TREATMENT OUTCOMES AND *CYP3A4*1G* GENOTYPE DISTRIBUTION IN CHILDREN WITH PEPTIC ULCERS

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*Many factors, including the role of the cytochrome P450 system such as CYP3A4*1G, may influence the effectiveness of peptic ulcer treatment. The study aimed to describe the treatment outcomes and distribution of CYP3A4*1G genotype in children with peptic ulcers. A descriptive cross-sectional study, longitudinal follow-up before and after six weeks of treatment was conducted on 100 children with peptic ulcers from October 2022 to February 2024. Children with peptic ulcers without H. pylori infection had a higher rate of anemia, and pyloric and duodenal deformation, compatible with children with H. pylori. The ulcer healing rate was lower in children with deep peptic ulcers, excess eosinophils in mucosal biopsies, and poor therapeutic compliance. The distribution of CYP3A4*1/*1, CYP3A4*1/CYP3A4*1G, CYP3A4*1G/CYP3A4*1G in the study was 43.2%, 48.1%, and 8.6%, respectively, with no difference by gender. There were no difference in ulcer healing rates according to the presence of the CYP3A4*1G variant. In conclusion, the degree of peptic ulcer healing in children varies according to several characteristics. However, there were no difference in ulcer healing rates according to the presence of the CYP3A4*1G variant.*

Keywords: Treatment outcome, peptic ulcer, children, *CYP3A4*1G*.

I. INTRODUCTION

Peptic ulcer disease is a common gastrointestinal condition affecting individuals of all ages. In 2019, it was estimated that there were approximately 8.09 million cases of peptic ulcer disease worldwide, an increase of 25.82% compared to 1990.[1] The prevalence of peptic ulcer disease in children ranges from 5% to 22% depending on various studies.[2] Since being discovered and announced by Warren J.R. and Marshall B.J. in 1983, many studies have demonstrated *Helicobacter pylori* (*H. pylori*) 's role in peptic ulcer disease. However, a study by Shu-Chung in Taiwan showed that only about 50% of children with peptic ulcer disease were infected with *H. pylori*.[3] The remaining cases, which are not related to *H. pylori* infection, can also cause many dangerous complications if not treated promptly, such as gastrointestinal bleeding, ulcer perforation, obstruction due to pyloric stenosis, and duodenal narrowing.

The effectiveness of peptic ulcer treatment is influenced by factors such as treatment adherence, antibiotic sensitivity, and the virulence factors of *H. pylori*. Additionally, the outcomes of ulcer healing and successful eradication of *H. pylori* are also affected by the cytochrome P450 (CYP450) system, which plays a crucial role in drug metabolism. Among these, the CYP3A enzyme is responsible for metabolizing a significant portion of major drug

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classes, including proton pump inhibitors (PPIs) and certain antibiotics. These drug-metabolizing genes are highly polymorphic.[4]

Depending on the presence of mutant alleles in the genotype, enzyme activity can be normal, reduced, increased, or absent. Therefore, these variations can influence and potentially play a role in predicting the outcomes of ulcer healing and *H. pylori* eradication. While the *CYP3A4*22* allele is associated with reduced enzyme activity and decreased substrate clearance, there are still controversies regarding the polymorphism of *CYP3A4*1G*, which has been linked to both decreased and increased enzyme activity.[5,6,7] Until now, limited data is available on the polymorphism of *CYP3A4*1G* in the context of Vietnamese children. Thus, our study was conducted to describe the treatment outcomes and the distribution of the *CYP3A4*1G* genotype in children with peptic ulcer disease.

II. SUBJECTS AND METHODOLOGY

1. Subjects

Criteria for patient selection

- Age: 2 - 17 years old.

- Diagnosed with peptic ulcers at the National Children's Hospital.

- Children and their families consent to participate in the study.

Diagnosis of peptic ulcers: There is a condition of surface mucosal necrosis of the stomach, duodenum with a minimum diameter of 0.5cm penetrating through the muscularis propria layer diagnosed in endoscopy.[8]

Diagnosis of *H. pylori* infection[9]- Histopathology confirms the presence of *H. pylori* and rapid urease test (+). Or

- Culture of gastric biopsy specimens shows the presence of *H. pylori*. Or

- If only one of the two histopathology and rapid urease tests (+), additional tests such as the UBT or stool antigen test should be performed. If the UBT or stool antigen test is positive, it confirms the *H. pylori* infection.

Diagnosis of non-*H. pylori* infection

- Culture of gastric biopsy specimens, histopathology shows no presence of *H. pylori*, and rapid urease test (-).

Exclusion criteria

- Taking antibiotics or bismuth within 4 weeks; using antacids, H2 receptor antagonists, or PPIs within 2 weeks before consultation.

- Contraindication for upper gastrointestinal endoscopy.

- History of allergy to one of the drugs used in the study.

2. Location and Duration of the Study

The study was conducted at the National Children's Hospital from October 1, 2022, to February 29, 2024. Additionally, the molecular biology testing procedure was conducted at the Vietnamese-German Center for Medical Research (VG-CARE) - 108 Military Central Hospital.

3. Study Design

The study is a descriptive cross-sectional, prospective, longitudinal design, with pre-and post-treatment follow-up over 6 weeks.

Sampling was conveniently selected. It involved 50 children diagnosed with peptic ulcer disease meeting the criteria for *H. pylori* infection and 50 children diagnosed with peptic ulcer disease meeting the criteria for non-*H. pylori* infection. The total sample size for the study is 100 children.

4. Variables and indices

- ***Degree of scarring:*** Sakita-Miwa classification[10]+ Scarred: ulcer scarring (S1-S2) or no longer ulcerated

+ Not scarred: still ulcerated (stage A1 to H2)

- ***Complications:*** anemia, upper gastrointestinal bleeding, ulcer perforation, pyloric deformation, duodenal deformation.

- ***Some factors related to the treatment outcomes of peptic ulcer disease***: *H. pylori* infection status, treatment adherence, lesion characteristics on endoscopy, and history of COVID-19 infection.

- ***Treatment adherence:*** After 6 weeks of treatment, the researcher checks the medication containers that the children have used.

+ Good treatment adherence: subjects who consumed greater than 85% of doses.[11]

+ Poor treatment adherence: subjects who consumed less than 85% of doses.

- ***Genotypes***

+ Wild type (*CYP3A4*1/*1*).

+ Mutant type: mutant heterozygote (*CYP3A4*1/*1G*), mutant homozygote (*CYP3A4*1G/*1G*).

- ***Alleles***: G allele (*CYP3A4*1*), A allele (*CYP3A4*1G*).

5. Treatment procedure

All children diagnosed with peptic ulcers are treated with PPI (Esomeprazole at a dose of 1.5-2 mg/kg/day and not exceeding 80 mg/day) for 6 weeks to suppress acid secretion.

When the bacterial culture results and antibiotic susceptibility test are available, the group of children with peptic ulcer disease and *H. pylori* infection are prescribed *H. pylori* eradication treatment by the researcher. The researcher explains the *H. pylori* eradication treatment regimen to the children's parents, based on the antibiotic susceptibility test and following the guidelines of ESPGHAN and NASPGHAN 2016.

6. Molecular biology testing procedure

DNA samples were extracted from gastric biopsy specimens obtained during endoscopy procedures. These DNA samples were then used to perform PCR (Polymerase Chain Reaction) and Sanger sequencing on the Beckman Counter 8800 system. The sequencing results were analyzed to determine the genotype of the *CYP3A4*1G* gene using Bioedit and MEGA7 software.

7. Data processing

The data were entered and processed using SPSS 16.0 software. Descriptive statistics were used for both quantitative and qualitative variables.

8. Research ethics

The study was conducted with ethical principles, and with the consent of the research participants. It was approved by the Ethics Committee of the National Children's Hospital and Hanoi Medical University (number: 3012/BVNTW-HĐĐĐ, December 15, 2022).

III. RESULTS

During the study period, 100 eligible children participated in the research, consisting of 73 boys and 27 girls, with a male-to-female ratio of 2.7/1. In detail, the group of children with *H. pylori* infection included 37 boys and 13 girls, while the group of children without H. pylori infection included 36 boys and 14 girls. The average age of the children in the study was 10.1 ± 3.0 years (ranging from 2 to 15 years). There was no difference in gender and age between the two groups of children with peptic ulcer disease, those without and those with *H. pylori* infection.

Table 1. Degree of ulcer healing and complications according to the *H. pylori* infection status

		Non *H. pylori* *n = 50*		*H. pylori* *n = 50*		p-value*
		n	%	n	%	
Ulcer healing	Scarred	38	76	31	62	0.194
	Not scarred	12	24	19	38	
Anemia		33	66	18	36	**0.005**
Gastrointestinal bleeding		17	34	11	22	0.265
Ulcer perforation		3	6	0	0	-
Pyloric deformation		25	50	11	22	**0.006**
Duodenal deformation		25	50	12	24	**0.012**

**Chi-square Test*

Children with peptic ulcer disease without *H. pylori* had a higher incidence of anemia, pyloric deformities (such as twisting and abnormal contraction), and duodenal deformities (such as distortion, constriction, diverticula, and narrowing of the descending intestinal) compared to children with peptic ulcer disease infected with *H. pylori*. This difference was statistically significant with a p-value of less than 0.05.

Table 2. Degree of ulcer scarring and some associated factors

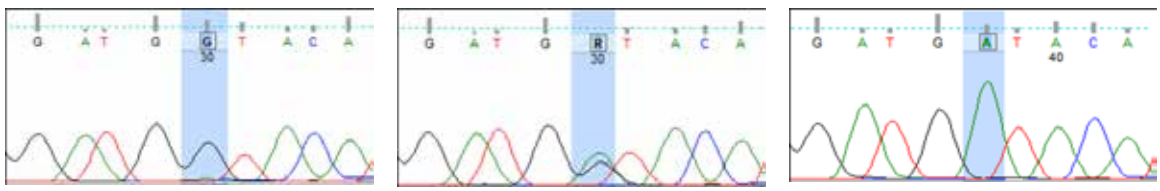
		Scarred n = 69		Not scarred n = 31		p-value*
		n	%	n	%	
Number of ulcers	1	40	71.4	16	28.6	0.664
	≥ 2	29	65.9	15	34.1	
Size of ulcers (cm)		1.35 ± 0.67		1.51 ± 0.69		0.294**
Deep ulcers		31	81.6	7	18.4	**0.045**
Eosinophilia in Gastrointestinal Biopsy		8	47.1	9	52.9	**0.044**
History of COVID-19		32	65.3	17	34.7	0.518
Treatment compliance	Yes	55	76.4	17	23.6	0.016
	No	14	50	14	50	

**Chi-square Test*

***T-Test*

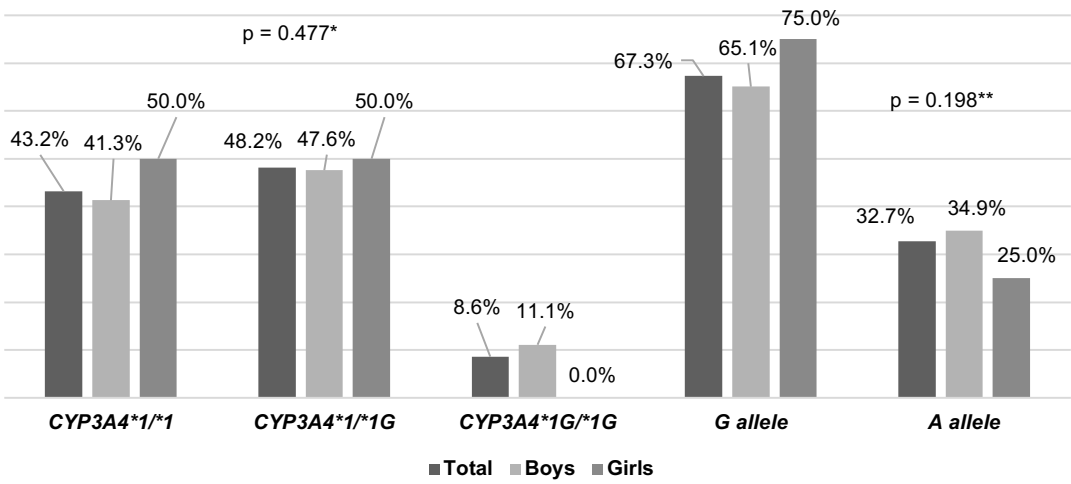
Children with deep ulcers, eosinophilic gastroenteritis on pathological examination, and poor adherence to treatment had a lower rate of ulcer healing compared to children with shallow ulcers, no eosinophilic gastroenteritis on pathological examination, and good adherence to treatment.

After excluding samples that did not meet the standard during the DNA extraction and purification process, we sequenced 81 out of the total 100 study samples, including 48 samples with *H. pylori* (37 boys, 11 girls) and 33 samples without *H. pylori* (26 boys, 7 girls)



Wild type **Mutant heterozygote** **Mutant homozygote**

Figure 1. Sequencing results of the PCR product carrying the *1026+12 G>A* gene segment of the *CYP3A4*1G* gene



*Fisher's Exact Test

**Chi-square Test

Chart 1. Frequency of genotypes and alleles of *CYP3A4*1G* according to gender

The frequencies of the *CYP3A4*1/*1*, *CYP3A4*1/*1G*, and *CYP3A4*1G/*1G* genotypes in the study population were 43.2%, 48.1%, and 8.6%, respectively. The frequencies of the G allele (*CYP3A4*1*) and the A allele (*CYP3A4*1G*) were 0.67 and 0.33, respectively. There was no difference in the distribution of the *CYP3A4*1G* genotype and alleles by gender.

Table 3. *CYP3A4*1G* genotype distribution and the degree of ulcer scarring

*CYP3A4*1G* genotype	Degree of ulcer scarring		Total	p-value
	Scarred	*Not scarred*		
Wild type	22 (62.9%)	13 (37.1%)	35 (100%)	0.635*
Mutant type	32 (69.6%)	14 (30.4%)	46 (100%)	
Mutant heterozygote	28 (71.8%)	11 (28.12%)	39 (100%)	0.065**
Mutant homozygote	4 (57.1%)	3 (42.9%)	7 (100%)	
Total	54 (66.7%)	27 (22.3%)	81 (100%)	

**Chi-square Test*

***Fisher's Exact Test*

There was no difference in the rate of scarring between the group of children without and with the presence of the *CYP3A4*1G* mutation.

IV. DISCUSSION

During the study period, we collected data from 100 children with peptic ulcer disease who met the criteria for inclusion, including 50 children infected with *H. pylori* and 50 children not infected with *H. pylori*. Among them, there were 73 boys and 27 girls, with a boy-to-girl ratio of 2.7/1. The average age of the children in the study was 10.1 ± 3 years (ranging from 2 to 15 years). There was no difference in gender and age between the two groups of children with peptic ulcer disease, whether they were infected with *H. pylori*.

Our study also recorded that 69% of children had ulcer healing after 6 weeks of treatment. The rates of complications such as anemia, gastrointestinal bleeding, pyloric deformity, and duodenal bulb deformity were 51%, 28%, 36%, and 37%, respectively. Notably, three children in our study experienced ulcer perforation, accounting for 3%. Children with peptic ulcer disease who were not infected with *H. pylori* had higher rates of anemia, pyloric deformity (abnormal contraction and distortion), and duodenal deformity (deformation, traction, diverticulum, and narrowing) compared to children with peptic ulcer disease who were infected with *H. pylori*. This indicates that in addition to the pathogenic role of *H. pylori*, other causes of peptic ulcer disease in children should be considered, such as the use of nonsteroidal anti-inflammatory drugs (NSAIDs), underlying conditions like inflammatory bowel disease, eosinophilic gastroenteritis, and *Schonlein-Henoch*...

Eosinophilic gastroenteritis is a disease caused by chronic, immune-mediated disorders characterized clinically by symptoms of gastrointestinal dysfunction and histologically by inflammation of the stomach, small intestine, or colon with a predominance of eosinophils. In our study, 17% of children had eosinophilia based on pathology results. Notably, we also observed that this group of children had a lower ulcer healing rate after treatment compared to the group without eosinophilia on pathology results. We suggest that treating peptic ulcer disease in children with eosinophilia requires not only PPIs and *H. pylori* eradication but also other combined treatments such as dietary modifications and immunosuppressive drugs.

Patient adherence to treatment plays a crucial role in determining the treatment outcome. Poor adherence is inversely related to the effectiveness of *H. pylori* eradication. Many factors affect patient adherence to treatment, such as complex treatment regimens, prolonged treatment duration, availability and cost of medication, and side effects of the drugs.[12] Most patients are accustomed to simpler treatment methods, such as taking acid-suppressing drugs once daily instead of twice or using three different drugs that have a synergistic effect. On the other hand, treatment regimens may not immediately improve clinical symptoms. The occurrence of side effects is also a reason for non-adherence to treatment. Diarrhea and an unpleasant taste are the most common side effects. Studies have reported a side effect rate of around 30%, which causes discomfort in patients' lives.[13] In a study monitoring treatment adherence in Switzerland, 11.5% of patients discontinued treatment early. The reason for early discontinuation in two-thirds of the cases was due to side effects.[11] Our study also recorded that the ulcer healing rate was higher in the group that adhered to treatment compared to the group that did not adhere to treatment.

In addition to factors such as treatment adherence, the characteristics of the ulcer, and the virulence factors *of H. pylori*, the outcomes of ulcer healing and successful eradication of *H. pylori* are also influenced by the CYP450 system, which plays a crucial role in the metabolism of PPIs and certain antibiotics. The CYP3A enzymes are responsible for metabolizing major drug classes. More than 40 polymorphisms of *CYP3A4* have been identified, and enzyme activities vary significantly among individuals. In some studies, the *CYP3A4*1B* and *CYP3A4*1G* alleles have been reported as polymorphisms that enhance enzyme function. The *CYP3A4*22* allele is associated with reduced enzyme activity and expression.[6] The frequencies of the **1B*, **1G*, and **22* alleles have been reported to be 5.4%, 8.9%, and approximately 5.0 - 7.0% in Caucasians, respectively.[14]The results of our study showed that the frequencies of the *CYP3A4*1/CYP3A4*1*, *CYP3A4*1/CYP3A4*1G*, and *CYP3A4*1G/CYP3A4*1G* genotypes were 43.2%, 48.1%, and 8.6%, respectively. There was no difference in the distribution of the *CYP3A4*1G* genotype by gender due to the location of the *CYP3A4* gene on chromosome 7q22.1. Our study also reported the allele frequencies of the G allele and A allele as 0.67 and 0.33, respectively. According to a study by Em Sutrisna et al on 60 individuals from Indonesia, the genotype frequencies of *CYP3A4*1G* were 25%, 55%, and 20% for *CYP3A4*1/*1*, *CYP3A4*1/*1G*, and *CYP3A4*1G/*1G*, respectively; the allele frequency of G allele was 0.53, while A allele was 0.47.[7]The polymorphism of the *CYP3A4*1G* gene may occur due to the substitution of guanine with adenine at position 82266 (*G82266A*). This variant is located in intron 10 of the *CYP3A4* gene. This leads to the amino acid alteration from isoleucine to valine (I369V). Both of these amino acids are polar and have different molecular weights. Amino acid alterations with different properties will modify the catalytic ability of the enzyme CYP3A4. This amino acid alteration will reduce the catalytic ability of the CYP3A4 enzyme, thereby potentially increasing the concentration of drugs in the bloodstream.[7]The drug-metabolizing genes of the CYP450 family exhibit high polymorphism. Depending on the presence of mutant alleles in the genotype, enzyme activity can be normal, reduced, increased, or absent. Therefore, they have the potential to influence and may play a role in predicting the outcomes of ulcer healing and *H.*

pylori eradication. The expression of genes and the activity of CYP3A4 in the liver vary greatly among individuals, and this variation affects the pharmacokinetics of drugs. However, the results of our study did not observe any difference in the rate of ulcer healing between the group of children with and without the presence of the *CYP3A4*1G* mutation. A study conducted by Karaca RO et al on 194 patients with peptic ulcer disease found no significant difference in pantoprazole serum concentrations between different *CYP3A* genotype groups. The mean pantoprazole concentrations for individuals with *CYP3A4*1/*1* (n = 85), *CYP3A4*1/*1G* (n = 12), and *CYP3A4*1G/*1G* (n = 4) were 1.88 (1.52–2.44) µg/ml, 0.62 (0.04–2.56) µg/ml, and 1.85 (0.04–2.24) µg/ml, respectively (p = 0.31). Additionally, the success rate of *H. pylori* treatment in this study was similar across different *CYP3A4* genotype groups.[5] Therefore, further studies on the role of *CYP3A4* gene polymorphism, along with the role of other important drug-metabolizing genes, as well as the influence of related factors such as different inhibitory and activating substances, are needed during research.

V. CONCLUSION

Peptic ulcers can cause many complications, even if they are without *H. pylori* infection. The treatment outcomes of peptic ulcers in children vary according to several characteristics.

All three genotypes of *CYP3A4*1G* appeared in this study with no gender difference observed. There were no difference in ulcer healing rates according to the presence of the *CYP3A4*1G* variant.

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