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Rapid Urease Test, Touch Cytology and Histopathologic Assessment in Determining Infection by *Helicobacter pylori* in Outpatient Setting

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Abstract: *Helicobacter pylori* (HP) is a common cause of gastric infection with serious consequences which is detected by different methods. This study aimed at comparing the diagnostic value of Rapid Urease Test (RUT), Touch Cytology (TC) and histopathologic assessment in outpatients setting. In this cross-sectional study, 51 candidates for upper gastrointestinal endoscopy were recruited in Tabriz Imam Khomeini Teaching Centre in a 24 month period of time. Three biopsy specimens were obtained from gastric antrum during endoscopic intervention. The RUT, TC and histopathologic assessment were performed on each biopsy specimen in each patient. Definite infection by HP was considered when at least 2 out of 3 tests indicated presence of infection. Fifty one patients, 29 females and 22 males with a mean age of 40.10±12.54 (range: 18-72) years enrolled in this study. Infection by HP was definite in 41 cases (80.4%). The infection rates by RUT, TC and histopathologic examination were 82.4, 82.4 and 76.5%, respectively. The sensitivity, specificity and accuracy of RUT, TC and histopathologic assessment were 92.7, 60 and 66.75%; 100, 90 and 98% and 95.1, 100 and 96.1%, respectively. There were significant agreements between outcomes of the three methods in diagnosis of infection by HP. In conclusion, TC was the most sensitive and histopathologic assessment was the most specific method in diagnosis of infection by HP in outpatient setting. The diagnostic value of RUT was rather low in this regard.

Key words: *Helicobacter pylori*, rapid urease test, touch cytology, histopathologic assessment, endoscopy

INTRODUCTION

Helicobacter pylori (HP) which was previously know as *Campylobacter* is a spiral gram-negative bacterium with high affinity to epithelium of human stomach (Sheikhian *et al*., 2011; Rasmi *et al*., 2009; Cotran *et al*., 1999; Day and Morson, 2003). Gastritis, Peptic ulcer and gastric malignancies have been attributed to infection by HP (Moghaddam *et al*., 2009; Khedmat *et al*., 2007; Nahaei *et al*., 2008). On the other hand, the prevalence of infection by HP is thought to be dramatically high in developing countries like Iran. Actually, it is assumed that almost half of the world's population is infected by HP (Zamani and Daneshjou, 2006; Moghaddam and Moghaddam, 2008). So, the diagnosis of infection by HP is a pivotal step in planning therapeutic approaches. Screening modalities are even of greater importance due to high prevalence of this infection in developing countries (Mahmood and Hamid, 2010). Different invasive and non-invasive screening methods have been ever introduced for detection of infection by HP with varying accuracies. There is no consensus on the most appropriate method in diagnosis of infection by HP in the literature. Diagnostic accuracy of many tests varies greatly in different settings (Sadeghifard *et al*., 2006; Suhaila *et al*., 2010). This microorganism colonizes in gastric mucosa, especially the antrum or cardia or lives freely on the gastric surface (Mills, 2007). HP could be seen after staining the infected specimens by Hematoxylin-Eosin (HandE), Giemsa, Wartin-Stary, Alchin yellow, Toluidin Blue or Silver dyes. Immunoreactive methods and Polymerase Chain Reaction (PCR) are also proposed in this regard (Rosai and Ackerman, 2004). During the upper gastrointestinal (GI) endoscopy, detection of HP is achievable by different methods including Rapid Urease Test (RUT) and Touch Cytology (TC). The latter is more effective when the number of microorganisms is limited (Cibas and Ducatman, 2009). This study aimed at comparing the diagnostic accuracies of three methods in detection of infection by HP including RUT, TC and histopathologic assessment in a group of patients undergoing upper GI endoscopy.

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MATERIALS AND METHODS

Subjects: In this cross-sectional study, 51 outpatient candidates of upper GI endoscopy were recruited in Tabriz Imam Reza Educational Centre in a 12-month period of time from June 2010 to June 2011. All the patients complained from GI symptoms and there were indications of upper GI endoscopy for further evaluation recommended by a skilled specialist in GI diseases.

There was no history of other diseases in target population and anti-HP treatments or bismuth subcitrate were discontinued at least 4 weeks before enrollment.

Procedures: During the endoscopic procedure, 3 biopsy specimens were obtained from antrum, 3-4 cm superior to the pylorus. The RUT, TC and histopathologic assessment were performed on each biopsy specimen in each patient.

***RUT:** The specimen was placed into a medium containing urea and phenol red. Change of the color from yellow to red was considered as a positive outcome; i.e., infection by HP.

***TC:** After spreading and drying the specimen on a slide, staining was performed by Giemsa and presence of the microorganism was investigated under magnification by light microscopy.

***Histopathologic (biopsy) assessment:** The specimen was placed in 10% formalin solution. Paraffin-embedded specimen was stained by HandE and Giemsa.

Definite infection by HP was considered when at least 2 out of 3 tests yielded positive (infected) outcome (Trevisani *et al*., 1997).

Study design and variables: Results of the triad tests with regard to detection of infection by HP were compared with definite outcome. Intra-tests agreements were also investigated. Other studied variables were the patients' demographics and underlying causes of upper GI endoscopy. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences.

Statistical analysis: Statistical evaluation was made using SPSS for Windows V 18.0 (SPSS Inc., Il, USA). Data were shown as frequency (percentage) or Mean±SD. Agreement between outcomes of different tests was defined by determining Kappa coefficient. The agreement was considered high when the kappa coefficient was >0.5. This agreement was considered intermediate when the kappa coefficient was between 0.3 and 0.5. The p values less than 0.05 were regarded as significant. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were calculated by the following formulas:

$$\text{Sensitivity}=\frac{\text{True positive}}{(\text{True positive}+\text{False negative})}$$

$$\text{Sensitivity}=\frac{\text{True negative}}{(\text{True negative}+\text{False positive})}$$

$$\text{PPV}=\frac{\text{True positive}}{\text{Positive cases}}$$

$$\text{NPV}=\frac{\text{True negative}}{\text{Negative cases}}$$

$$\text{Accuracy}=\frac{(\text{True positive}+\text{True negative})}{\text{Total cases}}$$

RESULTS

Fifty one patients including 29 females and 22 males with a mean age of 40.10±12.54 years were enrolled in this study. Underlying causes of upper GI endoscopy were heart burn in 31 patients, dyspepsia in 9 patients, nausea/vomiting in 5 patients, weight loss in 3 patients, dysphagia in 2 patients and diarrhea in 1 patient. Based on the diagnostic criterion, HP infection was present in 41 patients (80.4%) (Table 1).

The HP infection was present in 82.4% of the subjects by both the RUT and TC methods and 76.5% of the population by biopsy. The highest and lowest true positive results were by the TC (80.4%) and the RUT (74.5%), respectively. The highest and lowest true negative results were by the biopsy (19.6%) and the RUT (11.8%), respectively. The highest and lowest false positive results were by the RUT (7.8%) and biopsy (0%), respectively. The highest and lowest false negative results were by the RUT (5.9%) and the TC (0%), respectively. The TC and RUT were the most and the least sensitive methods in diagnosis of HP infection, respectively (100% vs. 92.7%). The biopsy and RUT were the most and the least specific methods in this regard, respectively (100% vs. 60%). The highest PPV was documented by biopsy and the lowest PPV by the RUT (100 and 90.5%, respectively). The highest NPV was seen by the TC and the lowest PPV by the RUT (100 and 66.7%, respectively). The most and the least accurate methods in detection of HP infection were the TC (98%) and RUT (82.3%), respectively (Table 2).

Considering the final diagnosis as the definite result in detection of the HP infection, there was a significant

Table 1: Demographics and general data of the studied population

Variable	Value
Gender	
Male	29 (56.7)
Female	22 (43.1)
Age (year)	40.10±12.54 (18-72)
Cause of endoscopy	
Heart burn	31 (60.8)
Dyspepsia	9 (17.6)
Nausea/vomiting	5 (9.8)
Weight loss	3 (5.9)
Dysphagia	2 (3.9)
Diarrhea	1 (2)
Helicobacter pylori infection	41 (80.4)

Data are presented as frequency (percentage) and Mean±SD (range)

Table 2: Outcomes of the triad tests in diagnosis infection with *Helicobacter pylori*

Variable test	Rapid urease	Touch cytology	Biopsy
Infection	42 (82.4%)	42 (82.4%)	39 (76.5%)
True positive	38 (74.5%)	41 (80.4%)	39 (76.5%)
True negative	6 (11.8%)	9 (17.6%)	10 (19.6%)
False positive	4 (7.8%)	1 (2%)	0 (0%)
False negative	3 (5.9%)	0 (0%)	2 (3.9%)
Sensitivity	92.7%	100%	95.1%
Specificity	60%	90.5%	100%
Positive predictive value	90.5%	97.6%	100%
Negative predictive value	66.7%	100%	83.3%
Accuracy	82.3%	98%	96.1%

Table 3: Agreements between the outcomes of the triad tests in diagnosis infection with *Helicobacter pylori*

Test	Kapppa	p-value
Rapid urease vs. final diagnosis	0.55	<0.001
Touch cytology vs. final diagnosis	0.94	<0.001
Biopsy vs. final diagnosis	0.88	<0.001
Rapid urease vs. Touch cytology	0.46	0.001
Touch cytology vs. Biopsy	0.82	<0.001
Rapid urease vs. Biopsy	0.46	0.001

p<.05 is considered statistically significant

intermediate agreement between the outcome of the RUT and the final diagnosis (kappa = 0.55, p<0.001). This agreement was also significant but high between the outcome of the TC and the final diagnosis (kappa = 0 .94, p<0.001). Finally, there was a significant high agreement between the outcome of the biopsy and the final diagnosis (kappa = 0.88, p<0.001). There was a significant intermediate agreement between the outcomes of RUT and TC (kappa = 0.46, p = 0.001). There was also a significant high agreement between the outcomes of TC and histopathologic assessment (kappa = 0.82, p<0.001). The agreement between the outcomes of RUT and histopathologic assessment was also significantly intermediate (kappa = 0.46, p = 0.001) (Table 3).

DISCUSSION

In this study, outcomes of three diagnostic tests in detection of infection with HP were compared. These triad tests were RUT, TC and histopathologic assessment (biopsy) on specimens collected from antrum. As there is not yet a mere gold standard method for diagnosis of infection with HP, the patients with at least 2 similar results out of 3 tests were considered as definitely infected cases (Trevisani *et al*., 1997). This limitation is mainly due to discrete infection of HP with consequent defective cultures, as well as errors of interpretation (Kolts *et al*., 1993). In the current study, the sensitivity, specificity and accuracy of the RUT were 92.7, 60 and 66.7%, respectively. Relevant percentages were 100, 90 and 98% for the TC and 95.1, 100 and 96.1% for the histopathologic assessment, respectively. In a series by Hashemi *et al*. (2008), 100 specimens from antrum were evaluated. They considered 100% conformity of three tests as the final outcome of infection. Accordingly, the infection was diagnosed in 46%, they used different staining methods in TC. The highest sensitivity and specificity were reported by the histopathologic assessment and RUT, respectively (100% for all the variables). Specificity of the TC varied between 70.4 and 89% according to the type of staining employed. They proposed the RUT as starting test and the TC in the second step if there was a negative result for the first test in spite of high suspicious. In our study the TC and biopsy were the most sensitive and specific methods, respectively. Despite the results of Hashemi *et al*. (2008), the RUT was not the method of choice in our series with rather low specificity. This conflict may be due different criteria employed for definite infection. We encountered two false negative results in the histopathologic assessments which both were positive in the other two tests. Retesting these two specimens by histopathologic assessment revealed that they were really infected with HP. This finding further confirms accuracy of our gold standard approach. Yamamoto (2001) reported high agreement between the results of culture, histopathologic assessment and TC in diagnosis of HP infection. In this study, the TC was introduced as a reliable, fast and cost-effective method in this regard. Present results are also in conformity with this report. Tokunaga *et al*. (2000) also concluded that the TC and a modified type of RUT are appropriate in detecting infection with HP. The sensitivity, specificity and accuracy of the TC were 91%, 100 and 95% in this series. Present results are very similar with outcomes of the mentioned study, too. Saksena *et al*. (2000) also proposed the TC as a reliable method in detecting HP in gastric specimens. We confirm this opinion. Overall, present results are very similar with those in the literature. However, apparently the accuracy of RUT is lower in present study comparing with similar reports. The criteria in selecting patients and their level of cooperation may justify this heterogeneity. Base on the results of present study, the TC is the most sensitive and

accurate approach in diagnosis of HP infection in appropriate patients. Although, its specificity was lower than that of the histopathologic approach, is still significantly high. Debongnie *et al*. (1994) also showed a high agreement between the results of biopsy and TC. Our findings are in line with this report. Presence of microorganisms in or under gastric superficial mucosal layer and preservation of this areas in specimens required for TC justify this high accuracy (Mendoza *et al*., 1993; Pinto *et al*., 1991). Furthermore, it is thought that the number of microorganisms may be insufficient for histopathologic assessment if the biopsy specimens are rather small or with low quantities of superficial epithelium. These specimens are sufficient for TC (Genta *et al*., 1994).

CONCLUSION

Comparing accuracies of the triad tests in detecting infection with HP, this study showed that the TC was the most sensitive and the histopathologic assessment was the most specific method. The RUT was not an appropriate method in this regard. The histopathologic assessment could be replaced by the TC in detecting infection with HP; however, biopsy is still mandatory for evaluating severity of mucosal lesion and presence of atypia.

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