A2143G POINT MUTATION IN THE 23S rRNA GENE A RISK FACTOR OF FAILURE OF HELICOBACTER PYLORI ERADICATION WITH TRIPLE THERAPY

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Abstract

Background: Point mutation A2143G in the 23SrRNA gene were noted as a common mutation in H.pylori resistant to clarithromycin, but data about this point mutation was still limited in Vietnam. This study is aimed at determining the rate of H.pylori eradication of triple therapy RAC and investigating A2143G point mutation in two groups of eradicated and non-eradicated H.pylori. **Patients and methods**: 90 patients with peptic ulcer or gastritis consulted at Hue University Hospital from Jan 2011 to Dec 2012. Triple therapy used to eradicate H.pylori was RAC in 10 days. A2143G point mutation in the 23S rRNA gene was tested by PCR- RFLP with BsaI enzyme. **Results**: Eradication rate of the triple therapy RAC in 10 days was 53.3%; the A2143G point mutations was found in 45.2% and 16.7% in two groups of having non-eradication and eradication of H.pylori, respectively. History of clarithromycin usage may be a risk factor of the presence of A2143G point mutation.**Conclusion**: A2143G point mutation in the 23SrRNA gene may be a risk factor of failure of H.pylori eradication by triple therapy RAC.

Key words: *A2143G point mutation; 23SrRNA gene; clarithromycin resistance.*

1. BACKGROUND

Helicobacter pylori is a very common cause of gastritis and peptic ulcer. H.pylori eradication faces to many challenges, but the most important problem was antibiotic resistance, in particularly resistance to clarithromycin. Point mutations A2143G in the 23SrRNA gene were noted as a common mutation in H.pylori resistant to clarithromycin, but data about this point mutations were still limited in Vietnam. This study is aimed at determining the rate of H.pylori eradication of a 10 day- triple therapy RAC and invesgating the frequency of A2143G point mutation in the 23SrRNA gene in two groups of eradicated and non-eradicated H.pylori .

2. PATIENTS AND METHOD

2.1. Patients

Patients with peptic ulcer or gastritis consulted at Hue University Hospital from Jan 2011 to Dec 2012.

- Criteria of inclusion:

o Peptic ulcer or gastritis diagnosed by clinical and endoscopic examinations

o Evidence of histopathology in case of gastric ulcer or chronic gastritis.

o Taking no antibiotics in the previous 4 weeks of no PPI in the previous 2 weeks.

o CLO-TEST positive.

- Criteria of exclusion:Non-adherence to the therapy, history of antibiotics hypersensitivity, evidence of gastric neoplasia

- Number of patients enrolled: 90 patients

2.2. Method

Study design: cross-sectional study

Data collection:

- History: history of peptic ulcer and gastritis, history of macrolide usage

- Physical examination

- Esogastroduodenal endoscopy: EGD Olympus GIF -150.

- Gastric biopsies: 2 biopsies for CLO-TEST (one from antrum and one from fundus). 4 biopsies

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for histopathological examination and PCR-RFLP.

- H.pylori were considered positive if one sample was positive (change of color from yellow to pink) and confirmed by PCR.

- Kits of CLO-TEST were from Viet A company.

- Therapy:

o Rabeprazole 20 mg, bid, 30 min before meals

o Amoxicillin: 1g, bid.

o Clarithromycin: 0.5 g, bid

o Duration of treatment: 10 days

- Eradication was confirmed by a negative CLO-TEST four weeks after stopping of antibiotics.

DNA extraction for diagnosis of H. pylori infection and detection of A2143G point mutation:

- Samples: gastric biopsies, conserved in TE, at -20^{o}C at the Department of Medical Genetics

- Bacterial DNA was extracted using standard protocol of kit wizard Genomic DNA purification (Promega).

- After extraction, DNA was measured in Nanodrop machine , then diluted by 100 ng/µl.

Detecting H.pylori by PCR:

- Extracted DNA, perform PCR with primer pair of 16S rRNA gene of H.pylori, designed by Bickley.

- Sequences of primers:

Hp-F:5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3'
Hp-R: 5'-AAGCTTACTTTCTAACACTAACGC-3'

- Reaction components included: 12.5 µl GoTaq Green MasterMix (Promega), 10 pmol/ each primer, 100 ng DNA template and water enough for 25 µl.

- PCR Cycling condition: 95^{o}C in 5 min, followed by 30 cycles, each cycle included: denaturation at 94^{o}C in 60 sec, 60 sec annealing at 52^{o}C and 60 sec extension at 72^{o}C. A final extension of 10 min at 72^{o}C.

- The reaction was performed by an Applied Biosystems 2720 Thermal cycling machine, at the Department of Medical Genetics, Hue University of Medicine and Pharmacy.

- PCR product waselectrophoresed on 0.8% agarose gel, at 80V. The results were read under ultraviolet light after being dyed with ethidium bromide. The size of products was 109 bp.

Detecting of A2143G point mutation by PCR-RFLP:

Step 1: Amplifying a fragment of 23S rRNA gene containing site 2143 by PCR. Pair of primers was designed by Menard (2002). The primer sequences was following:

HPY-S:5'-AGGTTAAGAGGGATGCGTCAGTC-3'.
HPY-A: 5'-CGCATGATATTCCCATTAGCAGT-3'

- Kit PCR: GoTaq Green MasterMix (Promega).

- Volume of reaction: 50 µl.

- Reaction components included: 25 µl GoTaq green MasterMix (Promega), 20 pmol/each primer, 200 ng DNA template and water enough for 50 µl.

- PCR Cycling condition: 95^{o}C in 5 min, followed by 30 cycles, each cycle included: denaturation at 94^{o}C in 60 sec, 60 sec annealing at 52^{o}C and 60 sec extension at 72^{o}C. A final extension of 10 min at 72^{o}C.

- The reaction was performed by an Applied Biosystems 2720 Thermal cycling machine, at the Department of Medical Genetics, Hue UMP.

- PCR product was electrophoresed on 0.8% agarose gel, at 80V, 30 min. The size of products was 267 bp.

Step 2: Digestion of PCR product by BsaI of Thermo Scientific.

The volume of each reaction of digestion was 20 µl, containing 2 µl buffer G 10X, 5µ PCR product, 10 U BsaI enzyme, water enough for 20 µl.

- Incubation in a water bath at 37^{o}C, in 20 hours.

- Electrophoresis of digested product on 2.5% agarose gel, in 1 h 40 min. We read the results under ultraviolet light after dying with ethidium bromide.

- Reading the results upon the appearance of bands correspond to digested product as following:

	Product after digestion by BsaI	
	Normal	A2143G
Number of band	1	2
Size	267 bp	208 bp and 59 bp

2.3. Statistical analysis*:* using Medcalc software; determination of OR and 95%CI. A value of $p < 0.05$ was considered significant.

3. RESULTS

3.1. Baseline characteristics

Characteristics		Patients	
		n	%
Age	<30 years old	42	46.7
	30-60 years old	32	35.6
	>60 years old	16	17.8
Gender	Male	55	61.1
	Female	35	38.9
Diseases	Gastric ulcer	18	20
	Duodenal ulcer	22	24.4
	Chronic gastritis	50	55.6

3.2. Rate of H. pylori eradication

Eradication		Non- eradication	
n	%	n	%
48	53.3	42	46.7

Rate of H.pylori eradication of triple therapy RAC 10 days was only 53.3%.

3.3. Frequency of A2143G point mutations in 2 groups of patients

	Eradicated (n=48)		Non-eradicated (n=42)		OR	95%CI
A2143G mutation	8	16.7%	19	45.2%	4.13	1.56- 10.92
No A2143G mutation	40	83.3%	23	54.8%		

The frequency of A2143G point mutation in the 23S rRNA gene was 45.2% in the group of non-eradicated H.pylori vs 16.7% in group of eradicated H.pylori ; OR = 4.13 with 95% CI = 1.56-10.92.

3.4. Factors involved to the frequency of A2143G point mutation

Factors		A2143G mutations		p
		n	%	
Age	<30 (n=42)	12	28.6	0.770
	30-60 (n=32)	9	28.1	
	>60 (n=16)	6	37.5	
Endoscopic lesion	Chronic gastritis (n=50)	13	26	0.638
	Gastric ulcer (n=18)	6	33.3	
	Duodenal ulcer (22)	8	36.4	
Gender	Male (n=55)	17	30.9	0.758
	Female (n=35)	10	28.6	
History of clarithromycin usage	Yes (n=31)	22	71.0	**0.039**
	No (n=59)	5	8.5	
History of PPI usage	Yes (n=72)	20	27.8	0.943
	No (n=18)	7	38.9	

A significant difference of A2143G point mutation was found between group having history of clarithromycin usage and group having no clarithromycin usage ($p<0.05$).

4. DISCUSSION

Triple therapy with one PPI and two antibiotics (amoxicillin and clarithromycin) was one standard therapy for H. pylori infection for a long time [6]. The antibiotic-resistant H.pylori, in particularly resistant to clarithromycin, has become more and more common in the world as well as in Vietnam [3,11,14]. With the antibiotic resistance, especially clarithromycin resistance, the rate of H.pylori eradication of this regimen has remarkly decreased, from more than 90% down to less than 40% [1,4,10].

In this study, the rate of H. pylori eradication by triple therapy RAC for 10 days was only 53.3%. Because all the patients enrolled in this study had acceptable adherence, this results suggested the importance of antibiotic resistance in our group of patients.

Many authors were agree that the resistance of Amoxicillin in South East Asia was very low, even less than 1%. So we focused to investigate clarithromycin resistance in our patients.

Phenotypic tests to detect sensibility to antibiotics were still gold standard in assessing antibiotic resistance, but the technique was

difficult and need to wait more than one week [5]. The discovery of a relationship between 23S rRNA mutation gene with clarithromycin resistance has led to a new approach to the diagnosis of clarithromycin resistance. Although sequencing is still considered as a test of reference, but some simpler techniques have applied in this purpose, such as PCR-RFLP, PCR-OLA, PCR-DEIA… [7, 10]. Among these techniques, PCR-RFLP is a relatively simple, rapid, less difficult and widely accepted by many authors. Accuracy rate of PCR-RFLP in the diagnosis of clarithromycin resistance H.pylori was also considered high in many studies [8,12,13].

In this study, we investigated the rate of A2143G point mutations, a common point mutations reported in may papers to be responsible to clarithromycin resistance. In our study, A2143G point mutations was found in 45.2% in group with failure to triple therapy and 16.7% in group with sucessful eradication. This result suggested that A2143G point mutations may be a risk factor of clarithromycin-resistant H. pylori (OR = 4.13; 95%CI = 1.56-10.92). According to Menard's protocol, the most important point mutations in the detection of clarithromycin resistance was A2142G, A2143G and A2142C [7].

The frequency of A2143G point mutation varies according to different countries. A2143G point mutations were found in 39-45% in America [9,10]; 44-67% in Europe [11] and 68.3-90% in some countries of Asia [11,12]. Generally, A2143G point mutation became a global problem that affect importantly the efficacy of classical triple therapy.

Some factors affecting A2143G point mutations of H.pylori. We also evaluated some factors affecting the rate of A2143G point mutation and found the significant difference between the groups having or not history of clarithromycin usage. This result was similar to other authors [14].

5. CONCLUSION

- Eradication rate of the triple therapy RAC in 10 days was only 53.3%

- The A2143G point mutation in the 23S rRNA gene was found in 45.2% and 16.7% in two groups of non-eradicated H.pylori and eradicated H.pylori, respectively.

- History of clarithromycin usage may be a risk factor of the presence of A2143G point mutation in the 23S rRNA gene.

REFERENCES

1. Binh, T.T., et al., *The Incidence of Primary Antibiotic Resistance of Helicobacter pylori in Vietnam.* J Clin Gastroenterol, 2012, Pubmed.
2. Nguyen Thi Nguyet et al, *Khảo sát tính kháng thuốc các chủng Helicobacter pylori phân lập từ các bệnh nhân viêm dạ dày mạn tính, loét dạ dày và ung thư dạ dày.* Tạp chí Y học thực hành, 2010. 712(4): trang 20-22.
3. Le Dinh Minh Nhan, Vo Thi Chi Mai, *Tính đề kháng kháng sinh của Helicobacter pylori trong bệnh viêm loét dạ dày tá tràng.* Tạp chí y học TP Hồ Chí Minh, 2006. 10(1): trang 73-75.
4. Nguyen Van Thinh et al, *Tình hình kháng kháng sinh của Helicobacter pylori ở những bệnh nhân loét hành tá tràng trong 6 tháng đầu năm 2009.* Tạp chí Y học thực hành, 2009. 669(8): trang 14-18.
5. Megraud, F. and P. Lehours, *Helicobacter pylori detection and antimicrobial susceptibility testing.* Clin Microbiol Rev, 2007. 20(2): p. 280-322.
6. Malfertheiner, P., et al., *Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report.* Gut, 2012. 61(5): p. 646-64.
7. Menard A., Santos A., Maraud F., Oleastro M. (2002), "PCR-Restriction Fragment Length Polymorphism can also detect point mutation A2142C in the 23S rRNA gene, associated with Helicobacter pylori resistance to clarithromycin", *Antimicrobial Agent and Chemotherapy,* 46(4):1156-1157.
8. Versalovic J., Shortridge D., Kibler K. Griffy M.V., Beyer J., Flamm R.K., Tanaka S. K., Graham D. Y., Go M. F. (1996), "Mutation in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori", *Antimicrobial Agent and Chemotherapy,*40(2):477-480.
9. Taylor D.E., Ge Z., Purych D., Lo T., Hiratsuka K. (1997), "Cloning and sequence analysis of two copies of 23S rRNA gene from Helicobacter pylori

and association of clarithromycin resistance with 23S rRNA mutations", *Antimicrobial Agent and Chemotherapy,* 41(12):2621-2628.
10. Xia H. H-X., Fan X-G., Talley N.J. (1999), "Clarithromycin resistance in Helicobacter pylori and its clinical relevance", *World Journal of Gastroenterology,* 5(3):263-266.
11. Megraud F. (1998), "Epidemiology and mechanism of antibiotic resistance in Helicobacter pylori", *Gastroenterology,* 115:1278-1282.
12. Kargar M., Ghorbani-Dalini S., Doosti A., Baghernejad M. (2011), "Molecular assessment of clarithromycin resistant Helicobacter pylori strains using rapid and accurate PCR-RFLP method in gastric specimens in Iran.
13. Abdollahi H., Savari M., Zahedi M.J., Moghadam S.D., Abasi M.H. (2011), "Detection of A2142C, A2142G and A2143G mutations in 23s rRNA gene conferring resistance to clarithromycin among Helicobacter pylori isolates in Kerman, Iran", *Iran J Med Sci,* 36(2):104-110.
14. De Francesco, V., et al., *Worldwide H. pylori antibiotic resistance: a systematic review.* J Gastrointestin Liver Dis, 2010. **19**(4): p. 409-14.