ORIGINAL ARTICLE

Interleukin-8 heterozygous polymorphism (-251 T/A and +781 C/T) increases the risk of *Helicobacter pylori*-infection gastritis in children: a case control study

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ABSTRACT

Aim To investigate the effects of interleukin-8 (IL-8) -251 T/A and +781 C/T polymorphism on the risk of *Helicobacter pylori*-infection gastritis in children, and the IL-8 level of children with or without gastritis *H. pylori* infection according to polymorphism.

Methods This prospective, case control clinical study included 64 children 2-18 years old. A disease group (32 gastritis patients with *H. pylori*-infection) was compared with a control group (32 gastritis patients without *H. pylori* infection). Demographic characteristics of patients were taken by a questionnaire; gastritis was confirmed by gastroscopy, *H. pylori* infection was confirmed with rapid urease test. Serum IL-8 level was measured by ELISA, and IL-8 -251 T/A and +781 C/T polymorphisms were analysed by RT-PCR. Demographic characteristics, IL-8 level, polymorphism of patients, and IL-8 level according to polymorphisms were compared between the groups.

Results Children with tobacco exposure were associated with an increased risk of *H. pylori*-infection gastritis by 3.4-fold. There was a higher IL-8 level in the disease group compared to the control group. The disease group with IL-8 -251 AT polymorphism had a higher risk compared to TT polymorphism by 8.7-fold, and with IL-8 +781 CT polymorphism had a higher risk compared to CC polymorphism by 10.7-fold. Children in the disease group with IL-8 -251 AT and TT, and +781 CT and CC polymorphisms produced a higher IL-8 level than the control group in respective polymorphisms.

Conclusion Children with *H. pylori*-infection gastritis have higher IL-8 production. There was an increased risk of developing *H. pylori*-infection in heterozygous -251 AT and +781 CT.

Key words: chemokine CXCL8, genetic heterogeneity, genetic predisposition, gastrointestinal disease

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INTRODUCTION

Gastritis is a disease characterized by the inflammation process in the mucosa layer of the stomach (1). It happened as a response to damage, either acute or chronic that causes mucosal atrophy and epithelial metaplasia (2). *Helicobacter pylori* (*H. pylori*) infection is the most common cause of gastritis globally, but it can also be caused by another microorganism infection, autoimmune, bile acid reflux, radiation exposure, and also the exposure to tobacco, consumption of alcohol, use of non-steroidal anti-inflammatory drugs (NSAIDs) or steroids (3).

Infection of *H. pylori* comprises approximately 50% of the world's population (4). Oral ingestion of *H. pylori* gives the bacteria access to gastric mucosa and induce immune responses, which cause tissue damage (5). Immune responses against *H. pylori* produce pro-inflammatory cytokines, especially interleukin 8 (IL-8) (6) through several pathways, including toll-like receptor 5 (TLR5), neutrophil activating protein A (NAP), heat shock protein 60 (Hsp60), cytotoxin-associated gene A (CagA), and vacuolating cytotoxin A (VacA) (4). A previous study (7) showed that the production of IL-8 genetically determined with homozygous for the AA genotype at -251 position demonstrated a higher level of IL-8 production, whereas other studies (5, 8-10) suggest IL-8 -251 A allele is an important risk factor for the development of *H. pylori*–associated gastric disease, and it is associated with increased inflammation and severity of the disease (11). Another important IL-8 polymorphism is IL-8 +781C allele, for which a previous study showed the association between IL-8 +781C allele with increased risk of non-small cell lung carcinoma (12), ovarian cancer (13), and also gastric cardiac cancer (14). However, some studies reported that IL-8 +781C was not associated with the incidence of gastric disease (15), glioma (16,17), and osteosarcoma (18). The role of IL-8 +781 polymorphism for gastric diseases is still not clear.

The aim of this study was to investigate the effects of IL-8 -251A and +781C polymorphisms on the risk of *H. pylori*-infection gastritis in children, and also the IL-8 levels of gastritis in children with *H. pylori* infection or without infection according to their genotype polymorphism.

PATIENTS AND METHODS

Patients and study design

This study was conducted as a prospective, case control clinical study at H. Adam Malik General Hospital, and Universitas Sumatera Utara Hospital, Medan, Indonesia, during the period between September 2019 and December 2019. All patients were 2 to 18 years old with gastritis confirmed by gastroscopy, who did not consume antibiotics, bismuth containing drugs, histamine H-2 receptor antagonists, proton pump inhibitor (PPI), and immunomodulatory drugs for the past 4 weeks before admission. Patients with malignancy, immunosuppression, metabolic disorders, upper gastrointestinal bleeding, or history of gastrointestinal surgery were excluded.

A written informed consent was taken from all subjects' legal guardians after providing sufficient information about the study prior to the study enrolment.

The study was approved by the Health Research Ethical Committee, School of Medicine, Universitas Sumatera Utara in accordance with the principles of the Helsinki Declaration.

A study group consisted of 34 gastritis children with the positive *H. pylori* infection (disease group), whereas a control group consisted of 34 gastritis children without *H. pylori* infection.

Methods

The infection of *H. pylori* was confirmed by using rapid urease test. All patients were interviewed using a structured questionnaire to note gender, age, socioeconomic status, and tobacco exposure. Weight and height of all patients were also measured, then Z-score of weight-for-height were calculated to determine nutritional status based on WHO paediatric growth indicators (19).

Six millilitres of blood sample were collected from all patients and were subjected to measuring serum IL-8 level by Quantikine ELISA Human CXCK8/IL-8 Immunoassay kit (R&D System, Minneapolis, USA), using ELISA technique. To extract DNA, high Pure PCR Template Preparation Kit (Roche Applied Science, Penzberg, Germany), using spin column method was used. Polymorphism of IL-8 -251 T/A and IL-8 +781 C/T was examined by using the

following primers: IL8 -251T/A forward primer 5'-ATTGGCTGGCTTATCTTCA-3'; reverse primer 5'-CAAATACGGAGTATGACGAAAG-3' and gene IL8 +781C/T forward primer 5'-GTGGTATCACAGAGGATTATGC-3'; reverse primer 5'-CAGTCATAACTGACAACATTGATC-3'. The PCR cycle conditions consisted of 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds and 60°C for 60 seconds. Amplification was done by using C1000 Thermal Cycler CFX96 Real Time System (Bio-Rad Laboratories, California, USA).

Statistical analysis

Demographic characteristics of the children were analysed for differences between gastritis with *H. pylori* infection and without *H. pylori* infection by using $\chi 2$ test for categorical data, independent t-test for normally distributed numerical data, and Mann-Whitney U test if the distribution was not normal. Serum IL-8 was compared between gastritis with *H. pylori* infection and without *H. pylori* infection by using independent t-test if data were normally distributed, otherwise Mann-Whitney U test was used. A comparison of genotype polymorphism between the groups was analysed by using $\chi 2$ test, or Freeman-Halton extension of Fisher's exact test if $\chi 2$ assumptions were violated. Serum IL-8 level was compared between the groups according to genotype polymorphism by using independent t-test for normally distributed data, and Mann-Whitney U test for non-normal distribution. Differences were considered statistically significant at $p<0.05$.

RESULTS

Gender, age, weight, height, nutritional status (weight-for-height) and socioeconomic status were similar among the disease and control group. Children with tobacco exposure were associated with increased risk of *H. pylori*-infection gastritis by 3.4-fold (95% CI: 1.18, 9.99) (Table 1).

There was a significantly higher IL-8 level in the disease group (median = 20.64) compared to the control group (median = 10.64), U=160.5, r = 0.62 ($p<0.001$) regardless of the genotype polymorphism (Figure 1).

The frequency of IL-8 -251 AT and AA polymorphism was significantly higher in the disease than in the control group, but only children with

Table 1. Demographic characteristics of 34 gastritis children with *H. pylori* (+) and without *H. pylori* (-) infection

Characteristic	*H. pylori* (+)	*H. pylori* (-)	p
Gender (No, %)			
Male	13 (52)	12 (48)	0.801
Female	21 (48.8)	22 (51.2)	
Age mean (SD) (years),	11.83 (3.52)	11.96 (3.37)	0.880
Weight mean (SD) (kg)	36.47 (10.91)	37.29 (9.91)	0.746
Height median (min. – max.) (cm)	141.5 (104 – 165)	141.5 (104 – 162)	0.825
BMI-for-age (Z-score) mean (SD)	0.37 (1.58)	0.52 (1.42)	0.689
BMI (No, %)			
Severely wasted	2 (100)	0	
Wasted	0	2 (100)	
Normal	29 (51.8)	27 (48.2)	
Overweight	1 (25)	3 (75)	
Obese	2 (50)	2 (50)	
Socioeconomic status (No, %)			
Moderate – high	28 (48.3)	30 (51.7)	0.493
Low	6 (60)	4 (40)	
Tobacco exposure (No, %)			
Yes	27 (60)	18 (40)	0.021
No	7 (30.4)	16 (69.6)	

BMI, body mass index;

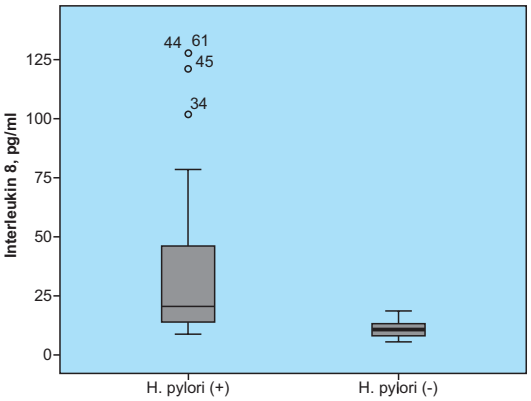

Figure 1. Interleukin-8 level in gastritis children with (+) or without (-) *H. pylori* infection

AT polymorphism had a significantly higher risk of *H. pylori*-infection gastritis compared to TT polymorphism, by 8.7-fold (95% CI: 2.55, 30.01). There was also a significant increase in the frequency of IL-8 +781 CT polymorphism between the disease and the control group, having CT polymorphism increase the risk of *H. pylori*-infection gastritis compared to CC polymorphism by 10.7-fold (Table 2).

When IL-8 level of each polymorphisms was compared between the groups, children in the disease group with -251 AT and TT, and +781 CT and CC polymorphisms were producing a signifi-

Table 2. Comparison of genotype polymorphism between gastritis children with *H. pylori* (+) or without *H. pylori* (-) infection

Polymorphism (No of patients)	No (%) of patients		p	Odds Ratio* (95% CI)
	H. pylori (+)	*H. pylori* (-)		
IL-8 (-251 T/A)				
AA (11)	6 (54.5)	5 (45.5)		4.56 (0.98-21.32)
AT (33)	23 (69.7)	10 (30.3)	0.001	8.74 (2.55-30.01)
TT (24)	5 (20.8)	19 (79.2)		---
IL-8 (+781 C/T)				
TT (4)	2 (50)	2 (50)		3.43 (0.41-28.94)
CT (33)	25 (75.8)	8 (24.2)	<0.001	10.71 (3.36-34.14)
CC (31)	7 (22.6)	24 (77.4)		---

*comparison of -251 TT and +781 CC polymorphisms

cantly higher IL-8 level than the control group in respective polymorphism (Table 3).

Table 3. Interleukin-8 levels of gastritis children with *H. pylori* (+) or without *H. pylori* (-) infection according to genotype polymorphism

Polymorphism	Mean (SD) or median (min.-max.) (pg/mL)		Mean difference (95% CI) or U	p
	H. pylori (+)	*H. pylori* (-)		
IL-8 (-251 T/A)				
AA	20.98 (8.20)	12.31 (5.27)	8.67 (-0.99, 18.33)	0.073
AT	19.01 (10.05–128)	10,69 (5.57 – 17.61)	41	0.004
TT	37.85 (19.32–121.05)	10.14 (5.88 – 17.21)	0.00	0.001
IL-8 (+781 C/T)				
TT	22.91 (20.84–24.98)	12,87 (7.02 – 18.71)	0.00	0,121
CT	19.01 (8.81–128)	9,96 (5.57 – 17.01)	24	0.001
CC	51.02 (42.81)	11.27 (3.50)	39.75 (0.16, 79.34)	0.049

DISCUSSION

Infection of *H. pylori* may be acquired during childhood and lasts for lifetime if not properly treated (20). It is known to have a strong association with socioeconomic status and poor personal and community hygiene, especially environmental tobacco exposure (21), which is in concordance with this study. Continuous exposure of *H. pylori* caused chronic gastritis, peptic ulcer disease, gastric cancer (4), and even a huge variety of extragastric diseases (22). High burden of the disease caused by this infection entices the mandatory measure in eradication and prevention strategy (23), especially in more susceptible population for developing a more severe disease (5).

Interleukin-8 is known as a potent inflammation mediator with eminent role in angiogenesis, tumour growth, invasion and metastases process in malignancies (24). Our study showed a higher value of serum IL-8 levels in children with *H. pylori*-infection gastritis compared to their counterparts. This finding is in concordance with a previous study that showed infection of *H. pylori* inducing IL-8 secretion by gastric cell by some mechanism, such as high levels of CagA expression (25,25) and via TLR4-dependent mechanism (26).

Production of IL-8 is also influenced by the polymorphism of IL-8 gene, such as -251 T/A gene and +781 C/T gene (15). The result of this study showed an increased risk of having *H. pylori*-infection gastritis in patients with -251 AT polymorphism than TT polymorphism and also +781 CC polymorphism than CC polymorphism. A previous study by Chang et al. (9) showed that having IL-8 -251 AT polymorphism increased risk in developing severe gastritis A study by Taguchi et al. (27) however did not find any association between IL-8 -251 AT polymorphism with atrophic gastritis and also gastric cancer. Previous studies (14,28) showed no significant relationship between IL-8 +781 CC polymorphisms and the incidence of gastric disease. In contrast, this study showed an increased risk of developing *H. pylori*-infection gastritis in patients with +781 CT polymorphism compared to CC polymorphism by around 11-fold higher.

This study finds significantly higher IL-8 production in *H. pylori*-infection gastritis patients with -251 AT, -251 TT, +781 CT, and +781 CC polymorphisms compared with gastritis patients without *H. pylori*-infection. These findings are consistent with a previous study (9) that shows significant differences in IL-8 levels between patients with *H. pylori*-infection compared with patients without *H. pylori*-infection in IL-8 -251 TT, AT, and AA polymorphisms. Our study did not find significant differences in IL-8 production in patients with -251 AA and +781 TT polymorphisms, which might be due to the limited number of patients enrolled in this category.

Some limitations of this study should be noted. This study protocol did not perform tissue biopsy for histologic and culture examinations due to resources constraint. Also, it did not perform any follow-up of esophagogastroduodenoscopy (EGD) after the establishment of the diagnosis and eradication therapeutic interventions. Patients with known gastric cancer were also excluded from this study to focus on the risk of

polymorphisms in gastritis patients, therefore it showed a limited number of patients especially in IL-8 -251 AA polymorphism and maybe in +781 TT polymorphism.

To the best of our knowledge, this study is the first study to report the association of IL-8 -251 T/A and IL-8 +781 C/T polymorphisms with serum IL-8 levels in children with *H. pylori*-positive gastritis. This study is also the first study that shows a significantly higher risk in patients with IL-8 -251 heterozygous AT and +781 heterozygous CT developing *H. pylori*-infection gastritis.

In conclusion, the presence of IL-8 -251 AT and TT, and +781 CT and CC polymorphism causes higher IL-8 production in gastritis children with *H. pylori* infection. Heterozygous gene polymorphisms (-251 AT and +781 CT) may be associated with the risk of developing gastritis in children with *H. pylori* infection.

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CONFLICTS OF INTEREST

Competing interests: None to declare

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