Original Article

Serologic Detection of *Helicobacter pylori* Infection and the Required Cautions

Maryam Esmaeili[1+], Samaneh Saberi[1+], Mehdi Alikhani[1], Mojgan Hatefi[1], Mohammad Tashakoripour[2], Mahmoud Eshagh Hosseini[2], Mohammad Ali Mohagheghi[3], Marjan Mohammadi[1*]

[1]HPGC Research Group, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran;
[2]Department of Gastroenterology, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran;
[3]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[+]Maryam Esmaeili and Samaneh Saberi share first authorship.

Received May 16, 2016; Accepted June 17, 2016

Introduction: *Helicobacter pylori*, as an etiologic cause of peptic ulcers and gastric cancer, should be tested and treated. The true state of infection can only be detected by isolation of gastric biopsies through the invasive method of gastroscopy. However, there are several non-invasive methods for detection of infection, the most common of which is serology. **Methods:** Here we have evaluated the efficacy of two commonly used commercial IgG-based ELISA kits (Kit-1 and Kit-2) against the endoscopy (biopsy)-based methods of rapid urease test (RUT) and bacterial culture. Our study population included 754 subjects categorized as having: 1) nonulcer dyspepsia (NUD, N=485), 2) peptic ulcer disease (PUD, N=65), and 3) gastric cancer (GC, N=204). **Results**: The rates of agreement between the results obtained by Kit-1, Kit-2 and both kits with culture/RUT were 62.4% (318 of 500, P=0.0001), 74.4% (183 of 246, P<0.0001), and 81.8% (117 of 143, P<0.0001), respectively. The agreement rate between the two kits, regardless of the results of the culture and RUT, was 83.6% (147 of 176, P<0.0001), leaving 16.4% of the subjects with discrepant results. The sensitivity rate and more drastically the specificity rates (against biopsy-based tests), claimed by Kit-1 (100% and 75%) and Kit-2 (>96% and >99%) were significantly reduced (Kit-1: 94.4% and 40.3%; Kit-2: 86.5% and 71%) for the Iranian population. **Conclusion**: Our data raises questions regarding the accuracy of commercial IgG-based ELISA kits for the detection of *H. pylori* infection. Therefore, caution should be practiced when such tests are used as the sole basis of medical decision making. *J Med Microbiol Infec Dis, 2016, 4 (1-2): 11-15.*

Keywords: Serology, ELISA, Sensitivity, Specificity, Gold Standard.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infect more than half of the adult population worldwide, causing a variety of gastrointestinal disorders, ranging from gastritis to peptic ulcer diseases and in the worst case scenario, the fatal cancer of the stomach [1]. Although the stomach is exposed to an array of ingested external ingredients, including toxins and carcinogens, it remains fairly secluded. Thus, any direct examination of its epithelial lining requires the invasive and uncomfortable procedure of endoscopy and collection of gastric biopsies. Due to its complexity and discomfort, most dyspeptic patients, in need of such an examination, prefer to avoid this invasive procedure and therefore, inadvertently remain at risk of the silent development of the mentioned gastrointestinal disorders [2].

H. pylori, being a type one carcinogen [3], colonize the gastric epithelial lining, and the only true indication of its existence is the actual recovery of this organism from the stomach [4]. There are, however, noninvasive means of detecting *H. pylori* infection of the stomach [5]. These include the urea breath test (UBT), serology and the stool antigen test, all of which encompass some shortcomings [1]. Of these, serology is the most convenient and most commonly used method [4]. Nowadays, physicians readily request serology for their dyspeptic patients and prescribe medications based on the pertinent results. But, rarely is the accuracy of such tests questioned. In this study, we have evaluated the efficacy of two commonly used commercial IgG-based ELISA kits against the endoscopy (biopsy)-based methods of rapid urease test (RUT) and bacterial culture. We have also assessed the rate of agreement between the results obtained by the two independent commercial kits.

MATERIAL AND METHODS

Subjects. Our study population included 754 subjects categorized as having: 1) nonulcer dyspepsia (NUD, N=485), 2) peptic ulcer disease (PUD, N=65), and 3) gastric cancer (GC, N=204).

*__Correspondence:__ Marjan Mohammadi
HPGC Research Group, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, No. 69, Pasteur Ave, Tehran, Iran, 1316943551.
Email: marjan.mohammadi@pasteur.ac.ir
Tel/Fax: +98 (21) 66480780

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Subjects have been sampled during endoscopy or gastric surgery. Gastric specimens were obtained for RUT and bacterial culture. Fasting blood samples were collected for serology (ELISA). Demographic factors were documented *via* personal interviews. Sample collection was carried out according to protocols approved by the National Committee on Ethical Issues in Medical Research, Ministry of Health and Medical Education of Iran; Ref No. 315.

***H. pylori* Status**

- **Bacterial Culture.** Gastric specimens (C1, I, and A2) were homogenized and cultured onto *H. pylori* special peptone agar (HPSPA) medium [6], supplemented with 7% defibrinated sheep blood. Plates were incubated under microaerobic conditions (10% CO2, 5% O2, and 85% N2), at 37°C for 5-7 days. The identity of the grown *H. pylori* colonies was confirmed by wet mount, urease, catalase, and oxidase tests.
- **RUT.** RUT was carried out by placing fresh gastric tissue (I) into RUT media (Avaco, Iran) and documenting its yellow-to-purple color transformation within 4 h.
- **Serology (ELISA).** The presence of serum IgG antibodies against *H. pylori* was detected by two commercial kits (Kit-1 and Kit-2), according to the manufacturers' instructions. Sera with titers above and below the defined cut-off values were indicated as positive and negative, respectively. Those at borderline (determined by the kit) were considered equivocal.
- For the purposes of this study culture/RUT double positive and double negative subjects were assigned as *H. pylori*-positive and -negative, respectively and were used as "gold standards" (GS).

Statistical analysis. Continuous and categorical variables were described as mean (± SD) and number (%), respectively. MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium) was used to calculate the sensitivity and specificity rates. The values are given with 95% confidence interval. Each method was tested against the GS. In all analyses, a two-sided value of less than 0.05 was regarded as significant. Statistical analyses were done using IBM SPSS statistics software (version 23).

RESULTS

Table 1 presents the demographic characteristics of the studied subjects, the test results for each group and their statistical differences. The age range and gender distribution varied between the two groups. As expected, the GC patients were older, with a male gender dominance (P<0.0001).

Gastric biopsies were obtained from every included subject and underwent bacterial culture and RUT. The GS for the status of *H. pylori* infection were defined as culture/RUT double-positive or -negative, against which the efficacies of the two commercial serologic (ELISA) kits were evaluated.

Table 1. Demographic characteristics of the study population and *H. pylori* test results

Demographic and test variables		Clinical Groups				*P* values
		NUD	PUD	GC	Total	
Number of Subjects		485	65	204	754	-
Age		48.2 ± 13.4	51.9 ± 11.6	61.7 ± 11.4	52.1 ± 13.9	0.0001
Gender	*Female*	276	28	57	361	0.0001
	Male	209	37	147	393	
	Total	485	65	204	754	
Culture/RUT	*Double Negative*	242	18	48	308	0.011
	Double Positive	192	35	45	272	
	Pos/Neg	26	7	9	42	
	*ND**	25	5	102	132	
	Total	485	65	204	754	
Serology (Kit-1)	*Negative*	93	11	34	138	0.804
	Positive	327	51	122	500	
	Border	28	2	10	40	
	ND	37	1	38	76	
	Total	485	65	204	754	
Serology (Kit-2)	*Negative*	85	5	20	110	0.426
	Positive	101	12	17	130	
	Border	10	-	2	12	
	ND	289	48	165	502	
	Total	485	65	204	754	
Both kits (Kit-1 & Kit-2)	*Double Negative*	52	3	-	55	0.956
	Double Positive	78	9	1	88	
	Neg/Pos	11	2	-	13	
	Neg/Border	10	1	-	11	
	Positive/Border	4	1	-	5	
	Border/Border	4	0	-	4	
	ND	326	49	203	578	
	Total	485	65	204	754	

*ND= Not Determined

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We primarily evaluated the serostatus of 510 subjects by commercial Kit-1 (Fig. 1). This kit was able to correctly detect 89.4% (220 of 246) of GS-positive and 37.1% (98 of 264) of GS-negative subjects. A small fraction (6.7%, 34 of 510) yielded equivocal (borderline) results and was excluded from the analyses. The total rate of agreement was 62.4% (318 of 500, *P*=0.0001). The sensitivity and specificity rates were determined as 94.4% (95% CI: 90.7% - 97%) and 40.3% (95% CI: 34.1% - 46.8%), respectively. When this analysis was repeated in the three substrata of subjects, namely NUD, PUD and GC patients, the rate of agreement (NUD=60.5%, PUD=82.3%, GC=57.4%) was found higher in the PUD group.

Secondly, 246 of our subjects were evaluated by commercial Kit-2 (Fig. 2), which was able to correctly detect 83.3% (90 of 108) of GS-positive and 67.4% (93 of 138) of GS-negative subjects. A small fraction (4.5%, 11 of 246) yielded equivocal (borderline) results and was excluded from the analyses. The total rate of agreement was 74.4% (183 of 246, *P*=0.0001). The sensitivity and specificity rates were determined as 86.5% (95% CI: 78.5% - 92.4%) and 71% (95% CI: 62.4% - 78.6%), respectively. When this analysis was repeated in the three substrata of subjects, namely NUD, PUD and GC patients, the rate of agreement (NUD=72.7%, PUD=93.8%, GC=71.9%) was found higher in the PUD group.

A subgroup of these subjects (N=176) was serologically assessed by both kits (Kit-1 and Kit-2). A significant fraction (18.8%, 33 of 176) yielded equivocal (borderline) results and was excluded from the analyses (Fig. 3). The remaining double-positive and double-negative results were evaluated against the GS biopsy-based tests. The combination of the two commercial kits successfully detected 91.9% (68 of 74) of GS-positive and 71% (49/69) of GS-negative subjects. The combined rate of agreement between the two kits and the GS tests was 81.8% (117 of 143, *P*=0.0001). Subsequent to the exclusions of the Kit-1/Kit-2 discrepant results (18.8%), the combination of the two kits resulted in the improvement of the sensitivity rates up to 91.9% (95% CI: 83.2% - 97%), as compared to the commercial Kit-2 (86.5%). But the specificity rate remained unchanged (71%, 95% CI: 62.4% - 78.6%). When this analysis was repeated in the three substrata of subjects, namely NUD, PUD and GC patients, the rate of agreement (NUD=80.4%, PUD=91.7%, GC=NA) was again found higher in the PUD group.

The agreement rate between the two kits, regardless of the GS test results, was 83.6% (147 of 176, *P*<0.0001) leaving 16.4% of the subjects with discrepant results (Table 2). When this analysis was repeated in the three substrata of subjects, namely NUD, PUD and GC patients, the rate of agreement (NUD=81.5%, PUD=80%, GC=NA) was found similar amongst different clinical groups.

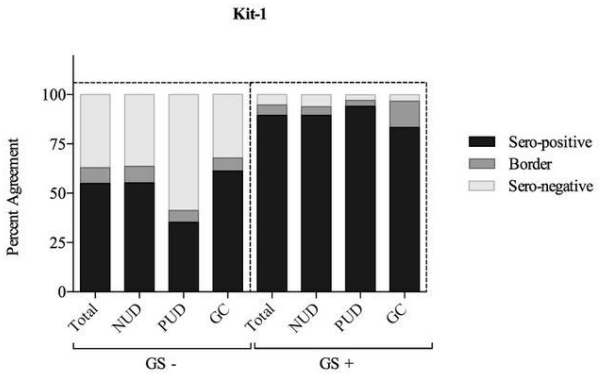


Fig. 1. The rate of performance of Kit-1 against GS tests

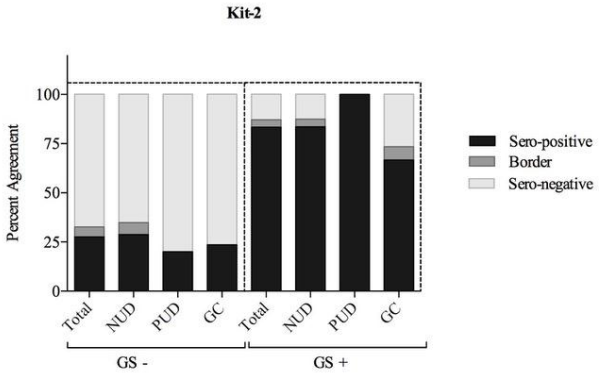


Fig. 2. The rate of performance of Kit-2 against GS tests

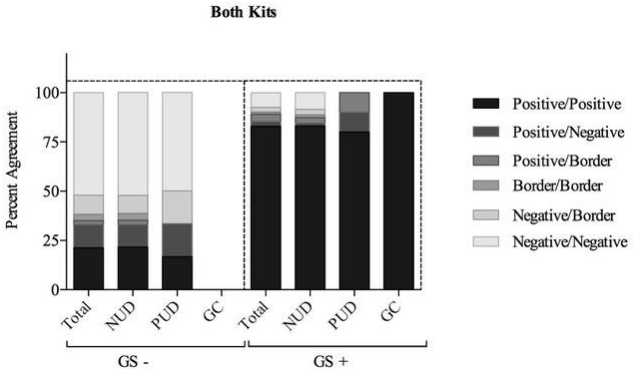


Fig. 3. The rate of performance of both kits (Kit-1 and Kit-2) against GS tests

Table 2. The rate of agreement between Kit-1and Kit-2

Commercial ELISA Kit-2	**Commercial ELISA Kit-1** N (% total)			
	Negative	*Border*	*Positive*	*Total*
Negative	55 (31.3)	10 (5.7)	10 (5.7)	75 (42.6)
Border	1 (0.6)	4 (2.3)	2 (1.1)	7 (4.0)
Positive	3 (1.7)	3 (1.7)	88 (50.0)	94 (53.4)
Total	59 (33.5)	17 (9.7)	100 (56.8)	176 (100)

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DISCUSSION

Serology, as a non-invasive and inexpensive means of detecting *H. pylori* infection, is quite commonly used by the medical community [7]. However, a positive serology assay alone is not indicative of an active *H. pylori* infection of the stomach [5]. The true state of infection is determined by the actual recovery of the bacterium from the gastric mucosa, by the invasive means of endoscopy [4]. Keeping in mind, however, that even then, false negative results can occur due to the patchy distribution of *H. pylori* among the gastric lining [8]. Therefore, non-invasive methods such as UBT [9], if accurately performed, can sometimes better reflect the true state of infection.

In this study, we have assessed the accuracy of two different widely used commercial IgG-based ELISA kits in reflecting the state of *H. pylori* infection, as assessed against the two biopsy-based GS methods of culture and RUT. In order to avoid undue errors, *H. pylori*-positive and *H. pylori*-negative subjects were defined as subjects who were found double-positive or double-negative for both tests of culture and RUT, respectively. Having done so, we discovered a serious decline in the performance criteria of the two kits, one more than the other. The sensitivity and specificity rates against biopsy based GS, claimed by Kit-1, was 100% and 75%, respectively. In our population, however, the sensitivity rate of the kit declined down to 94.4% and the specificity rate was nearly halved to a low rate of 40.3%. A similar, but less drastic reduction appeared for Kit-2, whose sensitivity and specificity rates were claimed as >96% and >99%, respectively. The former rate was reduced to 86.5% and the latter to 71%, suffering an approximate 30% reduction. These observations could be due to a number of reasons including the existing heterogeneity among *H. pylori* strains infecting patients from different geographic regions [10]. As a result, the *H. pylori* antigens used for these foreign kits, may fail to correctly reflect the serostatus of Iranian patients. However, it may also be argued that the biopsy-based (GS) tests are error-prone. These errors include 1) isolation of gastric biopsies from locations, where *H. pylori* is falsely absent [8, 11], 2) external contamination of the RUT test with formalin [12], blood [13], *etc.*, and 3) inadequate bacterial culture facilities, either of which may mount to false positive and/or negative test results. In order to address these issues and determine the consistency of the commercial kits, we have assessed their agreements against each other, regardless of the GS test results. This analysis showed that the two widely used commercial IgG-based ELISA kits bore only 83.6% rate of agreement. In other words, in 16.4 percent of the cases, the two kits produced discrepant results. The latter findings may partly be explained by the source of *H. pylori* strains (antigens), used for each kit, being heterogenic and highlights the crucial need for the development of local ELISA kits, incorporating antigens collected from local *H. pylori* strains. There are, however, other reasons, due to which serum IgG-based ELISA may fail to accurately reflect the state of *H. pylori* infection. These include a possible lack of seroconversion from IgM to IgG following *H. pylori* infection [14], yielding false negative serology results in *H. pylori*-infected subjects. On the other hand, serum antibodies to *H. pylori* infection are long lasting and may persist long after the infection has been eradicated and therefore produce false positive serology results, in *H. pylori*-negative subjects. Our observation of the higher accuracy of the tested ELISA kits in PUD patients in comparison with the other two clinical groups could originate from the higher rates of bacterial colonization [15] and persistence [16] in the gastric epithelium of these subjects, as opposed to those who may suffer from gastric atrophy.

Noninvasive tests for detection of *H. pylori* infection, although not highly accurate, enjoy the advantage of low cost and accessibility [15]. Therefore, despite the many errors that IgG-based ELISA tests may bear in reflecting the correct status of *H. pylori* infection, they are still used as a very common noninvasive means of detecting *H. pylori* infection, based on which eradication regimens are prescribed. It is essential to keep in mind that any kind of serology results should only be taken into consideration in association with patients medical history and symptoms [5]. Nevertheless, our data raises questions regarding the accuracy of foreign ELISA kits in the detection of *H. pylori* infection and calls for the development and validation of local kits [6, 17, 18], using antigens from local *H. pylori* strains.

ACKNOWLEDGEMENT

This study was supported by a technology development grant, funded by Pasteur Institute of Iran.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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