Molecular genetic analysis of 23S RNA mutations associated with clarythromycin resistance in Helicobacter pylori strains isolated in St.Petersburg, Russia

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Objectives

Resistance to antibiotics quite often interferes with effective eradication of Helicobacter pylori. It is known that resistance to clarythromycin which interferes with ribosomal synthesis can be achieved by point mutations in 23S ribosomal RNA. Most of the claR mutations described in the scientific literature are the transitions of adenine to guanine in the positions A2142G or A2143G. Recently other mutations of such kind have been described (Garrido L, 2007; Toracchio S, 2004; Umegaki N, 2000). After the analysis of H. pylori clinical strains collection isolated in St.Petersburg we have determined that most common claR mutations were A2142G, A2143G and T2717C. All these mutations are possible to determine by PCR with the following restriction analysis. However, in some cases of claR strains those mutations were not found.

The aim of present work was to analyze the structure of mutations in 23S RNA in H. pylori strains isolated in St.Petersburg, Russia.

Materials and methods

20 patients with chronic gastritis associated with H. pylori infection were selected. Clarythromicin resistance of the strains was tested by the disk diffusion method. Region corresponding to 23S RNA was amplified by PCR, digested with MboII, BsaI and HhaI and subjected to DNA sequencing.

Results

The presence of H. pylori among the group of patients under study was determined by urease test and by PCR employing the primers corresponding to several H. pylori genes (UreC, UreI, CagA). 7 strains under study were found to be resistant to clarythromicin and 4 out of 7 were resistant to digestion with MboII, BsaI and HhaI, which suggested the absence of mutations A2142G, A2143G and T2717C. After DNA sequencing it was determined that the rest of the strains carried different claR mutations: T2182C, C2195T or C2288T. Mutation T2182C have been described previously (Khan R, 2004; Kim, 2002; Posteraro R, 2006; Chihu L, 2005) but mutations C2195T and C2288T were found only together with A2142G or A2143G (Posteraro P, 2006; Pimbara E, 2007).

Conclusions

Molecular study of the structure of clarythromicin resistant 23S RNA mutations in St.Petersburg Russia allowed determining the broad spectrum of mutations some of which were determined for the first time. This fact leads to conclusion that commonly used for such diagnostics PCR with the following restriction analysis might be not appropriate for determining clarythromicin resistant H. pylori strains in Russia.