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One piece biopsy for both rapid urease test and cultivation of *Helicobacter pylori*

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ABSTRACT

Background & aims: Increasing antibiotic resistance among *Helicobacter pylori* isolates and the unsuccessful attempts at eradication can impose many costs to both healthcare systems and patients. The present study intended to find a way from which *H. pylori* could be isolated from biopsies with less invasive procedures undertaken on infected patients.

Methods: A biopsy specimen from each patient with gastric disorders was put into urea-containing agar. After two hours, the specimens were removed from agar and placed into tubes containing 1 ml 20% glucose solution. Then, the specimens were inoculated onto the Columbia agar and incubated under microaerophilic conditions. The grown colonies were identified as *H.pylori* based on the microbiology tests and PCR.

Results: Overall, 449 biopsy specimens were collected from the patients. Of all biopsies, 219 (48.8%) revealed positive results in the rapid urease test. Using the aforementioned method, 158 (35.2%) culture positive biopsy specimens were obtained.

Conclusion: The researchers attempted to use one biopsy specimen for both rapid urease and culture tests. This method causes fewer injuries of gastric tissue and allows antimicrobial susceptibility testing and characterization in detail of the isolated organism.

1. Introduction

Stomach infection with *Helicobacter pylori* is the second most common infectious disease in humans (Hussain et al., 2004). Colonization of the bacterium in the gastric epithelium predisposes the individual to severe diseases such as duodenal ulcer and gastric cancer (Kusters, van Vliet, and Kuipers, 2006). This association is extremely important since gastric cancer is the second leading cause of cancer death in the world (Hamashima, 2014). Meanwhile, cases of *H. pylori* infection represent more than half of the world population (Hooi et al., 2017) and the International Agency for Research on Cancer classifies this bacterium as a class I carcinogen (Wang, Meng, Wang, and Qiao, 2014). At present, there are both invasive (e.g. culture, a rapid urease test and histology) and non-invasive (e.g. urea breath test, serology and a stool antigen test) diagnostic assays for detection of *H. pylori* that they should be reliable, simple and quick to perform. However, high-resolution endoscopic technologies enable increased diagnostic accuracy for the detection of *H. pylori* infections (Serrano, Kikuste, and Dinis-Ribeiro, 2014). The study was designed for the detection of the microorganism from one biopsy specimen through the performance of a rapid urease test and culture to inflict minimum harm to gastric tissue of the patients especially in whom two or more attempts at eradication was unsuccessful.

2. Materials and methods

In this study, biopsy specimens were obtained from patients with gastric disorders, who underwent gastro-duodenoscopy in Imam Reza University teaching hospital affiliated with Tabriz University of Medical Sciences. Informed written consent was obtained from each patient. Using sterilized standard-sized biopsy forceps (Boston Scientific, Costa Rica), the endoscopist took a biopsy specimen from gastric antrum (size: 3 mm in diameter) and transferred it into rapid urease medium (Bahar Afshan, Iran). The medium yields an immediate (within 2 h)

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Table 1
Distribution of *H. pylori* infection among different age groups.

Age ranging	Rapid urease test; No (%)		Culture results; No (%)		Total number of patients in each group No (%)	Culture positive, RUT negative cases No (%)	RUT Positive, culture negative cases No (%)
	Positive	Negative	Positive	negative			
G1 = 1–5	6 (1.3)	17 (3.8)	3 (0.7)	20 (4.4)	23 (5.1)	1 (0.2)	4 (0.9)
G2 = 6–10	16 (3.6)	38 (8.4)	11 (2.4)	43 (9.6)	54 (12)	0 (0)	5 (1.1)
G3 = 11–15	15 (3.4)	19 (4.2)	12 (2.7)	22 (4.9)	34 (7.6)	0 (0)	3 (0.7)
G4 = 16–30	50 (11.1)	31 (6.9)	33 (7.3)	48 (10.7)	81 (18)	4 (0.9)	21(4.7)
G5 = 31–45	72 (16)	57 (12.7)	52 (11.6)	77 (17.1)	129 (28.7)	9 (2.0)	29 (6.5)
G6 = 46–60	41 (9.1)	38 (8.5)	31 (6.9)	48 (10.7)	79 (17.6)	4 (0.9)	14 (3.1)
G7 = 61–75	16 (3.6)	19 (4.2)	13 (2.9)	22 (4.9)	35 (7.8)	2 (0.45)	5 (1.1)
G8 = 76–90	3 (0.7)	11 (2.5)	3 (0.7)	11 (2.5)	14 (3.2)	2 (0.45)	2 (0.45)
Total	219 (48.8)	230 (51.2)	158 (35.2)	291 (64.8)	449 (100)	22 (4.9%)	83 (18.55)

G: Group; RUT: Rapid urease test.

color change from yellow to pink at room temperature, allowing a rapid identification (Connie, Mahon, and Manuselis, 2015). After two hours, under the sterile conditions, the specimens were removed by an inoculating loop from urea-containing agar and replaced into tubes containing 1 ml of filtrate 20% solution of glucose. The specimens were homogenized with a glass rod, then inoculated onto the fresh Columbia agar plates (Oxoid, England) supplemented with 5% sheep blood and antibiotics (vancomycin 6 μg/ml, amphotericin B 2.5 μg/ml, and trimethoprim 20 μg/ml). The plates were incubated under microaerophilic conditions (Anoxomat; Mart, Lichtenvoorde, the Netherlands) at 37 °C and high humidity for 5–7 days. To remove possible contaminating microorganisms from culture plates, we initially identified based on their typical colony morphologies, Gram staining, catalase, oxidase and urease tests. The results were also confirmed by PCR. After DNA extraction by the CTBA-based method (Russell and Sambrook, 2001) from the colonies, PCR was performed to detect the *glmM* gene using suggested primers by Kaplan et al. (Kaplan et al., 2006). For setting up the PCR, we had to carry out some modifications in the mixture of reagents and optimize program by a thermal gradient PCR. Briefly, the 50 μL PCR mixture(in DNase/RNase free 200 μL microtube) contained 2.5 μL of bacterial DNA, 25 pmol of each primer (F: 5′ AAG CTT TTA GGG GTG TTA GGG GTT 3′; R: 5′ AAG CTT ACT TTC TAA CAC TAA CGC 3′), 10 mmol/L Tris-HCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, and 2.5 units of Taq DNA polymerase(Ampliqon, Denmark). Reactions were performed in a thermal cycler (ASTEC, Nagano-Japan) with an initial denaturation step of 3 min at 94 °C; 35 cycles of 30 s at 94 °C, 60 s at 58 °C, and 30 s at 72 °C; and a final extension step of 3 min at 72 °C. The presence of the *glmM* gene was determined in each sample by gel electrophoresis. Each PCR assay included internal control to indicate the absence of inhibitors, *glmM* positive control strain (provided from the microbiological collection of the Department of Microbiology, Tabriz University of Medical Sciences) and negative control, which contained all the reagents but not the template DNA. The data analysis was conducted by means of SPSS (Inc., Version 16.0. Chicago, USA).

3. Results

In total, 449 biopsy specimens were collected from patients who underwent gastro-duodenoscopy. Of these patients, 221 were males (49.2%) and 228 were females (50.8%). Of all biopsies, 219 (48.8%) revealed positive results in the rapid urease test. Using the culture method described, 158 (35.2%) culture positive biopsies were obtained, while 22 (4.9%) of which had shown the negative rapid urease test. When we tested again obtained colonies of these 22 isolates for urease production, the test tube (routinely used urea agar not rapid urease medium) was incubated in 37 °C and all revealed the positive urease reaction within 24 h. However, in most cases the colony count of growing bacteria were too numerous to count. Meanwhile, 83 (18.55%) of rapid urease test positive isolates were culture negative. Distribution of *H. pylori* infection among different ages (ranging 1–90) was outlined in Table 1.

PCR revealed the *glmM* gene in all culture and urease positive specimens, which depicted in Fig. 1.

4. Discussion

High incidence of *H. pylori* infections and growing antibiotic resistance among the isolates (Milani et al., 2012) has made this documented class I carcinogen microorganism (Wang, Meng, Wang, and Qiao, 2014), a permanent threat to humans. To accurately detect *H. pylori*, gastric biopsies have been taken at endoscopy for rapid urease test, culture, histopathology, and PCR (Ricci, Holton, and Vaira, 2007). On the other hand, to perform antimicrobial susceptibility tests, assess the efficacy of treatment, biopsies are preferable specimens, since efforts to culture from other specimens have a low success rate (Wang

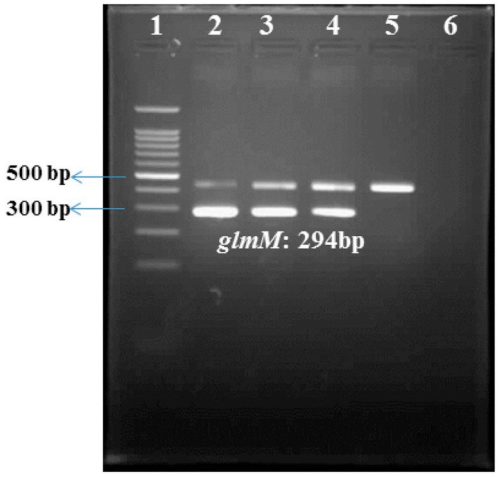

Fig. 1. Agarose gel electrophoresis of the amplified *glmM* gene by a polymerase chain reaction. Lane 1: 100-bp DNA size marker; Lanes 2–4: *glmM* (294 bp) positive strains along with Internal control (human growth hormone gene: 426 bp); Lane 5: *glmM* negative sample and lane 6: negative control (containing all of the reagents without any DNA).

et al., 2015). It is not acceptable for patients to undergo gastro-duodenoscopy for more biopsies, because the preparation of gastric biopsy specimens is an invasive method and this operation may damage the gastric tissue. In this study, in order to avoid the necessity for more biopsy specimens, after performing a rapid urease test, we strived to reuse the same biopsy specimen for bacterial culture. We began to think that most of the gastroenterologists (at least in our region) prefer to screen for *H. pylori* infections using a rapid urease test with one piece of gastric biopsy at their clinics instead of sending more pieces for cultivation or other tests in the laboratory.

The isolation of the organism by culture has been highly variable. It is common for laboratories that accomplish this function regularly to fail to isolate *H. pylori* even when the rapid urease test is positive (Blanchard and Nedrud, 2006). Success rates of cultivation depend on the endoscopist's expertise in the preparation of gastric biopsy and the technical expertise of the microbiology laboratory (Destura et al., 2004), and range from 30% to 73%. Using the aforementioned method, we had 48.8% (n: 219/449) and 35.2% (n: 158/449) rapid urease and culture positive specimens, respectively. Currently, with pure bacterial colonies, more details such as resistance patterns can be derived from isolated bacteria. As shown in Table 1, the peak of infection has occurred in G5 (age: 31–45) group, and the decreasing shift observed toward the oldest and youngest one, except G2 (age: 6–10) group. Regarding the presence of 22 cultures positive biopsies with negative rapid urease tests; endoscopists' inattention to previous treatments of patients with a proton pump inhibitor or any other medication and active bleeding may produce a false negative result for the rapid urease test (Uotani and Graham, 2015). Although, the most critical cause of gastroduodenal disorders related to *H. pylori* infection among the world population, there is also an increasing awareness of *H. pylori*-negative gastroduodenal disorders (Sugano et al., 2015).

In this study, the colonies were identified as *H. pylori* using conventional microbiology tests followed by PCR. Since in some cases, the genes may be silent and the phenotypic characteristics may change, a molecular method can be verified. The use of both phenotypic and genotypic methods will likely achieve more accurate results. For PCR we used the *glmM* gene, as the most promising target for the detection of *H. pylori* by PCR amplification (Shahamat et al., 2004). The results of Jang-Jih Lu et al. indicated that of five PCR methods examined (16 s rRNA gene, Random sequence, *ssa* gene, *ureA* gene and *glmM* gene), the *glmM* gene PCR is the most sensitive and specific for the detection of *H. pylori* in gastric biopsy specimens (Lu et al., 1999). To the best of our knowledge, this is the first report of using one biopsy specimen for two target applications and it has two significant advantages: First, it allows antimicrobial susceptibility testing and characterization in detail of the isolated organism; second, it causes less injuries in the gastric tissue.

Author's contribution

Yaeghob Sharifi has contributed in study design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; administrative, technical, or material support; study supervision.

Yaghuob **Moaddab** has contributed in samples preparation; acquisition of data; drafting of the manuscript; administrative, technical, or material support.

Morteza Milani has contributed in study design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; administrative, technical, or material support.

Declaration of Competing Interest

The authors disclose no conflicts.

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References

Blanchard, T.G., Nedrud, J.G., 2006. Laboratory maintenance of *Helicobacter* species. Curr Protoc Microbiol. https://doi.org/10.1002/9780471729259.mc08b01s00. Chapter 8 Unit8B.1.

Connie, R., Mahon, D.C.L., Manuselis, George, 2015. Textbook of Diagnostic Microbiology. (Saunders).

Destura, R.V., Labio, E.D., Barrett, L.J., Alcantara, C.S., Gloria, V.I., Daez, M.L., Guerrant, R.L., 2004. Laboratory diagnosis and susceptibility profile of *Helicobacter pylori* infection in the Philippines. Ann. Clin. Microbiol. Antimicrob. 3, 25.

Hamashima, C., 2014. Current issues and future perspectives of gastric cancer screening. World J. Gastroenterol. 20, 13767–13774.

Hooi, J.K.Y., Lai, W.Y., Ng, W.K., Suen, M.M.Y., Underwood, F.E., Tanyingoh, D., Malfertheiner, P., Graham, D.Y., Wong, V.W.S., Wu, J.C.Y., Chan, F.K.L., Sung, J.J.Y., Kaplan, G.G., Ng, S.C., 2017. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterol. 153, 420–429.

Hussain, M.A., Kauser, F., Khan, A.A., Tiwari, S., Habibullah, C.M., Ahmed, N., 2004. Implications of molecular genotyping of *Helicobacter pylori* isolates from different human populations by genomic fingerprinting of enterobacterial repetitive intergenic consensus regions for strain identification and geographic evolution. J. Clin. Microbiol. 42, 2372–2378.

Kaplan, M., Yavuz, S.S., Cinar, B., Koksal, V., Kut, M.S., Yapici, F., Gercekoglu, H., Demirtas, M.M., 2006. Detection of *Chlamydia pneumoniae* and *Helicobacter pylori* in atherosclerotic plaques of carotid artery by polymerase chain reaction. Int. J. Infect. Dis. 10, 116–123.

Kusters, J.G., van Vliet, A.H., Kuipers, E.J., 2006. Pathogenesis of *Helicobacter pylori* infection. Clin. Microbiol. Rev. 19, 449–490.

Lu, J.J., Perng, C.L., Shyu, R.Y., Chen, C.H., Lou, Q., Chong, S.K., Lee, C.H., 1999. Comparison of five PCR methods for detection of *Helicobacter pylori* DNA in gastric tissues. J. Clin. Microbiol. 37, 772–774.

Milani, M., Ghotaslou, R., Somi, M.H., Rafeey, M., Akhi, M.T., Nahaei, M.R., Hasani, A., Sharifi, Y., 2012. The status of antimicrobial resistance of *Helicobacter pylori* in eastern Azerbaijan, Iran: comparative study according to demographics. J. Infect. Chemother. 18, 848–852.

Ricci, C., Holton, J., Vaira, D., 2007. Diagnosis of Helicobacter pylori: invasive and non-invasive tests. Best Pract. Res. Clin. Gastroenterol. 21, 299–313.

Russell, D.W., Sambrook, J., 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Cold Spring Harbor, NY.

Serrano, M., Kikuste, I., Dinis-Ribeiro, M., 2014. Advanced endoscopic imaging for gastric cancer assessment: new insights with new optics? Best Pract. Res. Clin. Gastroenterol. 28, 1079–1091.

Shahamat, M., Alavi, M., Watts, J.E., Gonzalez, J.M., Sowers, K.R., Maeder, D.W., Robb, F.T., 2004. Development of two PCR-based techniques for detecting helical and coccoid forms of *Helicobacter pylori*. J. Clin. Microbiol. 42, 3613–3619.

Sugano, K., Tack, J., Kuipers, E.J., Graham, D.Y., El-Omar, E.M., Miura, S., Haruma, K., Asaka, M., Uemura, N., Malfertheiner, P., 2015. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 64, 1353–1367.

Uotani, T., Graham, D.Y., 2015. Diagnosis of *Helicobacter pylori* using the rapid urease test. Ann Transl Med. 3, 9. https://doi.org/10.3978/j.issn.2305-5839.2014.12.04.

Wang, F., Meng, W., Wang, B., Qiao, L., 2014. *Helicobacter pylori*-induced gastric inflammation and gastric cancer. Cancer Lett. 345, 196–202.

Wang, Y.-K., Kuo, F.-C., Liu, C.-J., Wu, M.-C., Shih, H.-Y., Wang, S.S., Wu, J.-Y., Kuo, C.-H., Huang, Y.-K., Wu, D.-C., 2015. Diagnosis of *Helicobacter pylori* infection: current options and developments. World J. Gastroenterol. 21, 11221.