

RESULTS OF THE STUDY OF RESISTANCE TO CLARITHROMYCIN IN PATIENTS WITH HELICOBACTER PYLORI-ASSOCIATED GASTRIC DISEASES IN UZBEKISTAN

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Abstract

The authors studied the resistance of bacteria to clarithromycin in 194 patients with a positive CagA gene of *H. pylori*-associated stomach diseases. The study is based on the results of molecular genetic research methods and statistical data. According to the study, the studied population was characterized by the presence of three types of gene mutations in the 23S-rRNA gene: 2142 G, 2142C and 2143 G. Wild type Wt type *H. pylori* was detected in 58.8% of cases.

Keywords: *H. pylori*-associated diseases of the stomach, molecular genetic study, resistance to clarithromycin, PCR diagnostics, CagA gene, amplification, primers and mutations of the 23S-rRNA encoded gene at [A/G, C, G] 2142 and 2143 plots.

INTRODUCTION

To achieve the optimally stable effectiveness of the ongoing therapeutic measures, it was important to choose a treatment strategy with the determination of the tactics of eradication therapy, including the need to study the resistance of *Helicobacter pylori* (*H. pylori*.) to clarithromycin. Resistance to clarithromycin *H. pylori* significantly reduces the effectiveness of treatment and may even be ineffective [1].

The study of *H. pylori* resistance to antimicrobials is an urgent problem in modern medicine. Thus, 700 thousand people die every year from the ineffectiveness of antibiotics, 100 trillion dollars are spent on treatment [2].

The irrational use of antimicrobials often leads to the formation of molecular genetic mechanisms of bacterial resistance [5].

Eradication therapy for *H. pylori* dramatically reduces the likelihood of developing inflammation of the gastric mucosa, precancerous conditions, atrophic gastritis and intestinal metaplasia [4, 7, 8, 9].

According to the WHO recommendations, it is expedient to determine *H. pylori* infection in case of stomach diseases, followed by the inclusion of eradication therapy in the arsenal of therapeutic measures [3]. Undoubtedly, the success of treatment depends on a number of factors, such as the virulence of the *H. pylori* pathogen, the degree of contamination and, most importantly, the sensitivity of the bacterium to the therapy. Therefore, the presence of *H. pylori* resistance to eradication therapy plays a significant role not only in the effectiveness of the therapy, but also in the development of its long-term results. In particular, *H. pylori* resistance is the reason for the unsatisfactory achievement of a full cure, the development of relapses, and even a decrease in the effectiveness of specialized treatment in patients with gastric cancer and MALT lymphoma [6].

The aim of this work was to study the resistance of *H. pylori* to clarithromycin.

Material and methods

It was examined that out of 194 patients with a positive CagA gene. Our studies were carried out on isolated bacterial isolates from each patient. To achieve the goal, we used the method of multiplex real-time PCR, which made it possible to simultaneously determine mutations in two regions. At the same time, based on the data of modern literature [3], We chose mutations of the 23S-rRNA molecule of the encoded gene at [A/G, C, G] 2142 and 2143 regions as conferring resistance to clarithromycin.

Results

When studying mutations in the H. Pylori genes in the A2142G/C and A2143G regions of the 23S rRNA gene, which provide resistance to clarithromycin, the following primers and probes were used (Table 1).

Table 1 Primers and probes used for detection and mutation of H. pylori genes in the A2142G/C and A2143G regions of the 23S rRNA molecule

Molecule	Name of coding sections	Sequence of primers and probes 5' - 3'	Paired nucleotides (bp)
23S-rRNA	23SScWT	(FAM) aa - ggt - agg - tga - aaa - ttc - ctc - cta - cc (BHQ 2)-(Spacer 18) gg - acc - acg - ggg - tct - tt	114
	23SScA2142G	(Cy3)aa-ggt-agg-tga-aaa-ttc-ctc-cta-cc(BHQ2)- (Spacer18)gg-acc-acg-ggg-tct-tc	
	23SScA2143G	(ROX)aa-ggt-agg-tga-aaa-ttc-ctc-cta-cc(BHQ2)- (Spacer18)gg-acc-acg-ggg-tct-c	
	23scA2142C	(Cy5)aa-ggt-agg-tga-aaa-ttc-ctc-cta-cc(BHQ2)- (Spacer18)gg-acc-acg-ggg-tct-tg	
	23S - F - 2	TGCGAAATTCCTTGTCGG	

The results obtained by us are documented in graphical form. At the same time, fixation of the amplification reaction graphs for the FAM, Cy3, ROX and Cy5 detectors was established. After determining the presence of H. pylori bacteria on the Real-Time PCR diagnostic charts, studied their resistance to the antibiotic clarithromycin. To do this, samples of H. pylori bacteria, using specific primers and probes, were examined by Real-Time PCR diagnostics with the detection of mutations in the CagA gene in the A2142 G / C and A 2143 G regions in the V functional domain of the 23S-rRNA molecule. In the A2142 G / C and A 2143 G regions of the 23S-rRNA gene, we added the following components to sterile tubes in a total volume of 20 µl: of which DNA - 100 ng / µl, 23 SF 2 - 1 µ M , A 2142 G - 0, 14 µ M , A 2143 G - 0.18 µ M , A 2142 C - 0.1 µ M , 23 Scwt - 0.08 µ M . The reaction was carried out with a PCR kit core (Isogen, Russia).

In the course of amplification, repetition of DNA thermal denaturation cycles, sequential annealing of the oligonucleotide probe and allele-specific primers on complementary DNA sequences, and subsequent completion of polynucleotide chains with thermostable DNA polymerase primers were observed. For the detection of a specifically formed PCR product, both reaction mixtures contained a probe that specifically binds to the studied DNA region, labeled at the 5' / -end with the fluorescent dye ROX or Cy3, FAM, Cy5, and at the 3' / -end with a fluorescence quencher (BHQ2). The quencher absorbed the radiation emitted by the fluorescent label until the probe was cleaved by DNA polymerase due to 5' / -exonuclease activity. After the DNA probe was cleaved, the dye was separated from the quencher and, as amplification accumulated, its fluorescence level increased. This process was monitored at the end of each amplification cycle in real time.

Fluorescent probes were attached to each reverse primer. This multiplex reaction made it possible in one test tube, due to a single reaction, to detect mutations in two regions A2142 G / C and A 2143 G simultaneously. When performing real-mode PCR diagnostics, in cases where A-adenin was present in the A2142 G / C and A 2143 G regions of the 23S-rRNA molecule, the FAM probe fluorescence was started earlier than that of the Cy3, Cy5, and ROX probes. And in cases where there was a

mutation of guanine or cytosine (G, C), respectively, the fluorescence of the probes Cy3, Cy5, ROX was observed earlier than in FAM.

Analysis of the obtained results of multiplex PCR showed that out of 194 patients with a positive CagA gene, *H. pylori* were detected in 80 (41.2%) in regions 2142 and 2143 of the encoded gene of the 23S-rRNA molecule, which confirmed resistance to clarithromycin in these patients. According to the monitoring of the PCR product accumulation graph (Fig. 1), in some cases there was no mutation, which starts from the 11th cycle - Wt (Wild-type - wild (non-mutant type)) of the encoded gene of the 23 S - rRNA molecule.

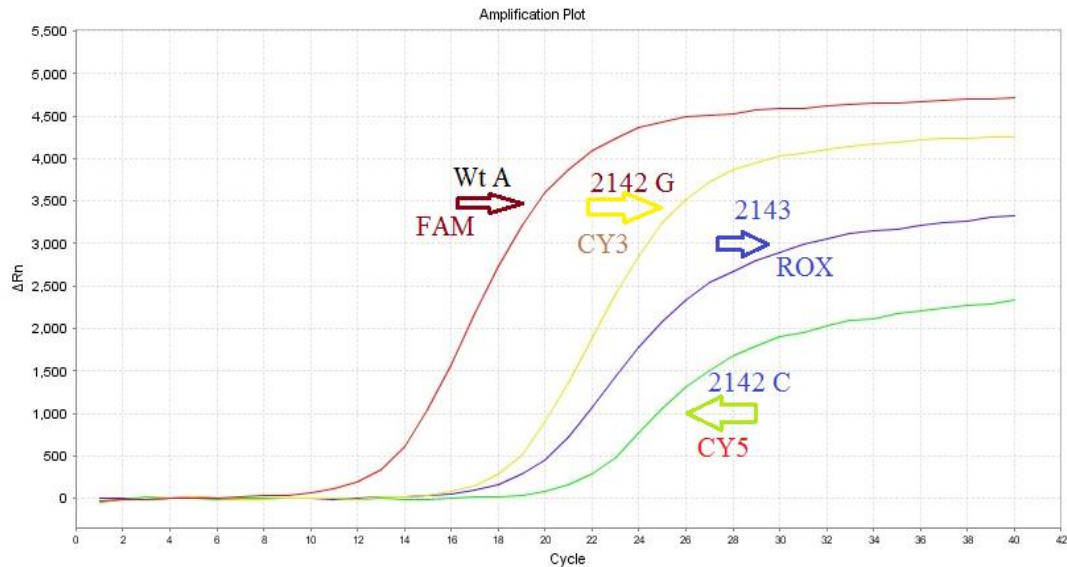


Fig. 1. Graph of PCR product accumulation in regions [A/G, C, G]

2142 and 2143 with no mutations: Wt (beginning from cycle 11), region 2142 (beginning from cycle 18 to cycle 21), region 2143 (beginning from 19 cycles).

In contrast to the wild, non-mutant gene, the presence of a mutation in the 2142 G region of the encoded gene of the 23 S rRNA molecule was noted from 17 to 25 amplification cycles (Fig. 2).

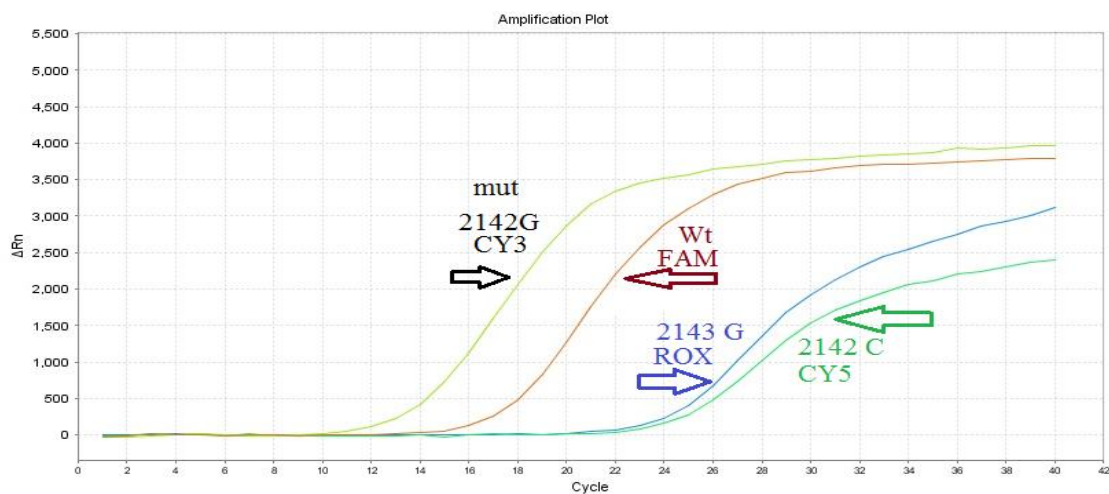


Fig. 2. Graph of the presence of the [A/G, C, G] mutation in the encoded gene of the 23S rRNA molecule at region 2142. Amplification curve of the mutation at region 2142 (beginning from cycle 12). Amplification curve of wild-type Wt (beginning at cycle 17). Amplification curve of the mutation in the 2142C region (rise from cycle 25).

Also, during the decrease in the PCR product, a mutation was found in the coding region 2142C (cytosine) of the gene of the 23S-rRNA molecule. At the same time, the amplification curve in the 2142C region had an increase before the 14th cycle against the background of the absence of a mutation in the 2143 region (beginning from the 24th cycle) (Fig. 3).

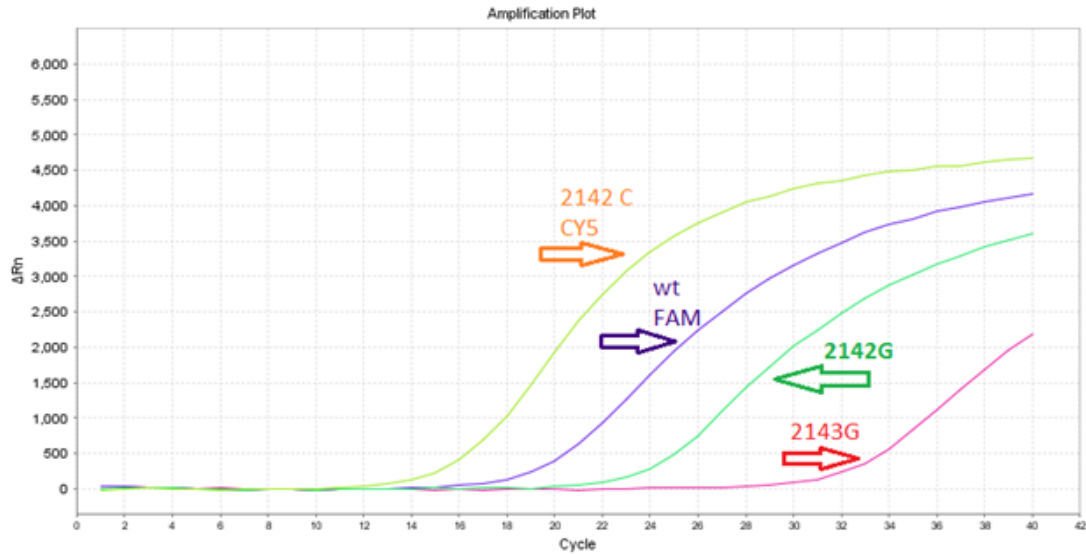


Fig. 3. Graph of the presence of a mutation in the 2142C (cytosine) coding region of the 23S rRNA molecule gene. Amplification curve of the mutation in the 2142C region (rise before cycle 14). Amplification curve of the Wt mutation (rise before cycle 19) and in the 2142G region (rise before cycle 23). No mutation in the 2143G region (from cycle 24).

In our studies, mutations in the encoded gene of the 23S-rRNA molecule in the 2143 G region were identified. At the same time, we observed an increase in Wt in the 2143G region from the 10th cycle, and an increase in the amplification of the mutant 2143G - from the 18th cycle. The graph shows the late rise of the encoded gene of the 23S-rRNA molecule at region 2142 (Fig. 4).

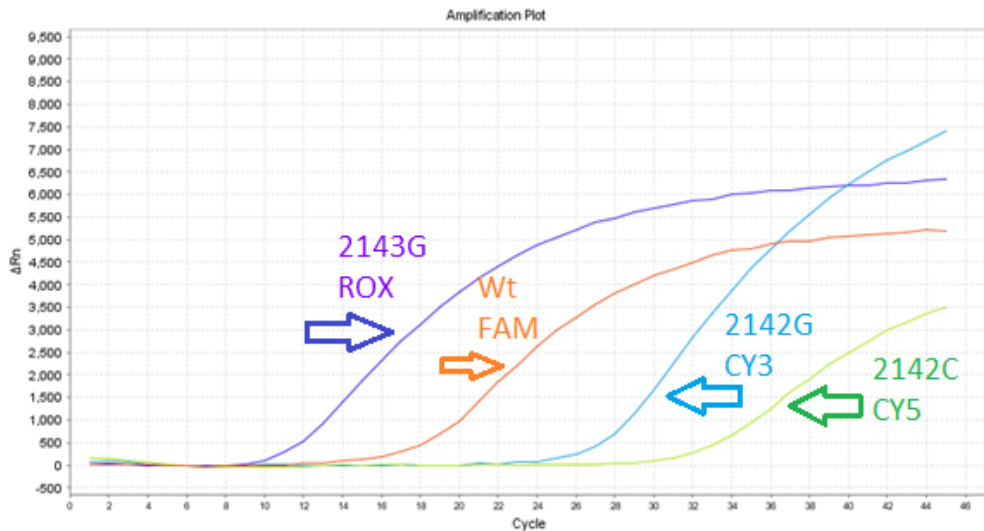


Fig. 4. Graph of the presence of mutation G (Guanine) in the coding region 2143 of the 23S-rRNA molecule gene. Amplification curve of the mutation in the 2143G region (beginning from cycle 10). Wt mutation amplification curve (rise from cycle 18). Mutation amplification curve in 2142 G/C regions (late rise).

The results showed that out of 81 patients in the 2142 G mutation of the 23S-rRNA coding gene was detected in 53 (66.3%), in the region 2142C - in 21 (25%) and in section 214 3 G - only in 7 (8.7%) isolates. It should be noted that according to the

biopsy samples studied by us, the mutation of the caodirovannoe gene of the 23S-rRNA molecule in the 2142 G region had a frequency of detection 2.5 and 7.5 times higher than the mutations of the isolates in the 2143 G and 2142C regions (Fig. 5).

This indicates the presence of resistance *H. pylori* to clarithromycin in association with other bacteria, which requires sample sequencing. Taken together, it should be noted that the study of the 23S rRNA gene responsible for *H. pylori* to clarithromycin showed the frequency of its occurrence in 41.2% of cases.

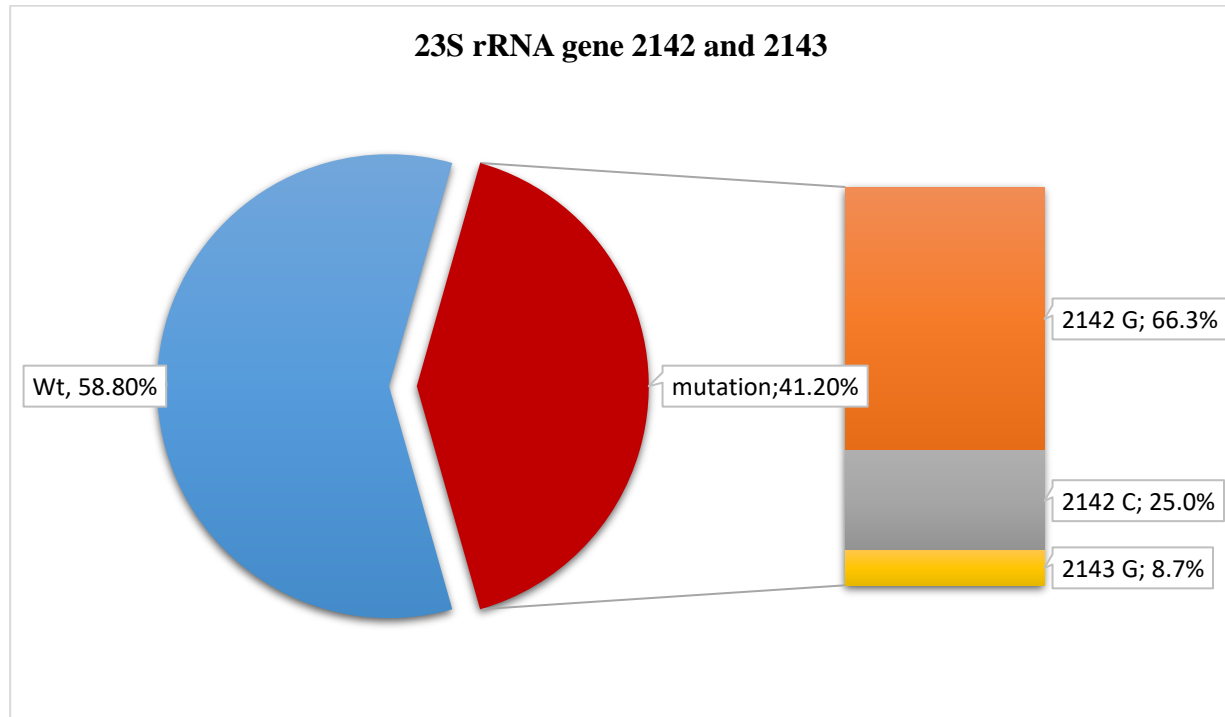


Fig 5. Expression of the 23S-rRNA gene.

This circumstance led to unsuccessful traditional eradication therapy in almost every second patient. At the same time, every third patient had mutations in the 2142 G region.

In general, the population studied by us was characterized by the presence of three types of gene mutations in the 23S-rRNA gene: 2142 G , 2142C and 2143 G. Wild type Wt type *H. pylori* was detected in 58.8% of cases.

Conclusions

Our results indicated the presence of bacterial isolates in patients with *H. pylori*-associated diseases of the stomach, which have a fairly pronounced resistance to clarithromycin (CLR). Effect of resistance *H. pylori* on the overall effectiveness of eradication therapy is undeniable.

It should be noted that in Uzbekistan, in the treatment of patients with *H. pylori* -associated diseases of the stomach, it is important to identify the resistance of *H. pylori* strains to clarithromycin and other antibiotics. In turn, this will open up new opportunities for the optimal choice of treatment tactics and achieve the full success of eradication therapy. In the future, for a broader study of the molecular genetic aspects of the resistance of *H. pylori* strains to clarithromycin, it is necessary to sequence the 23S-rRNA gene in the V domain, as well as to introduce Real Time PCR test systems in the most common mutations of *H. pylori* bacteria in Uzbekistan.

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