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Research Article

PRIMARY CLARITHROMYCIN RESISTENCE OF HELICOBACTER PYLORI BY FLUORESCENT IN SITU HYBRIDIZATION IN ALEPPO-SYRIA

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ABSTRACT

Helicobacter pylori is a human pathogen responsible for serious diseases including peptic ulcer disease and gastric cancer. The triple therapy included clarithromycin is the recommended first choice treatment, but primary clarithromycin resistance markedly reduces H.pylori eradication rate. The aim of this study to assess the prevalence of primary clarithromycin resistence in Aleppo of Syria by fluorescent in situ hybridization, which is a simple , rapid assay and permits detection of H. pylori and clarithromycin resistance simultaneously.

From September 2011 to May 2012, 130 H.pylori strains were isolated, among these, 16 strains were resistant to clarithromycin (12.3%). Of these, one were found to contain a mixed population of both sensitive and resistant H.pylori. Clarithromycin resistance rate was higher in normal endoscopic findings patients as compared to other findings patients (30.4% vs. 11.5%, p=0.04).

Based on the resistance rate we observed, it could be suggested that an empirical choice of a clarithromycin containing regimen based therapy could still remain the first-line therapeutic approach in clinical practice in Aleppo.

Keywords: Helicobacter pylori, Clarithromycin, Fluorescent in situ hybridization.

INTRODUCTION

Helicobacter pylori associated with many digestive disorders such as gastritis[1-2], peptic ulcer disease[3-4], gastric mucosa-associated lymphoid tissue (MALT) lymphoma[5-6], and gastric adenocarcinoma[7-8-9]. The role of H. pylori in extra intestinal diseases has also been suggested.[10-11-12-13]

Has proved that eradication of H.pylori enhances healing of peptic ulcers[14-15] reduces its recurrence [16-17], induces regression of preneoplastic lesions[18] and decreases gastric cancer risk[19-20]. According to the last Maastricht III consensus report, PPI- clarithromycin-amoxicillin or metronidazole is the recommended first choice treatment [21]. However, these treatment may fail for several reasons, particularly the growing resistance of H.pylori to clarithromycin.[22-23] A major difference in eradication rates was found: 87.8% when strains were clarithromycin susceptible against 18.3% when strains were clarithromycin resistant.[24]

It would be impractical and costly to test clarithromycin susceptibility for each individual seeking anti H.pylori treatment , so it is important for clinicians to know the local resistance rate. This is which is confirmed by Maastricht III Consensus report as recommended that the threshold of clarithromycin resistance at which this antibiotic should not be used, or clarithromycin susceptibility testing performed, is 15–20%.[21]

The most frequent point mutations responsible for clarithromycin resistance are A2142G, A2143G, and A2142C within the H. pylori 23S rRNA gene[25-26], so that, molecular methods can be used for their identification, such as fluorescent in situ hybridization (FISH) which is a simple and rapid assay. Moreover it permits detection of H. pylori and clarithromycin resistance simultaneously.[27-28]

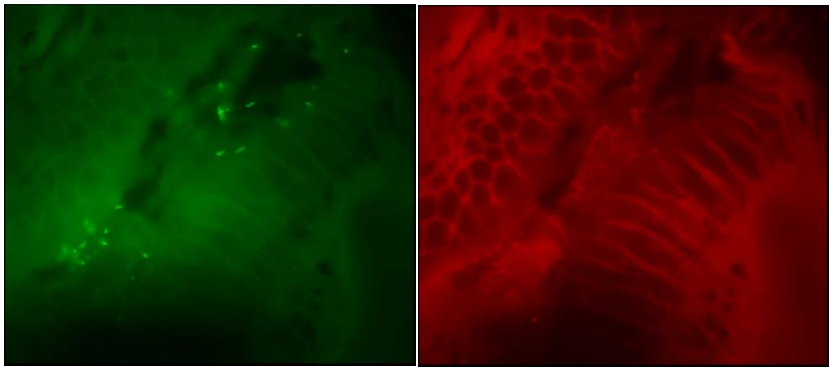
The aim of this study is to determine the primary clarithromycin resistance rate of H.pylori isolated from adults patients in the Aleppo city of Syria, by fluorescent in situ hybridization .

MATERIAS AND METHODS

From September 2011 to May 2012, gastric biopsy specimens were collected of adults patients (≥ 18 years old) who underwent upper gastrointestinal endoscopy for different indications at gastroenterology department in Aleppo University Hospital. All patients had never been treated for H.pylori infection, and the following data were also registered: age, gender, smoking habit, and endoscopic findings.

Fluorescent in situ hybridization

The biopsies were immediately fixed in formalin, embedded in paraffin, sectioned (4µm slice thickness) and dehydrated in an exylol and 96% ethanol. The deparaffinised, air –dried slides were incubated in the microwave oven at 400W for 10 min , this procedure greatly increases signal/noise ratio and renders largely insensitive to sample/fixation variations.



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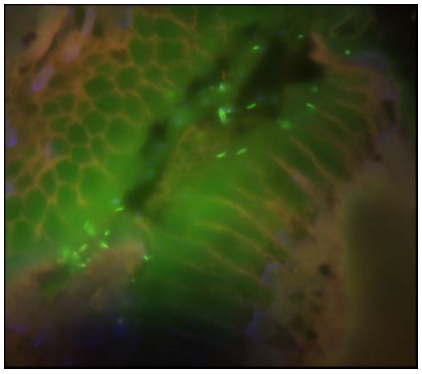

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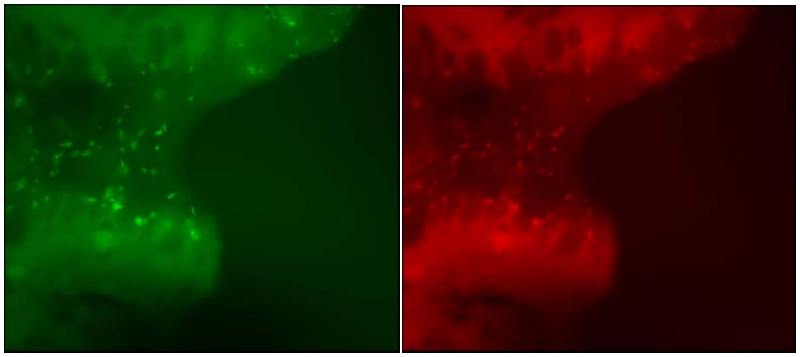
Fig. 1: It shows the detection of clarithromycin-sensitive H. pylori in the same section (a) green signal due to hybridization with H. pylori-specific, fluorescein-labelled probe (b) no red signal (c) green signal under dual band filter



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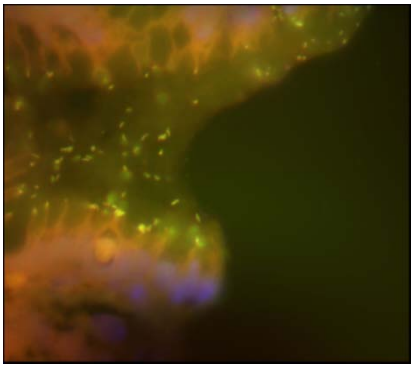

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Fig. 2: It shows the detection of clarithromycin-resistent H. pylori in the same section (a) green signal due to hybridization with H. pylori-specific, fluorescein-labelled probe (b) red signal due to hybridization with a Cy3-labelled mixture of probes specific for the most frequent mutations associated with clarithromycin resistance (c) yellow signal under dual band filter. Note the yellow appearance of resistant bacteria that results from an additive mixture of red and green fluorescence.

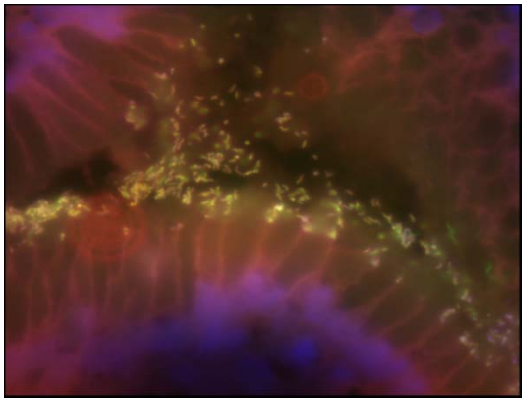

Fig. 3: It shows the detection of mixed population of both sensitive (green signal) and resistant (yellow signal) H.pylori

The hybridization were done at research laboratory in Faculty of medicine of Aleppo University using the commercially available test Bactfish™ Helicobacter pylori Combi kit. The probe for H.pylori identification was labeled with fluorescein (green signal) and the probes which detect tho most prevalent point mutations for clarithromycin resistance were labeled with fluorochrome Cy3 (red signal). Following hybridization for 90 min at 46ºC, the sections were washed at 46ºC for 2*15 min in wash buffer. The air dried sections were counterstained with 4`,6`-diamidino-2-phenylindole (DAPI) which binds to the DNA of the bacteria. Slides were inspected with Ziess Axioskope 40 microscope equipped with a standard fluorescence filters set. (figure1,2,3)

Statistical analysis

Logistic regression analysis was performed in order to identify possible risk factors (age, gender, endoscopic findings, smoking habit) for clarithromycin resistance. The odds ratio (OR) and 95% confidence intervals (CI) were calculated. P value of < 0.05 indicated significant differences. Statistical analysis was performed using a specific software (SPSS 13.0 for windows)

RESULTS

During the study period 130 H.pylori strains were isolated. The patients distributed according to the clinical and personal specifications as the following (table 1)

Table 1: Shows the clinical and personal specifications of the patients

	Number of patients
total	130
Age : 18-39	76
40-59	46
≥60	8
Sex : male	66
female	64
Smoking habit : smoking	43
no smoking	87
Endoscopic findings : gastritis	45
peptic ulcer	36
normal	23
others	26

The clarithromycin resistant strains were detected in 12.3% of patients (n=16). Of these, one were found to contain a mixed population of both sensitive and resistant H.pylori

Data for the analysis of risk factors associated with clarithromycin resistance are shown in table 2.

Table 2: Shows the association of risk factors with clarithromycin resistence rate

Risk factor	Clarithromycin resistence rate	OR (95% CI)	P value
Age : 18-39	14.4% (11/76)	1.2(0.14-10.76)	0.6
40-59	8.7% (4/46)	0.67(0.65-6.87)	
≥60	12.5% (1/8)	1.0 reference	
Sex : male	7.8% (5/66)	1.0 reference	0.1
female	17.2% (11/64)	2.5(0.83-7.76)	
Smoking habit :smoking	9.3% (4/43)	0.7(0.21-2.37)	0.5
no smoking	13.8% (12/87)	1.0 reference	
Endoscopic findings : gastritis	2.2% (1/45)	0.18(0.02-1.81)	0.04
peptic ulcer	13.9% (5/36)	1.24(0.27-5.7)	
normal	30.4% (7/23)	3.35(0.75-5.7)	
others	11.5% (3/26)	1.0 reference	

A distinctly higher prevalence of clarithromycin resistance was observed in normal endoscopic findings patients (30.4%) as compared to other findings patients (11.5%), the difference being statistically significant (OR: 3.35; 95% CI: 0.75 -5.7; P=0.04). Similarly clarithromycin resistance was observed in female (17.2%) was twice as much compared to male patients (7.8%), although the difference failed to reach a statistical significance (OR: 2.5; 95% CI: 0.83 -7.76; P=0.1).

The statistical analysis excluded any significant association between the clarithromycin resistance and both the age and smoking habit of patients

DISCUSSION

The method approved by the Clinical and Laboratory Standards Institute (CLSI) to test for clarithromycin susceptibility of H.pylori is agar dilution29. Due to the complexities of this method, many studies proved an alternative reliable method to detect clarithromycin resistant H.pylori is fluorescent in situ hybridization (FISH)30-31-32 . The advantage of FISH method is the rapid detection of H.pylori and its susceptibility to clarithromycin. In addition, this technique is able to detect mixed populations of clarithromycin-sensitive and resistant organisms which is may explain some of clarithromycin based treatment failures that occur in persons infected with clarithromycin-sensitive isolates as determined by culture and agar dilution24.

The primary clarithromycin resistance rate in the present study is 12.3%, which is similar to that was found in Tunisia (14.6%)[33], and Iran (14.3%)[34]. This prevalence is higher than that was found in Brazil (8%)[35], and Malaysia (0%)[36], but is lower than that was detected in France (26%)[37], and Turkey (41.9%)[38].This difference in clarithromycin resistance rates between countries might be due to the different prescription and administration of this antibiotic. Therefore, the selection of a regimen among those recommended for H.pylori eradication, must consider the local data of antimicrobial resistance.

Many studies which have assessed the correlation of clarithromycin resistance of H.pylori with risk factors have been controversial, for example, Wueppenhorst N[39] didn't find correlation of clarithromycin resistance with both the sex and disease status of patients , this findings contrast with the results were reported by De Francesco V[40] who disclosed a higher clarithromycin resistance rate for strains isolated from female and non ulcer dyspepsia patients, but failed identify as an independent risk factor for primary clarithromycin resistance. Whereas, Meyer JM[41] found that clarithromycin resistance was significantly associated with female sex and older age. Anyway, both last studies failed to provide logical interpretation of high clarithromycin resistance rate in female.

The recent study found that the clarithromycin resistance rate was higher in normal endoscopic findings patients (30.4%) as compared to other findings patients (11.5%). Such a phenomenon could play a role in the efficacy of standard eradication therapy observed according to gastroduodenal pathology, the cure rate being generally lower in non-ulcer dyspepsia patients (i.e., dyspepsia patients with normal endoscopic findings)[42-43]

In conclusion, Based on the resistance rate we observed, it could be suggested that an empirical choice of a clarithromycin containing regimen based therapy could still remain the first-line therapeutic approach in clinical practice in Aleppo, especially in non normal endoscopic findings patients.

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