null
include histology, rapid urease test, culture, and polymerase chain reaction (PCR); whereas, non-invasive tests include serology, urea breath test, and stool antigen test (Patel et al., 2014). Nowadays, researchers tend to develop more sensitive but less invasive diagnostic tests. Rapid urine test (RAPIRUN) was invented by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) using immunochromatographic assay for qualitative detection of urine \textit{H. pylori} antibody. Having been approved by FDA in 2006, RAPIRUN was evaluated in many countries and high sensitivity, specificity, and accuracy was reported (Graham and Reddy, 2001; Syam et al., 2015). However, the test needs validation in Thai population before its usage in clinical practice.

This study aimed to compare performance of different diagnostic tests for \textit{H. pylori} detection in Thai population. The investigated diagnostic test were rapid urease test, histopathology, and culture and rapid urine test.

**Materials and Methods**

**Patients**

Total of 94 patients with dyspepsia (50 males and 44 females, with the mean age of 43.2 years) who referred to Thammasat University Hospital, Pathumthani, Thailand, between December 2012 and April 2013 were enrolled in this study. Inclusion criteria were aging more than 18 years and lack of receiving \textit{H. pylori} treatment. Patients who received H2 receptor antagonists, proton pump inhibitor (PPI), bismuth compound, and antimicrobial agents in the past one month were excluded. Furthermore, we excluded patients receiving NSAIDs and anticoagulation drugs or had history of stomach surgery. Patients with significant co-morbid diseases such as renal failure and congestive heart failure, pregnant women, or breastfeeding women were not included in this study either. Informed consent was obtained from all patients before entering the study.

**H. pylori diagnosis**

Gastroscopy was carried out for all patients. During the endoscopy, 3 antral biopsies were obtained for rapid urease test (Pronto Dry, Eisai, Thailand), histopathology, and \textit{H. pylori} culture. Urine samples were also collected at the same time for rapid urine test (RAPIRUN by Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Positive gold standard tests were defined as (1) positive rapid urease test plus histopathology or (2) culture positive \textit{H. Pylori} infection.

**The Rapid Urine Test**

After collecting urine samples, they were immediately sent to the laboratory within 4 hours of collection. Next, 0.5 cc of each fresh urine was stirred with 0.5 cc of diluent by pipette. The test stick (contained dried antihuman IgG antibody-binding gold colloidal particles and nitrocellulose membrane) was then dipped into the mixture of urine and diluent. \textit{H. pylori} antigen and antihuman IgG antibodies were immobilized in the membrane to produce a test line and a control line, respectively. If patient had human IgG antibodies in urine sample, they will diffuse and form human IgG-antihuman IgG antibody complexes producing a red band by immunochromatographic technique. When two red bands were present at the test line and control line within 20 minutes, the test was considered positive. When only the control line was observed, the test was considered negative. If no line was observed, the test was regarded invalid caused by technical errors or extremely diluted urine.

**Statistical analysis**

All data were analysed by SPSS version 19 (SPSS Inc., Chicago, IL, USA). The demographic data were analysed by using the Fisher’s exact test or chi-squared where appropriate. P-values less than 0.05 were considered as statistically significance. The study protocol was in accordance with clinical practice guidelines and it was approved by our university ethics committee.

**Results**

A total of 94 patients with dyspeptic symptoms were enrolled in this study (50 males and 44 females with the mean age of 43.2 years old). Among 94 patients, 29 patients (30.9%) were diagnosed with \textit{H. pylori} infection by gold standard tests. Positive test was found in 24 patients (25.5%) for rapid urease test (RUT), 27 patients

Figure 1. Prevalence of \textit{H. pylori} Infection by Different Tests
Table 1. Efficacy of Rapid Urine Test Compare to Gold Standard for H. pylori Diagnosis

| Test          | Gold standard test + | Gold standard test - | Total |
|---------------|-----------------------|----------------------|-------|
| Rapid urine test + | 25                    | 6                    | 31    |
| Rapid urine test -  | 4                     | 59                   | 63    |
| Total         | 29                    | 65                   | 94    |

(28.7%) for histopathology, 28 patients (29.8%) for rapid urease test plus histopathology, 28 patients (29.8%) for culture, and 31 patients (32.9%) for rapid urine test. There was one patient who had negative culture but positive rapid urease test and histopathology and considered positive for gold standard tests. All data are demonstrated in the Figure 1.

Among 29 patients with positive gold standard tests, 25 had positive rapid urine test. There were 6 patients with positive rapid urine test, but they had negative gold standard tests (Table 1).

The rapid urine test had sensitivity of 86.2%, specificity of 90.8%, and accuracy of 89.4%, whereas, rapid urease test, histopathology, and culture had sensitivity of 82.8%, 93.1%, 93.1%; specificity of 100%, 100%, 100%; and accuracy of 95.7%, 97.9%, 97.9%, respectively. In our study population, the rapid urine test had negative predictive value of 93.7% and positive predictive value of 80.6%.

Discussion

So far, there has been much effort to develop ideal diagnostic tests for H. pylori detection. Several non-invasive diagnostic methods have been tested and compared with reference standard in search of the best non-invasive test for H. pylori infection. Current non-invasive tests consist of 13C or 14C urea breath test, stool antigen test, and antibody tests in serum, urine, and saliva (Patel et al., 2014; Tongtawee et al., 2016). In 2018, using Cochrane database of systematic reviews, researchers included 101 studies involving 11,003 participants to compare 3 non-invasive tests that were urea breath test, stool antigen test, and serology against the gold standard, but they excluded urine and saliva-based tests because they were not commonly used. They revealed that urea breath tests had the highest sensitivity for 13C or 14C urea breath test (94% and 92%, respectively), followed by 84% for serology and 93% for stool antigen test. They also concluded that urea breath test had higher diagnostic accuracy than serology and stool antigen test (Best et al., 2018). Although many studies have considered urea breath test as the mostly used and accurate non-invasive test, these tests have some limitation such as high cost and poor sensitivity in patients with gastric surgery history or those receiving PPI, H2 receptor antagonists, bismuth, and antibiotics (Gatta et al., 2004; Wang et al., 2015). The monoclonal stool antigen test was another proven method that had high sensitivity and accuracy and could be used both for diagnosis or evaluation after H. pylori eradication (Gisbert et al., 2006). However, a stool test result can be unreliable if a patient has gastrointestinal bleeding (Hanvivatvong et al., 2006). Another non-invasive test is serology which is not affected by gastrointestinal bleeding or the use of PPI or antibiotic. Nevertheless, it has limitation in distinguishing between active or past infection because of persistence of H. pylori antibodies up to 6-12 months after successful treatment (Gisbert and Abraira, 2006). Owing to some disadvantages of the above-mentioned non-invasive tests, the urine test has been verified in many countries to find whether it is comparable to current standard methods.

In our study, we validated rapid urine test for the diagnosis of H. pylori infection in Thai people and as a non-invasive method and meriting with high sensitivity, specificity, and accuracy according to previous studies (Graham and Reddy, 2001; Quach et al., 2014; Syam et al., 2015). There are two types of urine tests for diagnosis of H. pylori infection. The first one is urine ELISA for anti-H. pylori IgG discovered in 1993 with sensitivity of 95.9% and specificity of 90% (Alemohammad et al., 1993). The other type of urine-based test is rapid urine test which uses immunochromatographic method for detection of anti-H. pylori IgG in urine (Graham and Reddy, 2001). One study showed that accuracy of urine ELISA (93.2%) was slightly higher than that of the immunochromatographic method (91.2%), and both were comparable to serological tests but superior to stool antigen test (83.5%) (Adachi et al., 2002).

Results from our study showed that rapid urine test could detect H. Pylori approximately with the same prevalence (32.9%) as the gold standard tests (30.9%). Moreover, we also found that the rapid urine test had comparable sensitivity, specificity, and accuracy (86.2, 90.8, and 89.4%, respectively) to previous studies from other Southeast Asian countries such as Vietnam (84.7%, 89.9%, and 87.0%, respectively) and Indonesia (83.3%, 94.7%, and 93.2%, respectively) (Quach et al., 2014; Syam et al., 2015). However, our study revealed lower sensitivity, specificity, and accuracy of the rapid urine test than those reported in two studies from Japan (93.7%, 88.9%, and 92.2%, respectively) and China (96.7%, 95.2%, and 95.9%, respectively) (Fujisawa et al., 2001; Wong et al., 2002). This discrepancy maybe because of different H. pylori strains between Northeast and Southeast Asia. The vacA m1 type strains were predominant in Japan and South Korea while m2 type strains were more common in Southeast Asia such as Vietnam and Taiwan (Yamaoka et al., 2002). The urine test, which was developed from DNA analyses from Japanese H. pylori genes, could produce different test results. Another advantage of using rapid urine test is easily obtainable and non-invasive specimen collection. Moreover, given that the urine test does not require centrifugation, it takes less time.

Results from our study is considered applicable to Thai dyspeptic patients. Although there were few limitations in this study. In order to develop rapid urine test, Japanese H. Pylori strain OHPC-040 was used because it revealed the strongest reaction for positive specimen (Katsuura et al., 1998). As a result, different H. Pylori strains in Thai patients and divergent genetic background could induce varying antigen-antibody response and possibly led to
false negative results in our study (Uchida et al., 2015; Vilaichone et al., 2018). Six patients with false positive result could also be explained by extended positive urine test after \textit{H. pylori} eradication (Graham and Reddy, 2001). Moreover, the sample size in this study was relatively small. Larger studies may be needed to ascertain the validity of the rapid urine test in the future.

In conclusion, Rapid urine test (RAPIRUN) provides a reliable result for diagnosis of \textit{H. pylori} infection. Furthermore, this rapid urine test, according to our findings, has high accuracy and it was reliable, safe handle, and easy to use. The rapid urine test is not only comparable to gold standard tests but also non-invasive tests and it is suggested for Thai people.

Acknowledgements

This study was supported by the National Gastric Cancer and Gastrointestinal Diseases Research Center, Pathumthani, Thailand.

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