Clarithromycin-Resistant Helicobacter Pylori Strains among Dyspeptic Patients in Sudan

Nazar Abdalazeem*1, Hassan Abdul-Aziz1, Adam Ahmed Adam2, Waleed Hussein Omer2 and Ahmed Bolad2

*1Ahfad University for Women, Omdurman, Sudan
1Alribat University, Academic Affairs
2Al-Neelain University, Faculty of Medicine and Al-Neelain Medical Research Centre, Sudan

ABSTRACT

Introduction: The study aimed at characterizing the mutations in 23S rRNA gene related to Clarithromycin-resistant Helicobacter pylori strains among dyspeptic patients in Khartoum State.

Methods: Two hundred gastric biopsies were obtained by endoscopy from 200 patients with dyspepsia. DNA was extracted from culture isolated and relevant mutations in 23S rRNA gene were detected.

Results: Out of the 200 biopsies, H. pylori was isolated from 48 (24%) biopsies. Twelve of them were found to be resistant to Clarithromycin. Eight of the resistant strains had both A2143G and A2142G by using restriction enzymes Bsa1 and Bbs1. Sequencing the remaining four isolates by PCR detected A2140G mutation.

Conclusions: In conclusion, Clarithromycin-resistant H. pylori in Sudan may be the main cause of treatment failure aiming at eradication of the bacterium from patients. Such a finding may necessitate the need for other treatment regimens. More collaborated research in this field is needed.

Keywords: Helicobacter pylori, Clarithromycin resistance.

INTRODUCTION

Helicobacter pylori is a gram-negative bacterium that causes chronic gastritis and peptic ulcer disease (1,2). Infection with H. pylori can be effectively treated by the combination of a proton pump inhibitor in addition Clarithromycin and amoxicillin.

Emergence of Clarithromycin resistant strains is not well studied in Sudan.

Clarithromycin is a macrolide that binds to the 23S rRNA components of the bacterial ribosome. Resistant strains emerge due to failure of such binding because of modification of the target site by point
mutations in the peptidyl transferase region of domain V of the 23S rRNA \(^3\). H. pylori contains two copies of the 23S rRNA gene \(^8\). Several points mutations have been reported that are associated with macrolide resistance, but the most common is A-G transitions at position 2143 (A2143G) \(^3, 4\). The commonest mutation site worldwide is A2143G, constituting 69.8\% \(^4\).

**MATERIALS AND METHODS**

**Study Design:** The study is a descriptive analytic cross-sectional hospital based one.

**Study Objective:** The study aimed at characterizing the mutations in 23S rRNA gene related to Clarithromycin-resistant Helicobacter pylori strains among dyspeptic patients in Khartoum State.

**Specific Objectives:**

1. To estimate the prevalence of Helicobacter pylori detection among dyspeptic patients undergoing upper GIT endoscopy.

2. To estimate the prevalence of Clarithromycin-Resistant Helicobacter pylori among dyspeptic patients undergoing upper GIT endoscopy.

3. To characterize the mutation in 23S rRNA gene responsible for resistance of Helicobacter pylori to Clarithromycin.

**Study Area:** The study was carried out in the GIT Units in Alribat Teaching Hospital, Al Neelain Medical Centre and Asia Hospital in Khartoum State, Sudan.

**Study Population:** Any dyspeptic patient undergoing upper GIT endoscopy in the study area had an equal chance to be enrolled.

**Study Sample:** The sample included 200 patients from the study population.

**Selection Criteria:** A valid verbal consent was a prerequisite to participant’s enrollment in the study.

**Ethical Consideration:** Each participant was informed about his/her role in study and was only enrolled after giving a clear consent. The caretakers validated the consent of the insane and underage participants. The specimens collected were used only for the purpose of the study.

**METHODS**

**Collection and transportation of specimens:** Upper gastrointestinal endoscopic biopsies were taken from the affected sites. Each biopsy was transferred immediately to a buffered normal saline as a transport medium and processed in no more than four hours.

**Culturing of specimen:** A Specimen from each biopsy was inoculated in a medium containing agar base (brain heart infusion agar), selective supplement (Skirrow) and growth supplement (animal plasma). The inoculated plates were incubated in microaerophilic atmosphere using
microaerophilic kits (campylobacter system CN0025A oxoid) at 37 °C for 3-5 days.

**Identification of isolated bacterial growth:**
A- Macro examination: Growths of small, circular, smooth colonies were observed after 3 to 5 days on the brain heart infusion agar plated with specimens from gastric biopsies.

B- Microscopic examination: Microscopic examination of gram-stained films from the growths showed gram negative bacilli, straight and sometimes curved.

C- Biochemical test: Bacteria growths were further identified by the biochemical tests of cytochrome oxidase, catalase, and urease enzymes.

**Antimicrobial sensitivity test:** For this purpose, the Modified Disk Diffusion Method (MDDM) was used. Stored isolates of H. pylori were regrown on brain heart infusion agar supplemented with 7% fresh horse blood and Skirrow. A suspension from 3-day-old bacterial cells of each isolate was prepared in brain heart infusion broth (2 ml) equivalent to the McFarland turbidity standard. The suspensions were spread on the surface of the brain heart agar base with 5% sheep blood and Skirrow with sterile cotton swabs. The plates were briefly dried and then the antibiotic disks of clarithromycin (15 micrograms), were added to each plate and incubated microaerobically at 37 °C for 3-5 days. One plate without antibiotic disk was also incubated in each batch as a control.

The inhibition zone diameter was measured in millimeters, with a ruler. Resistance was determined by a zone of growth inhibition with a diameters ≤30 mm for clarithromycin. Greater zones of complete growth inhibition indicated the presence of susceptible strains. H. pylori strain ATCC 26695 was used as a reference strain for the quality control of antibiotic susceptibility testing.

**DNA Extraction:** DNA was extracted from endoscopic biopsies by Genomic DNA purification by DNA Preparation Kit according to manufacturer's instructions (Jena Bioscience).

1. Each specimen was put in lysis buffer and proteinase K and incubated overnight at 55°C.
2. 1.5 ml of the lysed specimen was transferred to epindorf tube containing Protein Precipitation Solution.
3. 1.5 ml of the supernatant was transferred to a clean epindorf tube containing 99% isopropanol.
4. 1.5 ml of 80% ethanol was and centrifuged at 15,000 g for 1 min.
5. The ethanol was carefully discarded and the tube was air dried at room temperature for 10-15 min

**To detect mutations related to Clarithromycin resistance in the 23S rRNA gene:**

**PCR Amplification:**

PCR amplification methods and oligonucleotide primers derived from a known sequence of the 23S rRNA gene were used

5_ -AGGTTAAGAGGATGCAGTCAGTC-3_and
5_- CGCATGATATCCATTAGC AGT-3

Amplification was carried out in a thermal cycler (Thermo Hybaid, MBS 0.5 S). PCR cycling conditions consisted of one cycle at 94°C for five min., 40 cycles at 94°C for one min, 55°C for one min. and 72°C for 1 min, and one cycle at 72°C for seven min.
**RFLP Analysis:** Restriction fragment length polymorphism (PCR-RFLP) was performed using BsaI, BbsI enzymes to detect restriction site that correspond to the point mutations at A2143G and A2142G respectively.

**Data Analysis:** All data were analyzed by SPSS software programme.

**RESULTS**

The male participants constituted 108 (54%) while the female were 92 (36%). The participants’ age ranged between 10 and 89 years with a mean age of 43.5 years. Out of the 200 gastric biopsies, 116 (58%) were positive for H. pylori. Out of the 200 cultured specimens, 48 showed growths; 12 of them were resistant to Clarithromycin. Eight of the resistant isolates showed point mutation at both positions of A2143G and A2142G by BsaI and BbsI restriction enzymes. The remaining four isolates were found to have point mutation at position 2140G by PCR sequencing.

<table>
<thead>
<tr>
<th>Table (1) Endoscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis</td>
</tr>
<tr>
<td>Duodenitis</td>
</tr>
<tr>
<td>Gastric ulcer</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table (2) Susceptibility of the culture isolates to Clarithromycin-by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 isolates by culture</td>
</tr>
<tr>
<td>Clarithromycin-resistant</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
(Figure-1) Detection of mutation A2143G by BSaI restriction digestion, the restriction Fragments of the 267-bp PCR products were analyzed by electrophoresis on a 3% agarose gel, Lanes (A) 50-bp DNA Step Ladder molecular size markers (Promega).

Lanes (B) and (C), PCR products of H. pylori strains digested with BSaI. Lanes (D), PCR products of the control strain A2143G H. pylori strains digested with BsaI restriction enzymes. Lanes (E) and (F), PCR products of H. pylori strains does not digest with BSaI.

(Figure.2) Detection of mutation A21432G by BbsI restriction digestion, the restriction Fragments of the 267-bp PCR products were analyzed by electrophoresis on a 3% agarose gel, Lanes (A) 50-bp DNA Step Ladder molecular size markers (Promega).

Lanes (B) and (C), PCR products of H. pylori strains digested with BbsI. Lanes (D), PCR products of the control strain A2143G H. pylori strains digested with BbsI restriction enzymes. Lanes (E) and (F), PCR products of H. pylori strains does not digest with BbsI.

DISCUSSION
The prevalence of Helicobacter pylori in this study was 24%. Azim et al in a similar study in Sudan reported a prevalence of 50% by the same method (5). Mohammad et al in Iran reported a culture isolate rate of 31.94% (6). It seems that the prevalence of Helicobacter pylori differs in different countries and even in the same country depending on factors that need to be elucidated. Such a difference may be related to the clinical differences of the patients and the detection procedure.

Twelve out of the 48 (25%) Helicobacter pylori isolates were found to be resistant to clarithromycin. As far as the published data can provide, this study was the first one in Sudan addressing Helicobacter pylori resistance to clarithromycin. A similar prevalence of resistance was reported from Israel (7). Mohammad et al in Iran reported 22.62% prevalence of Helicobacter pylori resistance to clarithromycin which was comparable to the finding in this study (6). Clarithromycin is an important component of the triple therapy aiming at eradication of Helicobacter pylori from infected patients.

Appearances of resistant strains are the main cause of treatment failure. Mutations in 23S rRNA gene are the well recognized aetiology in the emergence of Helicobacter pylori resistant strains and A2143G is the commonest. Eight clarithromycin-resistant isolates showed A2143G and A2142G mutations. More than one mutation in the 23S rRNA may make the situation worse. The DNAs of four resistant isolates were not digested by the restriction enzymes in use. Their sequencing by PCR showed A2140G mutation. This finding was not previously reported with the mutations of in the 23S rRNA gene. The liberal use of macrolides for treatment of different infections may urge emergence of resistant strains of many macrolides even clarithromycin. The primary risk factor for resistance to clarithromycin is previous excessive use of macrolides.

The possibility of the patient being infected with resistant or co-infected with resistant and sensitive strains should be considered if he/she does not respond successfully to the treatment.

PCR and RFLP techniques can offer a workable quick and accurate method for identification of Helicobacter pylori susceptibility to clarithromycin within 24 hours. In case of a clarithromycin resistance rate of more than 20% may change the treatment option to the quadruple therapy and substitution of clarithromycin with tetracycline.
Conclusion: Helicobacter pylori resistance to clarithromycin is becoming a real problem in treatment of patients with peptic ulcer disease. More studies in this field are needed to make the situation clear for treatment options.

Acknowledgement: We are grateful to the staff in Al-Neelain Medical Research centre and the endoscopic units of Al-Ribat Alwatani and Asia Hospitals for their great help.

Disclosure: There is nothing to disclose.

REFERENCE