Detection of point mutations on 23S rRNA of *Helicobacter pylori* and resistance to clarithromycin with PCR-RFLP in gastric biopsy specimens in Mersin, Turkey

Mersin'de mide biyopsi örneklerindeki *Helikobakter pilori*'nin 23S rRNA'sında nokta mutasyonu ve klaritromisin direncinin PCR-RFLP analizi ile gösterilmesi

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Background/aims: *Helicobacter pylori infection has a high prevalence and is considered an important health problem in Turkey. Unfortunately, an effective treatment has not yet been found for the eradication of Helicobacter pylori infection, at least in our country. Standard therapies recommended for the eradication of Helicobacter pylori have failed in the province of Mersin, Turkey. The rate of eradication with the standard triple treatment was only 45% in the province of Mersin. It may be that Helicobacter pylori has become resistant to antibiotics. Therefore, we aimed to determine the rate of resistance to clarithromycin in the province of Mersin.* ***Methods:*** *The study included 92 patients presenting with dyspepsia to the Gastroenterology Clinic of Mersin University Medical School and undergoing endoscopy. We obtained gastric biopsy specimens and investigated whether Helicobacter pylori was present and resistant to clarithromycin. We used polymerase chain reaction-restriction fragment length polymorphism to determine A2143G and A2144G mutations and resistance to clarithromycin.* ***Results:*** *Out of 92 specimens, 37 (40.2%) had Helicobacter pylori DNA. Out of 37 specimens with Helicobacter pylori DNA, 15 (40.5%) had point mutations. Eleven specimens (29.7%) had mutations on nucleotide 2144 and 4 specimens (10.8%) had mutations on 2143.* ***Conclusions:*** *Taking account of the failure of the treatment regimens used to eradicate Helicobacter pylori infection in the province of Mersin, the high rate of point mutations determined in this study was not surprising and the rate of resistance to clarithromycin was an important indicator for the failure in the eradication of Helicobacter pylori infection.*

Key words: *Helicobacter pylori,* resistance, clarithromycin

Amaç: *Helikobakter pilori infeksiyonu ülkemizde yüksek prevalansa sahip olup önemli bir sağlık sorunudur. Maalesef, Helikobakter pilori eradikasyonu için en azından ülkemizde halen etkin bir tedavi bulunamamıştır. Helikobakter pilori eradikasyonu için önerilen standart tedaviler bölgemizde ve ülkemizde başarılı olmamıştır. Standart üçlü tedavi ile ilimizde Helikobakter pilori eradikasyon başarısı %45'te kalmıştır. Bu Helikobakter pilori'nin antibiyotiklere karşı dirençli olmasına bağlı olabilir. Bu amaçla Mersin'de klaritromisine karşı gelişen direnç oranını saptamayı amaçladık.* ***Yöntem:*** *Mersin Üniversitesi Tıp Fakültesi Gastroenteroloji Bilim Dalında dispeptik yakınmalar nedeniyle endoskopi yapılan 92 hastadan alınan mide biyopsi örneklerinde Helikobakter pilori varlığı ve klaritromisin direnci araştırıldı. Bu çalışmada A2143G ve A2144G mutasyonu ve buna bağlı olarak ortaya çıkan klaritromisin direncinin sıklığını, polymerase chain reaction-restriction fragment length polymorphism ile inceledik.* ***Bulgular:*** *Doksaniki örneğin 37 (%40.2) tanesinde Helikobakter pilori DNA'sı tespit edildi. Bu 37 Helikobakter pilori PCR örneğinden 11'inde (%29.7) nükleotid 2144'te ve 4'ünde (%10.8) 2143'te olmak üzere toplam 15 (%40.5) hastada nokta mutasyonu saptandı.* ***Sonuç:*** *Bölgemizdeki eradikasyon tedavi yetersizliğini göz önüne aldığımızda, %40.5 gibi yüksek oranda saptadığımız Helikobakter pilori klaritromisin direnci beklendiği gibi yüksek çıkmış ve klaritromisin direncinin eradikasyon başarısızlığını açıklayabilecek önemli bir bulgu olduğunu göstermiştir.*

Anahtar kelimeler: *Helikobakter pilori,* direnç, klaritromisin

INTRODUCTION

Although there have been attempts to find an ideal treatment for *Helicobacter pylori (Hp)* infection for 20 years, there remains no treatment alternative that will eradicate the disease, at least in our country. At present, there are effective treatment regimens including proton pump inhibitors, ranitidine bismuth citrate and two additional antibiotics. However, the treatment fails and *Hp* infection persists in some 40-50% of the cases in Turkey (1, 2). This creates a serious health problem in our

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country, where the prevalence of *Hp* infection is high.

The major reason for the failure to treat *Hp* infection is the resistance of *Hp* to one or more of the antibiotics used. Two antibiotics to which *Hp* has most frequently become resistant are clarithromycin and metronidazole, which has a negative impact on treatment outcome. However, effect of clarithromycin resistance on the eradication treatment success is more prominent than of metronidazole resistance (3, 4). Therefore, we attempted to determine the rate of *Hp* resistance to clarithromycin in the province of Mersin.

MATERIALS AND METHODS

We investigated the *Hp* status and resistance to clarithromycin in gastric biopsy specimens obtained from 92 patients presenting with dyspepsia and undergoing endoscopy in the Department of Gastroenterology at Mersin University Medical School between June 2005 and March 2006. One biopsy specimen was taken from the antrum in each patient. Thirty-seven of those (40.2%) had *Hp* DNA. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine A2143G and A2144G mutations and the resistance to clarithromycin resulting from those mutations. The region 360 bp involving a fragment of V domain of *Hp* 23S rRNA gene was amplified with CRFL-1 (5'-ATGAATGGCGTAACGAGAT-3') and CRRL-2 (5'-ACACTCAACTTGCGATTCC-3') primers. Point mutations on the nt 2143 and 2144, which cause resistance to clarithromycin when A turns into G, were treated with *Mbo*II and BsaI restriction enzymes, respectively, and analyzed with RFLP. Detection of mutations was based on the number and size of bands.

RESULTS

Fifty-six of the patients were male and 36 were female. Median age of the patients was 46 years. On endoscopic examination, 70 antral gastritis, 10 pangastritis, 8 duodenal ulcer disease, and 4 gastric ulcer diseases were determined. Twenty-one of antral gastritis patients, 6 of pangastritis, 7 of duodenal ulcer diseases, and 3 of gastric ulcer diseases were *H. pylori* positive. Eradication treatment for *Hp* had not been previously performed in the patients. We determined point mutations in 15 (40.5%) from 37 *Hp* PCR specimens, of which 11 (29.7%) had mutations on nt 2144 and 4 (10.8%) had mutations on 2143. These resistant *Hp* strains with mutations on nt 2144 were determined from 3 duodenal ulcer, 1 gastric ulcer, 4 antral gastritis, and 3 pangastritis patients, and the other resistant strains with mutations on nt 2143 were determined from 1 duodenal ulcer and 3 antral gastritis patients.

DISCUSSION

Rates of resistance to clarithromycin vary widely from region to region. It has been reported that great variations in the resistance rates exist between the northern and southern parts of Europe. In fact, the rate of resistance to clarithromycin in adults is lower than 5% in northern Europe and higher than 20% in southern Europe (5). The resistance rate ranges from 12.4% to 23.5% among children throughout Europe (6).

Lower rates of resistance to clarithromycin have been reported from Canada. In fact, it was below 4% in Canada and reached only 10-15% in the United States prior to 2000 (7). The prevalence was reported to be 5.4% in Israel (8), 17% in Iran (9), 11-12% in Japan (10), 4.5% in Hong Kong (11), and 5-6% in Korea (12). High resistance rates of 23-44% were reported from Thailand in 2004 (13,14).

The primary risk factor for resistance to clarithromycin is previous use of macrolides. Excessive uses of macrolides for respiratory tract infections in children increase the risk of resistance. Several studies have shown a strong relationship between uses of macrolides and resistance to macrolides. Soon after the introduction of macrolides in 1997, resistance to these antibiotics was detected in Estonia in 1998 (15). Parallel with a four-fold increase in the use of clarithromycin in Japan, rates of resistance to this antibiotic have increased four-fold between 1993 and 2000 (10).

There can be cross-resistance to macrolides. This is particularly important for erythromycin. In fact, when the rate of resistance to clarithromycin was 17% in Iran, clarithromycin was not being used there, but erythromycin was being used (9). Similarly, the rate of resistance to clarithromycin (8%) in France in 1993 was attributed to excessive previous uses of erythromycin and josamine (16).

The cause of *Hp* resistance to clarithromycin is point mutations located on the peptidyl transferase region of the 23S rRNA gene. As a result of these mutations, clarithromycin cannot bind to 23S ribo-

some and cannot disrupt the protein synthesis of the bacterium. Various molecular techniques can help to detect those point mutations. The incidence of point mutations detected in various regions of the world is shown in Table 1. A2143G is considered the most frequent mutation in Europe (69.8%) (3). However, the frequency of these mutations varies with geographical regions. Furthermore, whichever mutation is sought in a study, the results of that study are directed towards the mutation sought. As a result, the obtained results may not show real mutation rates. Actually, if an appropriate restriction enzyme is not used, one cannot comment whether the mutation sought is the one responsible for resistance.

The results of the studies reported from Turkey on the rates of resistance to clarithromycin are summarized in Table 2. It is striking that the rate of resistance to clarithromycin has increased from 0% to 56% over the years (22-38).

Resistance to antibiotics is the cardinal factor that affects the outcome of treatment for eradication of *Hp*. In a study by Megraud (3) on the rates of resistance to antibiotics in the past five years, the rate of *Hp* eradication was 87.7% in the presence of sensitivity to clarithromycin and 18.3% in the presence of resistance to clarithromycin. This decrease in eradication rates (70%) is more striking than that reported in meta-analyses by Houben (53%) (39) and later Dore et al. (55%) (4). However, Megraud did not make a systematic evaluation, but evaluated the results of 20 studies on the same treatment. Those results showed striking clinical effects of resistance to clarithromycin.

In conclusion, the high rate of resistance to clarithromycin found in this study may clearly explain the reason why standard treatment regimens have failed to eradicate *Hp* infection. Multi-center studies on resistance to antibiotics including amoxicillin and tetracycline should be performed immediately. The results of those studies will help to determine national treatment regimens to eradicate *Hp* infection.

Table 1. Prevalence of mutations causing resistance to clarithromycin

Geographic area (reference)	A2142G (%)	A2142C (%)	A2143G (%)	A2143C (%)	A2144G (%)
Europe (3)	11.7	2.6	69.8		
Japan, Korea (17)					90-93
Brazil (18)	83		11.5		
Mersin (Turkey)			26.6		73.4
Adana (Turkey) (19)			15.3		46.1
Ankara (Turkey) (20)	100				
Ankara (Turkey) (21)					100

Table 2. Distribution of rates of resistance to clarithromycin in Turkey by years

Year	City	Method	Resistance %	Author (Reference)
1994	Ankara	DD	21	F. Şahin (22)
1996	İzmir	-	0	A. Aydın (23)
1997	İzmir	DD	5.4	A. Aydın (23)
1997	Ankara	DD	0	M. Palabıyıkoğlu (24)
1999	Diyarbakır	-	18.7	V. Göral (25)
2000	Malatya	DD	9.8	B. Kantarçeken (26)
2001	Ankara	E-test	11.4	D. Ergin (27)
2003	Adana	AD	24.5	F. Işıksal (28)
2004	Ankara	PCR-Beacon	16.9	M. Y. Çırak (29)
2004	Ankara	RT-PCR	16.2	M. Y. Çırak (30)
2004	Adana	-	21	S. Çolakoğlu (19)
2004	Kocaeli	DD	9	S. Hülagu (31)
2004	İzmir	RT-PCR	48.2	G. F. Önder (32)
2004	Ankara	AD, E-test	56	A. Özden (33)
2005	İzmir	RT-PCR	38.8	A. Aydın (34)
2005	Ankara	AD, E-test	55	P. Bağlan (35)
2006	Mersin	PCR-RFLP	40.5	O. Sezgin
2006	Ankara	FISH	35	P. Bağlan (36)
2006	Konya	PCR	35.7-64 (DM)	D. Akkaya (37)
2006	Diyarbakır	E-test	16.4	Y. Tüzün (38)

DD: Disc diffusion; AD: Agar dilution; RT-PCR: Reverse transcription-polymerase chain reaction; FISH: Fluorescent in situ hybridization; DM: Diabetes mellitus.

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