RESEARCH ARTICLE

Antibiotic Resistant Pattern of *Helicobacter Pylori* Infection Based on Molecular Tests in Laos

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Abstract

Background: The efficacy of standard treatment of *Helicobacter pylori* (*H. pylori*) is declining because of antibiotic resistance. Clarithromycin resistance is also increasing in many Asian countries. The aim of this study was to determine the antibiotic susceptibility patterns of *H. pylori* infection and clinical association in Laos. Materials and Methods: A total of 329 Lao dyspeptic patients who underwent gastroscopy at Mahosot Hospital, Vientiane, Laos during December 2010-March 2012 were enrolled in this study. During gastroscopy, 4 biopsies were collected (2 each from the antrum and body) for CLO-test and histopathology. Only the positive CLO-test gastric tissues was stored at -80°c in a freezer until DNA was extracted and a GenoType®HelicoDR test was conducted for detecting mutations in the rrl gene encoding 23S rRNA (clarithromycin resistance) and mutations in gyrA gene (fluoroquinolone resistance) . Results: Of the total, 119 Lao patients (36.2%) were infected with *H. pylori* including 59 males (49.6%) and 60 females (50.4%) with a mean age of 46 years. Clarithromycin and fluoroquinolone resistance of *H. pylori* infection was demonstrated in 15 (12.6%) and 16 strains (13.4%) respectively. In clarithromycin resistance, the number of patients who had education above primary school and BMI≥ 25 kg/m2 were significantly higher than those who had education below primary school and BMI< 25 kg/m² (23.1% *vs* 7.5%, P-value= 0.036 and 20.5% *vs* 8%, P-value= 0.048, respectively). In fluoroquinolone resistance, the number of lowland Lao was significantly higher than those of non- lowland (highland and midland) Lao ethnic groups (16.7% *vs* 0%, P-value= 0.039). Conclusions: *H. pylori* infections remain common in Laos. Clarithromycin and fluoroquinolone resistance with *H. pylori* infection are growing problems. Education above primary school and BMI ≥ 25 kg/m2 might be predictors for clarithromycin resistance and lowland Lao ethnicity might be predictors for fluoroquinolone resistance with *H. pylori* infection in Laos.

Keywords: Antibiotic resistance - *Helicobacter pylori* - Laos

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Introduction

Helicobacter pylori (*H. pylori*) was significantly associated with chronic gastritis, gastric ulcer (GU), duodenal ulcer (DU) (Dixon., 1991; Tytgat et al., 1993), MALT lymphoma (Wotherspoon et al., 1999) and gastric cancer (Parsonnet et al., 1991; Vilaichone et al., 2006; Srinarong et al., 2014; Vilaichone et al., 2014; Vilaichone et al., 2015). Eradication of *H. pylori* is also an important factor to prevent gastric carcinogenesis (Hopkins et al., 1996; Vilaichone et al, 2014). Base on the Maastricht IV consensus report, standard triple therapy with combination of proton pump inhibitor (PPI), clarithromycin and amoxicillin remains first line for *H. pylori* eradication in low clarithromycin resistance area (Malfertheiner et al., 2012).

An increase in clarithromycin resistant strains of *H. pylori* infection was impacted outcome of treatment regimens and reduces eradication rate up to 40-50% (Vilaichone et al., 2006). Fluoroquinolone such as levofloxacin and moxifloxacin, was introduced to be effective drugs for treating *H. pylori* infection. However, fluoroquinolone resistance is now a growing problem and impact on the eradication rate in many countries (Vilaichone et al., 2013; Prapitpaiboon et al., 2015). *H. pylori* resistance to clarithromycin and fluoroquinolone was related with mutation points that can be tested by molecular technique. Clarithromycine resistance was related to point mutations in rrl gene encording 23S rRNA (Megraud et al., 2007) and fluoroquinolone resistance was directed to gyrase A (gyrA) gene mutation point (Moore et al., 1995; Tankovic et al., 2003). Recently, a new

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molecular test (GenoType®HelicoDR) which detected these mutation points of antibiotic resistance in *H. pylori* for clarithromcin and fluoroquinolone was evaluated with reliable sensitivity and specificity (Cambau et al., 2009).

The Lao People Democratic Republic (Laos) is located in Southeast Asia; border with China and Burma (Myanmar) in North, Cambodia in South, Vietnam in East and Thailand in West. Laos has population of 6,500,000 people, classified in 3 major difference ethnic groups: Lao Lum (lowland Lao), Lao Thoeng (midland Lao) and Lao Sung (highland Lao). However, there have never been investigated the pattern of *H. pylori* antibiotic resistance in Laos. The aim of this study was to determine antibiotic susceptibility patterns of *H. pylori* infection and clinical association in Laos.

Materials and Methods

Patients

Total of 329 Lao dyspeptic patients who underwent gastroscopy at Mahosot Hospital, Vientiane, Laos during December 2010- March 2012 were enrolled in this study. Inclusion criteria was consist of (1)age more than 15 years old, (2)being scheduled for upper gastrointestinal endoscopy, (3)non gastrointestinal chronic medical conditions, (4)absence of contraindications to upper gastrointestinal endoscopy and (5) providing informed consent. Exclusions included (1) patient received antibiotic or PPI one month before study, (2) prior eradication of *H. pylori* infection, (3) upper gastroduodenal bleeding. Informed consent was obtained from each patient and the protocol was approved by the hospital ethics committee. During gastroscopy, endoscopic findings were recorded and 4 biopsies were collected (2 from antrum and 2 from body) for CLO test and histopathology. Only the positive CLO-test gastric tissues were stored at –80°c freezer until DNA was extracted and proceeded to do the molecular tests.

Determination of 23S rRNA and gyrA genes mutation

H. pylori DNA was extracted from CLO-test positive gastric tissue by using QIAamp DNA Mini Kit (QIAGEN, Inc. Santa Clarita, CA, USA). Polymerase chain reaction (PCR) was performed for DNA amplification. Identification of 23S rRNA gene mutation for clarithromycin resistance and gyrA gene mutation for fluoroquinolones resistance of *H. pylori* strain by using GenoType®HelicoDR test (Hain Lifescience GmbH, Germany) according to the manufacturer's instructions.

Data analyses

The demographic information and frequencies of adverse effects were compared using chi-squared and Fisher's exact test. The P-values <0.05 were considered to be statistically significant. The data analysis was performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). The study was conducted according to the good clinical practice guideline, as well as the Declaration of Helsinki and was approved by our local ethics committee.

Results

119 Lao patients (36.2%) were infected with *H. pylori* infection including 59 males (49.6%), 60 females (50.4%) with mean age of 46 years. There were 17 ethnic highland Lao (Lao Sung) (14.3%), 6 ethnic midland Lao (Lao Thueng) (5%), and 96 ethnic lowland Lao (Lao Lum) (80.7%). Patient had education above primary school and BMI ≥ 25 kg/m2 were 39 (32.8%) and 44(32.8%) respectively. Clinical presentation included 86 chronic gastritis (72.3%), 13 gastric ulcer (10.9%) and 20 duodenal ulcer (16.8%) (Table 1). Clarithromycin and fluoroquinolone resistance were demonstrated in 15 (12.6%) and in 16 strains (13.4%) respectively (Table2).

Antibiotic resistance and clinical outcomes

Clarithromycin resistance was more common in female than male patients (16.7% vs 8.5%; P-value= 0.18), whereas fluoroquinolone resistance was more common in male than female patients (15.3% vs 11.7%; P-value= 0.60). There was also no association between antibiotic resistance and age, smoking and endoscopic findings in Laos's patients. However, clarithromycin resistance had significant more common in patients with education above primary school and BMI ≥ 25 kg/m2 than those of education below primary school and BMI < 25 kg/m2 (23.1% vs 7.5%, P-value= 0.036 and 20.5% vs 8%, P-value= 0.048, respectively) as detail in table 3. Furthermore, fluoroquinolone resistance was significantly more common in lowland than those of non-lowland Laos ethnic group (16.7% vs 0%, P-value= 0.039) (table 4).

Discussion

Triple therapy consists of PPI, clarithromycin and amoxicillin remains commonly used for *H. pylori* eradication worldwide (Malfertheiner et al., 2007; Fock et al., 2009). However, the eradication rate of this standard triple therapy has been declined. Antibiotic resistance is one of the major causes of treatment failure and antibiotic resistance for *H. pylori* infection is globally increasing and varies in different areas. Clarithromycin resistance is also increasing and impacted outcome of standard triple therapy by reducing the eradication rate to approximately 50% (Megraud et al., 2004).

Laos has high prevalence of *H. pylori* infection (Rasachak el al., 2000; Vannarath et al., 2014) but never been investigated which regimen should be the first line or second line therapy. However, the most common regimen using for first line eradication in Laos is standard triple therapy containing of PPI, clarithromycin and amoxicillin. Clarithromycin and fluoroquinolone resistance are increasing in ASEAN countries such as in Vietnam (31% and 19%) (Nguyen et al., 2011) and Thailand (18%, and 13%) (Prapitpaiboon et al., 2015). In this study, clarithromycin and fluoroquinolone resistances were demonstrated in 12.6% and 13.4 %. The common use of these 2 antibiotics for respiratory, gastrointestinal and urinary tract diseases in Laos are likely caused of increasing resistance and might be becoming greater problems in the future. However, Maastricht IV consensus

report (Malfertheiner et al., 2012) remain suggested that area of low clartithromycin resistance (<15-20%), 10-14 days of standard triple therapy can be used as first line therapy for *H. pylori* eradication. This worldwide recommendation leads to the idea that standard triple therapy could be a good choice for first line therapy of *H. pylori* eradication in Laos. In Japan, history of prior clarithromycin consumption increase 4-fold chance of this drug resistance (Perez et al., 2002). We also demonstrated that education above primary school and BMI ≥ 25 kg/m^2 might be predictors for clarithromycin resistance and lowland Lao ethnic group might be predictors for fluoroquinolone resistance. These clinical factors could be predictive markers for these antibiotic resistances in Laos.

In summary, *H. pylori* infections remain common infection in Laos. More than one third of Laos's dyspeptic patients infected with this bacteria. The prevalence of clarithromycin and fluoroquinolone resistance was not really high and 10-14 days standard triple therapy could be reliable first line for *H. pylori* eradication in Laos. Fluoroquinolone resistance might be our future problems. Education above primary school and BMI ≥ 25 kg/m2 are predictors for clarithromycin resistance and lowland Lao ethnic group is a predictor for fluoroquinolone resistance in Laos.

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