	22 (37.3)	8.4 (3.5-20.5)	<.001*
Total n (%)	113(100)	54(100)	59(100)		

(*p* value was compared between IL-8 genotypes and *H. pylori* infection. Risk assessment was done by Binary logistic regression).
A carrier = A/A+A/T; **$p < .001$* [(a)],** df =2, χ^2 =26.2;
p^a was determined by χ^2 test & p^b was determined by binary logistic regression
*= statistically significant.

Table 2 shows the frequency of the polymorphisms of the host Interleukin-8 genes at -251 position in *H. pylori* positive cases and *H. pylori* negative cases. The presence of A allele at position -251 of IL-8 gene (A/A, A/T or A carriers) is significantly associated with susceptibility to *H. pylori* infection ($p < .001^a$, Odds Ratio of T/T=1.0, Odds Ratio of T/A=6.8, Odds Ratio of A/A=15.4 and Odds Ratio of A carrier = 8.4; *p* (T/A) <.001; *p* (A/A) <.001; *p* (A carrier) <.001 [b]).

Table 3 shows the frequency of host IL-8 genotypes in different gastroduodenal disease among *H. pylori* positive cases and *H. pylori* negative patients. the *H. pylori* infected patients and A allele carriers (T/A+A/A genotypes=75%) had an increased risk of developing gastritis(p=.003[a], *p* (T/A) =.064; *p* (A/A) =.003*; *p* (A carrier) =.005* [(b)]; OR of T/T=1.0, OR of T/A=3.1 and *p* =, OR of A/A=15.3 and OR of A carrier=4.9) and peptic ulcer disease (*p* =.002; *p* (T/A) =.005*; *p* (A/A) =.013*; *p*

(A carrier) =.002* [(b)]; OR of T/T=1.0, OR of T/A=22.0, OR of A/A=27.0 and OR of A carrier=23.8).

Table 3. Frequency of host IL-8 genotype in different gastrointestinal diseases among *H. pylori* positive and *H. pylori* negative cases (*n*=113)

Genotypes IL-8 (-251)	Histopathological findings											
	Gastritis *n*=61				Peptic ulcer disease *n*=29				Gastric carcinoma & pre-cancerous lesions *n*=23			
	H.pylori positive n(%)	*H.pylori* negative n(%)	Odds Ratio (95% CI)	*p* value[(b)]	*H.pylori* positive n(%)	*H.pylori* negative n(%)	Odds Ratio (95% CI)	*p* value[(b)]	*H.pylori* positive n(%)	*H.pylori* Negative n(%)	Odds Ratio (95% CI)	*p* value[(b)]
T/T	6(25)	23(62.2)	1.0	**Ref.**	2(13.3)	11(78.6)	1.0	**Ref.**	1(6.7)	3(37.5)	1.0	**Ref.**
T/A	10(41.7)	12(32.4)	3.2 (0.9-10.9)	**.064**	8(53.3)	2(14.3)	22.0 (2.5-191)	**.005***	12(80)	4(50)	9.0 (0.7-113.0)	**.089**
A/A	8(33.3)	2(5.4)	15.3 (2.6-91.9)	**.003***	5(33.3)	1(7.1)	27.5 (2.0-378.8)	**.013***	2(13.3)	1(12.5)	6.0 (0.2-162.5)	**.287**
A carrier	18(75)	14(37.8)	4.9 (1.6-15.4)	**.005***	13(86.7)	3(21.4)	23.8 (3.4-169.4)	**.002***	14(93.3)	5(62.5)	8.4 (0.7-100.6)	**.093**
Total	**24**	**37**			**15**	**14**			**15**	**8**		

(*p* value was compared between lL -8 genotypes*H. pylori* infection. Risk assessment was done by Binary logistic regression)
p^a was determined by χ^2 test & p^b was determined by binary logistic regression
In gastritis patients $\boldsymbol{p^a}$**=.003***, df=2, χ^2=11.5 ; In Peptic ulcer disease patients $\boldsymbol{p^a}$**=.002***, *df* =2, χ^2=12.5
In Gastric carcinoma patients p^a=.171, *df* =2, χ^2 =3.5 ; *df* = degrees of freedom, *= statistically significant.

4. Discussion

The variation of clinical outcomes after exposure to *H. pylori* depends on host pathogen interaction. Moreover, Rad et al. [22] observed that, the cytokine gene expression and susceptibility to infectious disease is influenced by allelic variation in cytokine gene. So determination of allelic variation of host interleukin-8 gene has been suggested by some researchers as a way to predict clinical outcomes of *H. pylori* associated pathologies ([13,17]).

The current study has explored the relation between host Interleukin- 8 gene and different gastroduodenal diseases in Bangladeshi patients. A significant association was found between host IL-8 genotypes (T/T, T/A, A/A and A carrier) and the presence of *H. pylori* infection (p <.001[a], Odds Ratio of T/T=1.0, Odds Ratio of T/A=6.8, Odds Ratio of A/A=15.4 and Odds Ratio of A carrier=8.4[b]) in the present study. This suggests that the presence of T/A and A/A genotypes may be a risk factor of *H. pylori* infection whereas T/T genotype may act as a protective factor against *H. pylori*. Similar findings were observed in Brazil ([11,13]) whereas, no significant association was found between IL-8-251 T / A polymorphism and *H. pylori* infection in some Brazilian and Iranian patients ([23,24]). Xue et al. [25] showed in a meta-analysis that the IL-8 -251 A/A genotype is not associated with the *H. pylori* infection status. A genome wide linkage analysis to identify the host genetic factor for the prevalence of *H. pylori* infection was performed by Thye et al. [26] who observed the possible linkage of the host factors with chromosomes 4 and 6. As the human IL-8 gene is located on chromosome 4 (4q13–q21), the result of their study may support the finding that the IL-8 gene polymorphism is actually associated with *H. pylori* infection.

The IL-8 A carrier was significantly associated with *H. pylori* infected gastritis patients (p=.003[a]) in the present study. Similar association was found in Japan [12] and Hungary ([27,28]) whereas Ramis et al. [13] and Kamali-Servestani et al. [29] found no significant association among gastritis patients.

Several studies showed that the *H. pylori*-induced gastric mucosal inflammation is flared up 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 [30]. Ulcer occurs because of a disequilibrium between defensive mucosa-protective factors and aggressive injurious factors [31].

Current study shows that, the patients infected with *H. pylori* and carriers of A allele at position -251 of the IL-8 gene had an increased risk of peptic ulcer disease (p=.002[a]). In accordance with the above findings, Ohayuchi et al. [12] found that *H. pylori*-positive patients and carriers of the A/A genotype at position -251 of the IL-8 gene had an increased risk of peptic ulcer disease in Japan. Ramis et al. [13] observed same finding in Brazilian population.

Hofner et al. [27] and Gyulai et al. [28] found significant association of A/T genotype and *H. pylori* infected duodenal ulcer disease patients in Hungarian population, whereas the IL-8-251 A/A genotype was significantly more common in *H. pylori*-positive patients with benign gastric ulcer than among controls in Korea [2]. But Kamali-Servestani et al. [29] and Farshad et al. [24] failed to found any significant relationship between IL-8 genotypes and peptic ulcer disease in Iran.

Despite of a high frequency of A allele carriers among *H. pylori* infected patients in gastric cancer and or precancerous lesion group, the current study did not find a correlation between polymorphism in the IL-8 gene and a higher risk of gastric carcinoma (p>.05) as reported in other studies ([13,15,16,24]). However, a positive correlation was reported by some other authors ([2,9,12,14,17]) who found that The IL-8 -251A allele seemed to increase the risk of gastric adenocarcinoma in *H. pylori*-infected patients in Korea, Japan and in China, which was in agreement with the concept that IL-8 may influence the risk of developing gastric cancer by altering the magnitude of inflammatory responses by the host after exposure to *H pylori*. These contradictory results may be related with differences in the study designs, in the sample size used, in the patients' age at diagnosis, in the dietary habits and genetic and ethnic differences in population [32].

5. Conclusion

The present study demonstrated that the IL-8 A allele carriers (T/A+A/A genotype) may increase the risk of

developing gastritis and peptic ulcer diseases in presence of *H. pylori* infection whereas the T/T genotype is protective for *H. pylori* induced gastroduodenal disease progression. So host IL-8 gene polymorphism plays an important role in the development of gastritis and peptic ulcer disease in *H. pylori* infected Bangladeshi patients. The IL-8 genotypes identified by PCR-RFLP cannot be confirmed by sequencing due to limitation of time, budget and resources. Another limitation of the study is the low number of gastric carcinoma population failed to find significant association with host IL-8 polymorphism. Moreover, *H. pylori* colonized individuals cannot be assessed as asymptomatic individuals were not included in the study. However, a multicentre evaluation of the association of various pro-inflammatory and anti-inflammatory cytokine gene polymorphisms in *H. pylori* infection in different ethnicities could prove more reliable correlation and also establish the role of host factors in developing gastroduodenal diseases.

References

[1] Montecucco C and Rappuoli R., "Living dangerously: how *Helicobacter pylori* survives in the human stomach", *Nature Reviews Molecular Cell. Biology*, 2, 457-466, 2001.

[2] Kang DW, Hwang WC, Park MH, Ko GH, Ha WS, Kim KS et al., "Rebamipide abolishes *Helicobacter pylori* CagA-induced phospholipase D1 expression via inhibition of NFκB and suppresses invasion of gastric cancer cells", *Oncogene*, 32, 3531-3542, 2013.

[3] Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY et al, "Effect of Interleukin 1 Polymorphisms on Gastric Mucosal Interleukin 1beta Production in *Helicobacter pylori* Infection", *Gastroenterology*, 123 (6), 1793-1803, 2002.

[4] Hatz RA, Brooks WP, Kra mling HJ, and Enders G, "Stomach immunology and *Helicobacter pylori* infection", *Current Opinion in Gastroenterology*, 8, 993-1001, 1992.

[5] Rieder G, Hatz RA, Moran AP, Walz A, Stolte M, "Role of Adherence in Interleukin-8 Induction in *Helicobacter pylori* - Associated Gastritis", *Infection and Immunity*, *65*(9), 3622-3630, 1997.

[6] Yamaoka Y, Kita M, Kodama T, Sawai N, Tanahashi T, Kashima K et al., "Chemokines in the gastric mucosa in *Helicobacter pylori* infection", *Gut*, 42, 609-617, 1998.

[7] Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY, "Relation between clinical presentation, *Helicobacter pylori* density, interleukin 1beta and 8 production, and cagA status", *Gut*, 45, 804-811, 1999.

[8] Crabtree JE, Lindley IJ, "Mucosal interleukin-8 and Helicobacter pylori-associated gastroduodenal disease", *European Journal of Gastroenterology & Hepatology*, 6(suppl 1), S33-S38, 1994.

[9] Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, Goto H, "Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan", *Cancer Epidemiol Biomarkers Prev*, 14, 2487-2493, 2005.

[10] Vinagre RMDF, Corvelo TCO, Arnaud VC, Leite ACK, Barile KAS, Martins LC, "Determination of strains of Helicobacter pylori and of polymorphism in the interleukin-8 gene in patients with stomach cancer", *Arq Gastroenterol*, 48(1),46-51, 2011.

[11] Caleman Neto A, Rasmussen LT, de Labio RW, deQueiroz VF, Smith M de A, Viani GA et al, "Gene Polymorphism of Interleukin 1 and 8 in Chronic Gastritis Patients Infected with *Helicobacter pylori", Journal of Venomous Animals and Toxins Including Tropical Diseases*, 20 (17), 1-5, 2014.

[12] Ohyauchi M, Imatani A, Yonechi M, Asano N, Miura A, Iijima K et al, "The Polymorphism Interleukin 8 -251 A/T Influences the Susceptibility of *Helicobacter pylori* Related Gastric Diseases in the Japanese Population", *Gut* , 54 (3), 330-335, 2005.

[13] Ramis IB, Vianna JS, Gonçalves CV, Groll AV, Dellagostin OA, da Silva PE, "Polymorphisms of the IL-6, IL-8 and IL-10 Genes and the Risk of Gastric Pathology in Patients Infected with *Helicobacter pylori", Journal of Microbiology, Immunology and Infection*, Mar 24, 1-7, 2015.

[14] Zhang L, Du C, Guo X, Yuan L, Niu W, Yu W et al., "Interleukin-8-251 A/T Polymorphism and *Helicobacter pylori* Infection Influence Risk for the Development of Gastric Cardiac Adenocarcinoma in a High-Incidence Area of China", *Molecular Biology Reports*, 37 (8), 3983-3989, 2010.

[15] Savage SA, Hou L, Lissowska J, Chow WH, Zatonski W, Chanock SJ et al, "Interleukin-8 polymorphisms are not associated with gastric cancer risk in a Polish population", *Cancer Epidemiology ang Biomarkers Preview*, 15, 589-591, 2006.

[16] Kamangar F, Abnet CC, Hutchinson AA, et al., "Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland)", *Cancer Causes Control*,17, 117-125, 2006.

[17] Ye BD, Kim SG, Park JH, Kim JS, Jung HC, Song IS, "The interleukin-8-251 A allele is associated with increased risk of noncardia gastric adenocarcinoma in Helicobacter pylori infected Koreans", *J Clin Gastroenterol*, 43, 233e9, 2009.

[18] Tongtawee T, Kaewpitoon S, Kaewpitoon N, Dechsukhum C, Loyd RA, Matrakool L, "Correlation between Gastric Mucosal Morphologic Patterns and Histopathological Severity of *Helicobacter pylori* Associated Gastritis Using Conventional Narrow Band Imaging Gastroscopy", *Biomedical Research International*, 2015, 1-7, 2015.

[19] Akada JK., Ogura K, Dailidiene D, Dailide G, Cheverud JM, Berg DE, "*Helicobacter pylori* tissue tropism: mouse-colonizing strains can target different gastric niches", *Microbiology*, 149: 1901-1909, 2003.

[20] Salih BA, Bolek BK, Arikan S, "DNA sequence analysis of cagA 3 ‘ motifs of Helicobacter pylori strains from patients with peptic ulcer diseases", *J Med Microbiol*, 59(2), 144-148, 2010.

[21] Lu JJ, Perng CL, Shyu RY, Chen CH, Lou Q, Chong KF et al, "Comparison of Five PCR Methods for Detection of *Helicobacter pylori* DNA in Gastric Tissues", *Journal of Clinical Microbiology*, 37 (3), 772-774, 1999.

[22] Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D et al., "Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection", *Gut*, 53(8), 1082-1089, 2004.

[23] Fabris RD, Rasmussen LT, Caleman Neto A, Lábio RW, Orcini W, Ximenez JP et al, "Polimorfismo da Interleucina-8-251T> A e *Helicobacter pylori", ACM arq. catarin. med*, *40*(3), 25-29, 2011.

[24] Farshad S, Rasouli M, Jamshidzadeh A, Hosseinkhani A, Japoni A, Alborzi A et al., "IL-1ß (+3953 C/T) and IL-8 (-251 A/T) Gene Polymorphisms in *H. pylori* Mediated Gastric Disorders", *Iranian Journal of Immunology, IJI* 7 (2), 96-108, 2010.

[25] Xue H, Liu J, Lin B, Wang Z, Sun J, and Huang G, "A Meta-Analysis of Interleukin-8 -251 Promoter Polymorphism Associated with Gastric Cancer Risk", *PLoS ONE*, 7 (1), 2012.

[26] Thye T, Burchard GD, Nilius M, Müller-Myhsok B, Horstmann RD, "Genomewide linkage analysis identifies polymorphism in the human interferon-γ receptor affecting *Helicobacter pylori* infection", *The American Journal of Human Genetics*, 72(2), 448-453, 2003.

[27] Hofner P, Gyulai Z, Kiss ZF, Tiszai A, Tiszlavicz L, Tóth G, Szõke D, Molnár B, Lonovics J, Tulassay Z, Mándi Y, "Genetic Polymorphisms of NOD1 and IL-8, but not Polymorphisms of TLR4 Genes, Are Associated with Helicobacter pylori-Induced Duodenal Ulcer and Gastritis" *Helicobacter*, 12(2),124-31, 2007.

[28] Gyulai Z, Klausz G, Tiszai A, Lénárt Z, Kása IT, Lonovics J, Mándi Y, "Genetic polymorphism of interleukin-8 (IL-8) is associated with Helicobacter pylori-induced duodenal ulcer. European cytokine network"., 15(4), 353-8, 2004.

[29] Kamali-Sarvestani E, Bazargani A, Masoudian, Lankarani K, Taghavi AR, Saberifiroozi M, "Association of *H. pylori* cagA and vacA Genotypes and IL-8 Gene Polymorphisms with Clinical Outcome of Infection in Iranian Patients with Gastrointestinal Diseases", *World Journal of Gastroenterology*, 12 (32), 5205-5210, 2006.

[30] Sugimoto M, Yamaoka Y, and Furutan T, "Influence of Interleukin Polymorphisms on Development of Gastric Cancer and Peptic Ulcer", *World Journal of Gastroenterology*, 16 (10), 1188-1200, 2010.

[31] Costa A, Figueiredo C, Touati E, "Pathogenesis of *Helicobacter pylori* Infection", *Helicobacter*, *14*, 15-20, 2009.

[32] McLean M & El-Omar E, "Genetic aspects of inflammation", *Current Opinion In Pharmacology*, *9*(4), 370-374, 2009.