Molecular prevalence of point mutations conferring resistance to clarithromycin in Helicobacter pylori in the United Arab Emirates

Mubarak S. Alfaresi, *MBBS, FACHARZT,* ***Abdulla A. Eltom,*** *FRCP (Edin), FRCP (Glasg),* ***Saeed S. Alshaikh,*** *LRCSxPI, MD,* ***Elwin H. Buchel,*** *MMED (Int), FCP (SA),* ***Lamya S. Saeed,*** *MBBS, MSc,* ***Monther M. Aljenaibi,*** *MBBS, MRCP (UK).*

ABSTRACT

Objective: The aim of the study was to find the prevalence of point mutations conferring resistance to clarithromycin in *Helicobacter pylori* (*H. pylori*) in the United Arab Emirates.

Methods: Gastric biopsy samples were obtained from 9 dyspeptic patients attending the endoscopy department in Zayed Military Hospital, Abu-Dhabi, UAE during the period from January to September 2004. Real-time polymerase chain reaction (PCR) was carried out for these biopsies to determine the point mutations.

Results: Of the 55 (60%) real-time biopsies that were PCR positive for *H. pylori*, 36 (65.5%) were found to have mutant genes. The A(2142/43)G mutation was more common (63.6%) than the A(2142)C mutation (18.2%).

Conclusion: Point mutations A(2442/43)G and A (2142)C and the combination of both were common among our patients. Perhaps the highest reported yet. Mutation at A(2142/43)G is far more common than mutation at A(2142)C.

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Helicobacter pylori (*H. pylori*) is a gram-negative spiral bacterium that colonizes and persists in human gastric mucosa. It infects more than half of the world's population and implicated as a causative agent of gastritis, peptic ulcer disease, gastric carcinoma, and lymphoma.[1]

To date, there is no single standardized treatment regimen, and treatment failure are still reported to be highly frequent. Many factors have been implicated as causes of treatment failure, including ineffective penetration of antibiotics into the gastric mucosa, antibiotic inactivation by low stomach pH, lack of patient compliance, and the emergence of acquired resistance to antibiotics by *H. pylori*.[2-4] The first line regimen consists of clarithromycin, ampicillin and a proton pump inhibitor for the duration of one week.

Poor compliance, side effects and resistance to antibiotics are the common cause of treatment failure. The increasing use of clarithromycin has resulted in development of resistance. Resistance to this antibiotic is due to point mutations within the peptidyltransferase-encoding region of the 23S rRNA.[5] Three major point mutations in 2 positions on the 23S rRNA (equivalent to *Escherichia coli* coordinates 2058 and 2059) have been described in which an adenine residue is replaced by a guanine or a cytosine residue in different positions: A2142C, A2142G and A2143G.[5-8] Mutations A2142G and A2143G are the most frequently reported, whereas

From the Department of Microbiology (Alfaresi), Department of Gastroenterology and Endoscopy (Eltom, Alshaikh, Buchel, Saeed, Aljenaibi), Zayed Military Hospital, Abu-Dhabi, *United Arab Emirates.*

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Address correspondence and reprint request to: Dr. Mubarak S. Alfaresi, Clinical Microbiologist, Zayed Military Hospital, PO Box 3740, Abu-Dhabi, *United Arab Emirates.* Tel. +9712 4055863. Fax. +9712 4492075. E-mail: uaenow@emirates.net.ae

mutation A2142C is less common.[9] Point mutations at 2 additional sites, A2115G and G2141A, have been described as occurring in the same strain,[10] although these mutations have never been subsequently reported.

The prevalence of primary and acquired clarithromycin resistance is also increasing worldwide, the consequence of this is less successful treatment.[11-13] Several reports have shown that the cure rate is between 0 and 50% when *H. pylori* strain is resistant to clarithromycin, whereas it is approximately 90% when the strain is susceptible.[14] More recently, real-time polymerase chain reaction (PCR) methods were developed that are based on amplification of a fragment of the 23S rRNA gene of *H. pylori* followed by melting curve analysis. The first attempt was performed by Gibson et al[15] on *H. pylori* strains.

The purpose of this study was to determine the prevalence of A2142G, A2143G and A214C point mutations in the 23S rRNA gene found to be associated with *H. pylori* resistance to clarithromycin. In this study we tried to determine the molecular prevalence of point mutations conferring resistance to clarithromycin in *H. pylori* in the United Arab Emirates (UAE) for the first time by using a published method in which the real-time PCR technology used.

Methods.

Study design. Gastric biopsy samples were obtained from 91 dyspeptic patients referred for endoscopy at Endoscopy Department in Zayed Military Hospital, Abu Dhabi, UAE. The mean age of the patients was 36 years (range, 16 - 65 years), and 70% of the patients were men. Patients were included in the study if they had a positive *Campylobacter*-like organism (CLO) test or not. One endoscopic biopsy specimen taken from antrum. The biopsies were immediately sent in normal saline to our department and were frozen at –8ºC for further processing.

DNA isolation. High pure PCR template preparation kit from Roche Diagnostics, Germany was used according to the manufacturer (Cat. No. 1 796 828). The isolated DNA was eluted in 200-ul elution buffer and then stored in –8ºC for further processing.

Detection of point mutations in the 23S rRNA gene of H. pylori by real-time PCR. As described earlier by Oleastro et al,[16] a real-time PCR-based PCR-hybridization assay was used directly on DNA obtained from gastric biopsies to detect point mutations conferring resistance to clarithromycin. The method included amplification of a fragment of the 23S rRNA gene of *H. pylori* coupled with simultaneous detection of the product by probe hybridization and analysis of the melting curve by using real-time PCR.[16,17] A 267-bp fragment of the 23S rRNA gene of *H. pylori* was amplified using primers HPYS and HPYA as previously described.[16] The amplified product was detected with 2 probes: the sensor probe, 5' labeled with LC-Red 640 and 3' phosphorylated (5'-GGCAAGACGGAAAGAACC-3'; nucleotides 2504 - 2520), which hybridized with the region containing the mutation sites, and the anchor probe, which hybridized 3 bases upstream from the former and was 3' labeled with fluorescein (5'-TGTAGTGGAGGTGAAAATTCCTCCTACCC-3'; nucleotides 2473 - 2501).

Using the light cycler thermocycler (Roche Diagnostics, Germany) the PCR and hybridization reactions were carried out in glass capillaries in a volume of 20 µl containing 3 µl of template DNA, 1.6 µl of MgC12 (25 mM), 0.4 µl of forward and reverse primers (20 µM each), 0.2 µl of sensor and anchor probes (20 µM each), and 2 µl of Fast Start DNA master hybridization probes (Roche Diagnostics). Polymerase chain reaction amplification comprised an initial denaturation cycle at 95ºC for 10 minutes, followed by 50 amplification cycles (with a temperature transition rate of 20ºC/s) consisting of 95ºC for 0 s, annealing at 60ºC for 10 s, and extension at 72ºC for 17 s. After amplification a melting step was performed, consisting of 95ºC for 0 s, cooling to 45ºC for 30 s (with a temperature transition rate of 20ºC/s), and finally a slow rise in the temperature to 85ºC at a rate of 0.1ºC/s with continuous acquisition of fluorescence decline.

Data analysis. Data were analyzed with the computer software program Statistical Package for Social Sciences version 10.0.1 (Windows version). The *p* value was calculated using the Chi-square method. Any *p* value less than 0.05 were reported as statistically significant.

Results.

Subject description. Total of 91 samples were studied. Seventy-six (74%) samples were from UAE national patients. Sixty-four (70%) were from male patients. Urease test was positive in 40 (44%) samples. Positive real-time PCR (positive considered when any of the 3 mutations or any combination of them were found) was found in 55 (60%) samples. From those real-time PCR positive for *H. pylori*, 36 (65.5%) found to have mutation of the A(2142/43)G or A(2142)C, or both types. The A(2142/43)G mutation type presented in 35 (63.6%) of the samples, 10 (18.2%) samples presented with A(2142)C mutation type. Combinations of both types were found in 9 (16.3%) of the samples.

Prevalence of A2142/43G and A2142C mutant genes. Of the 55 (60%), real-time PCR positive for *H. pylori*, 36 (65.5%) were found to have mutant genes. The A(2142/43)G mutation is more common (63.6%) than the A(2142)C mutation (18.2%). From the 39 UAE national patients, there were 26 (66.7%)

with mutant genes. Similarly, 10 (62.5%) patients were positive for mutant genes in non-UAE national group. In female group, there were 13 (81.3%) patients with mutant genes. In male group, there were 23 (59%) patients with mutations.

The results showed up no correlation between the point mutation and patient gender ($p>0.1$). Similarly, there was also no significant difference between patient nationalities either ($p>0.5$). The p value calculated using the Chi-square method. Any p value less than 0.05 were reported as statistically significant.

Discussion. Eradication of *H. pylori* is effectively achieved by the combination of a proton pump inhibitor and antibiotics. The first line regimen consists of clarithromycin, ampicillin and a proton pump inhibitor for the duration of one week. Treatment failure is increasingly reported worldwide. Poor compliance, side effects and resistance to antibiotics are common cause of failure. The increasing use of clarithromycin has resulted in development of resistance. The prevalence of clarithromycin resistance varies from 1% in Norway,[18] to 29% in Japan.[19] The mechanism of resistance to clarithromycin is associated with the point mutation within the peptidyltransferase encoding region of 23S rRNA gene.[20] Three major point mutations in 2 positions have been described in which adenine residue is replaced by guanine or a cytosine residue at adjacent positions: A2142C, A2142G and A2143G.[20]

The purpose of our study is to determine the prevalence of each mutation in endoscopic gastric biopsies tested positive for *H. pylori* by PCR. A second biopsy taken at the same time was immediately tested for *H. pylori* by rapid CLO test. Out of 91 biopsies studied, 55 were real-time PCR positive (44%); while only 40 were CLO test positive (44%).

Thirty six of the PCR positive demonstrated the A2142G, A2143G or A2142C point mutation (65.5%). The A2142/43G mutations were more common than the A2142C mutation, while the combination of both mutations was also present in 16.3% of the samples. We presume that *H. pylori* eradication failure among our patients is probably more frequently related to clarithromycin resistance than we initially believed. The wide use of clarithromycin in the UAE, other Gulf States, neighboring countries and possibly worldwide is most likely responsible for the development of *H. pylori* strain resistant to clarithromycin.

The prevalence of the point mutation A2142/43G and A2142C among our patients (65 point 5%), is the highest compared with figures reported from various other countries, namely, 29% in Japan.[19] Recent data from Western Europe indicate that greater than 60% of treatment failure with clarithromycin-based protocols are associated with the presence of clarithromycin-resistance *H. pylori* isolates after therapy. Fifteen CLO negative specimens tested positive by real-time PCR. We assume that the bacteria were present in too low count to be detected by this method and we concluded that real-time PCR is more sensitive than CLO test in detecting *H. pylori.* Kobayashi et al[21] compared real-time PCR results to those of cultures, Histopathology and rapid urease test. They found that real-time PCR had the highest sensitivity and specificity, 100% for both. Three patients were CLO test positive while in real-time PCR was negative for *H. pylori.* We assumed that this results was due to PCR inhibition in these samples.

In conclusion, real-time PCR is more sensitive than CLO test in detection of *H. pylori.* Point mutations A(2442/43)G and A(2142)C and the combination of both are common among our patients. Perhaps the highest reported yet. Mutation at A(2142/43)G is far more common than mutation at A(2142)C. We presume that *H. pylori* with mutant genes resistant to clarithromycin as been proved by several studies worldwide and may be responsible for treatment failure among our patients. Real-time PCR is a rapid and sensitive means for *H. pylori* detection and for determination of possible resistance to clarithromycin and accordingly helps in treating patients who failed to eradicate the bacteria. No correlation was found between prevalence of genes mutation and gender or nationality. Finally, the question is, should this method be used before the administration of an eradication course without having to wait for cases of failure? Would this strategy reduce the proportion of treatment failure and is it cost-effective?

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