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Comparative Efficacy Sensitivity and Specificity of the Tests used for the Diagnosis of *Helicobacter pylori*

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Abstract: A total number of 157 samples were examined by 4 different tests-In-house rapid urease (iRUT), Culture, Histopathology and Immunochromatography (Immuno CardSTAT) for the detection of Helicobacter pylori from the patients reported to Hospital Kuala Lumpur, Malaysia during 2007 to 2008. Out of the samples examined 47 (29.9%) were positive for *H. pylori* by the tests used in the laboratory. Efficacy of detection of the bacteria by the tests- In-house rapid urease, Culture, Histopathology and Immuno CardSTAT were 31.8, 13.9, 30.3 and 32.8%, respectively. However, sensitivity and specificity of the iRUT were 91.5 and 93.6%, respectively and the Positive Predictive Value (PPV) was 86% and the Negative Predictive Value (NPV) was 96.3%. The sensitivity for Immuno CardSTAT rapid test was 100% and the specificity was 79.3%. The PPV was 50% and the NPV was 100%. Convenient methods to the authors were 'In house rapid urease test and Immunochromatography though variability of specificities were observed.

Key words: Immunochromatogrphy, in house urease test, microaerophilic, histopathology, *Helicobacter pylori*

INTRODUCTION

H. pylori infection occurs worldwide. It is one of the commonest human infections in the world and it is estimated that more than 50% of the world population is infected with this organism; however, the prevalence may vary significantly within and between countries influenced by age, ethnicity, gender, geography and socio-economic status (OMGE Practice Guideline, 2006). The highest rates are found in populations of low socioeconomic status with poor educational level, living in overcrowded dwellings (Brown, 2000). The prevalence in the developed world is declining (25 to 50%) and the prevalence in developing countries is high (70 to 90%). Most of the infection is acquired in childhood and in the developing countries almost all are acquired before the age of 10 (Dunn *et al*., 1997). In Asia, the prevalence varies between 50-80% (OMGE Practice Guideline, 2006).

Persistent infection with *H. pylori* is strongly associated with duodenal and gastric ulcers. *H. pylori* have been described as the cause of more than 90% of duodenal ulcers and up to 80% of gastric ulcers (Nomura *et al*., 2002). Infected persons have a two to six fold increased risk for developing gastric cancer and mucosa-associated gastric lymphoma compared with their uninfected counterparts (Linke *et al*., 2010). It is accepted to be a major factor for chronic atrophic gastritis which is a precursor lesion for gastric cancer (National Cancer Institute, 2001). Accurate diagnosis is essential for the effective treatment and management of infections caused by *H. pylori* (Collins *et al*., 2006). Several methods for detecting *H. pylori* infection have been developed and of them iRUT is found to be sensitive, specific and very rapid (within 20 min) method of detecting *H. pylori* infection (Nakata *et al*., 2004). Similarly significant percent of sensitivity and specificity of ImmunoCard STAT for the detection *H. pylori* has also been claimed (Xavier *et al*., 2010).

Therefore, the present study was to undertaken to diagnose *H. pylori* from patients reported to Hospital Kuala Lumpur, Malaysia, by different methods and compare their efficacy, sensitivity and specificity.

MATERIALS AND METHODS

Study population and selection of subjects: The study population is all patients undergo in oesophagogastroduodenoscopy (OGDS) for the first time during June 2007 to September 2008 under the Gastroenterology unit of Hospital Kuala Lumpur, Malaysia. The patients were selected with the indications of dyspepsia, epigastric pain, nausea, vomiting, loss of appetite and bleeding or anaemia. During the 15 months of the study period, a total of 157 dyspeptic patients were included for the study.

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Samples

Biopsy: These were taken from the patients with written consent at the Gastroenterology unit of Hospital Kuala Lumpur, Malaysia those have showed the symptoms of suffering from *Helicobacter pylori* infection.

Stool: Samples collected from patients for the detection of antigen of *H. pylori* from stool.

Tests used

In-house rapid urease test (iRUT): The in-house RUT was prepared by the department of Medical Microbiology and Immunology, Hospital Kuala Lumpur using 50 g urea mixed with 500 mL distilled water and 8 mL of 1% phenol red was added as indicator. The biopsies were immediately immersed in a tube containing the solution media. The test was considered positive when the color changed from yellow to red within 24 h.

Culture: Biopsies were processed immediately for culture on a blood agar and incubated in miroaerophilic condition using Campygen Gas Pak (Oxoid) at 37°C for 6 days. The plate was checked for presence of growth every 2 days. The Campygen gas pack was replaced with a new one after each inspection of plate for growth. *H. pylori* produces small, transluscent, grey colonies on a nonselective blood-containing media. Positive culture was identified by gram stain as gram negative, curved, rods and biochemical tests were performed and *H. pylori* was identified by positive urease, oxidase and catalase test.

Histopathology: Biopsies were taken for histopathological examination. The specimens were put in 10% formalin as preservative and processed as routinely to look for presence of Helicobacter-like organisms. The specimens were stained with Giemsa, or Warthin Starry when necessary. The slides were confirmed by Histopathologists.

Stool antigen detection by immunochromatography: Stool samples collected from the suspected patients in air tight container for the detection of antigen were processed according to manufacturer's instructions using immunochromatography technique with ImmunoCard STAT (Meridian Bioscience, Inc.) rapid Kits.

RESULTS AND DISCUSSION

Out of 157 samples collected from the patients 29.9% were positive for the prevalence of *H. pylori*. The sensitivity and specificity of each test as presented in Table 1.

The in-house rapid urease test (iRUT) was positive in 50/157 (31.8%) and negative in 107/157 (68.2%) of patients. The true positive results were 43 and false positive were 7, the false negative were 4 and true negative were 103 (Table 1). Thus, the sensitivity and specificity of the iRUT were 91.5% and 93.6%, respectively and the positive predictive value (PPV) was 86% and the Negative Predictive Value (NPV) was 96.3%.

Fifty eight specimens were tested for *H. pylori* antigen using ImmunoCard STAT rapid test. The test showed positive in 19/58 (32.8%) and negative in 39/58 (67.2%) (Table 2). However, only 35 specimens for which the gold standard (culture or histopathology) were available were analyzed in this study. The true positive were 6, false positive were 6, false negative was 0 and true negative were 23 (Table 2). Thus, the sensitivity for Immuno CardSTAT rapid test was 100% and the specificity was 79.3%. The PPV was 50% and the NPV was 100%. Table 4 shows number of positive results obtained by ImmunoCard STAT compared to other tests.

Table 3 shows that iRUT was found to be 91.5% sensitivity, 93.6% specificity and its positive predictive value and negative values were 86 and 96.3%, respectively. On the other hand, immunoCardSTAT showed 100% sensitivity and 79.3% specificity and its positive predictive values and negative values were 50% and 100%, respectively.

Table 1: Sensitivity and specificity of in-house rapid urease test

	H. pylori		
Results	Positive	Negative	Total
IRUT positive	43	7	50
Negative	4	103	107
Total	47	110	157

Table 2: Sensitivity and specificity of ImmunoCard STAT

	H. pylori		
Results	Positive	Negative	Total
ImmunoCard STAT Positive	6	6	12
Negative	0	23	23
Total	6	29	35

Table 3: Summary of results of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of iRUT and ImmunoCard STAT

	Sensitivity	Specificity	PPV	NPV
Test	(%)			
iRUT	91.5	93.6	86	96.3
ImmunoCard STAT	100.0	79.3	50	100.0

Table 4: Comparison of ImmunoCard STAT with the results of other tests

Positive test	No. of positive result (%)
UHCS	4 (11.4)
UHS	2 (5.7)
US	1 (2.9)
ImmunoCard STAT	5 (14.3)
All negative	23 (65.7)
Total	35 (100)

U: iRUT; H: Histopathology; C: Culture; S: ImmunoCard STAT

This study observed that the iRUT has a high sensitivity (91.5%) and specificity (93.6%) with PPV of 86% and NPV of 96.3%. One patient had positive urease after 24 h; this could be due to presence of low bacterial density. Otherwise, all the positive specimens gave results within half an hour. Therefore, the iRUT is a good screening tool for diagnosis of *H. pylori* infection with a much lower cost compared to commercial RUT available. Being a 'home-made' test, the performance of the iRUT may vary according to centers depending on the preparation of the media and also other factors. Factors such as buffered or unbuffered, solid, semi-solid or liquid media, concentration of the phenol red indicator, incubation time and temperature and number of biopsies taken may all play a role (Chomvarin *et al*., 2006).

There are various methods available to diagnose *H. pylori* infection; however, there is not a single method that can be absolutely reliable to detect *H. pylori* infection. Culture, although is truly specific and the sensitivity in an experienced laboratories may be greater than 95%, may have a low sensitivity due to the fastidious nature of the bacteria requiring appropriate transport condition to ensure viability of the bacteria and laboratory culture must adhere to the strict growth requirement. Other methods of diagnosis of *H. pylori* are also simpler, less prone to variability and timelier. There is also no real consensus of what should be the gold standard test for diagnosis but as a general rule, *H. pylori* is considered positive when culture is positive or in the event of negative culture, both histopathology and urease test are positive appears to be a good compromise (Megraud and Lehours, 2007).

In this study, positive culture or histopathology was chosen as the gold standard of diagnosis. Better detection rate was obtained in histopathological specimens in this study because it is not subjected to transport problems. However, it is dependent on the quality of the specimens, the expertise of the pathologist and efforts made at finding the organism. Choice of appropriate stain is also important.

The rapid urease test is not taken as a gold standard because we were using an in-house rapid urease test which has never been validated before. In this study, a total of 151 cultures were performed but only 21 (13.9%) were positive and 130 (86.1%) were negative. The positive rate was low compared to histopathology examination whereby out of 152 patients, 46 (30.3%) were positive and 106 (69.7%) were negative. The histopathology results were comparable with the iRUT in which out of 157 patients, 50 (31.8%) were positive and 107 (68.2%) were negative. The low rate of positive cultures in this study could be due to several reasons such as delay in transport and subsequently in processing the specimens, although it was clearly instructed that all specimens for culture should be immediately sent to the laboratory and processed immediately by the laboratory personnel but human factors does have an important role in a busy setting such as Hospital Kuala Lumpur being a tertiary referral centre for most disciplines. The choice of transport and culture media are also important. In this study, the specimens were transported in saline solution and cultured on blood agar in a microaerophilic condition for 6 days. The saline should be adequate in sustaining viability of the bacteria for 4 to 6 h but a more suitable media such as Stuart's medium or supplemented brain heart infusion broth is recommended for longer period (Han *et al*., 1995). The plates were also incubated for 6 days only; perhaps a better yield can be obtained if the cultures are incubated longer, such as 12 days as recommended to be optimal for detecting the bacteria (Kullavanijaya *et al*., 2004).

Foroutan *et al*. (2010) conducted an experiment to determine the sensitivity and specificity of *H. pylori* infection using RUT and observed that overall sensitivity, specificity and accuracy rate of RUT were 98.6, 99.29 and 99.04%, respectively. Our results are in close agreement with the findings described by the authors. Manes *et al*. (2005) performed an accuracy of a new monoclonal stool antigen test in post-eradication assessment of Helicobacter pylori infection: comparison with the polyclonal stool antigen test and urea breath test and observed that Urea breath test showed the best performances with sensitivity 98.9% and specificity 99.5% which are close agreement with the present findings.

Stool antigen detection is a fairly new non-invasive test introduced to the field of *H. pylori* detection. As mentioned earlier, there are many kits now available for this test. In this study, it was observed that the rapid stool antigen test using ImmunoCard STAT Immunochromatogrphy method had 100% sensitivity; however, the specificity was relatively low at 79.3%. The PPV was 50% and the NPV was 100%. Although, the test had a 100% sensitivity and NPV, the specificity and PPV were low which could be attributed to the small sample size in this study. Nevertheless, it is a good screening test because it is non-invasive and rapid; results can be obtained within 15 min. It is also easy to perform and can be used as an office-based test or in an epidemiological study especially in areas where endoscopic and laboratory access is difficult. However, in local settings, the use is perhaps limited due to the high cost for each test locally. In three studies in the United Kingdom and Italy, it was noted that the sensitivity and

the specificity of the test ranged from 85 to 91.3% and 89.4 and 93.5%, respectively (Krogfelt *et al*., 2005). Stool antigen tests have been an interesting choice to consider especially for pediatric patients and various studies have been done using different kits or techniques, however, the results were still inconsistent especially in the young age group (Elitsur and Yahav, 2005). Stool tests are more convenient particularly in paediatric patients because stool sample can be obtained without their active collaboration. The performance of ImmunoCard STAT was evaluated in stool samples from 159 children in Munich and Vienna and found that the pretreatment sensitivity and specificity to be 88.1 and 88.1% respectively with accuracy of 83.5% (Antos *et al*., 2005). Another study by Li *et al*. (2004) in China observed that from 53 stool samples tested using ImmunoCard STAT the sensitivity was 92.6%; specificity was 88.5%; PPV was 92% and NPV was 90.6%, respectively. They concluded that the ImmunoCard STAT is a simple noninvasive and accurate test for the diagnosis of *H. pylori* infection.

While most studies found a significant difference in the prevalence of *H. pylori* infection in relation to age, there is usually no significant difference in relation to gender (Mitchell and Megraud, 2002). This study also showed no significant difference in the prevalence of *H. pylori* infection between male and female patients ($p = 0.072$) consistent with other Malaysian study (Goh and Parasakhti, 2001), similar findings were also noted in Malaysian children (Boey *et al*., 1999). However, in a large French cross sectional study, a significant lower prevalence of *H. pylori* infection was found in females compared to males (Broutet *et al*., 2001).

The ImmunoCard STAT rapid test may have a potential role as an alternative noninvasive screening test for patients especially for paediatric age group. However, the performance in paediatric patients may need to be studied separately. Furthermore, this study only looked at a small number of patients. Study involving a larger sample may be needed in the future. The use of culture for detection of *H. pylori* infection can be improved by ensuring that there is no delay in transport of sample to the laboratory and in the processing of specimen or a more lasting transport media can be used. Culture is important for obtaining isolates for study of antibiotic sensitivity pattern peculiar to local settings and also to allow molecular studies on pathogenicity and mutations conferring resistance to commonly used *H. pylori* eradication therapy.

Mutaz (2010) gave a comprehensive idea about the detection and treatment of *H. pylori*. The author mentioned that the sensitivity, specificity and positive and negative predictive values of the same test kit may differ in different ethnic or geographic populations.

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